ABSTRACT

Herbal dietary supplements (HDS) are widely used in complementary, alternative, and integrative medicine, but data on attitudes, behavior, safety, and efficacy are lacking. Using mixed methods, we administered an online survey to >1,000 breast cancer survivors to investigate HDS practices and perceptions and performed in vitro studies assessing the efficacy and toxicity of actein, a bioactive component of the HDS black cohosh (Actaea racemosa). Among cancer survivors, curcumin, flaxseed, and green tea were reported as the most frequently used HDS. Many subjects increased HDS intake after diagnosis and sought web-based information on HDS. In human breast cancer (MCF-7) and liver (HepG2/C3A) cell lines, actein had anti-proliferative and anti-estrogenic effects and did not exhibit hepatotoxicity or affect the action of tamoxifen and raloxifene.
HERBAL DIETARY SUPPLEMENTS: SAFETY, EFFICACY, AND USE BY BREAST CANCER SURVIVORS

By

Team IMAC
(Integrative Medicine And Cancer)

Peter Frechette
Stephanie Galanie
Anna Hung
Sarah Kim
Kelsey Merrick
Krupa Nataraj
Jessica Nooralian
Mihir Patel
Jessica Stevens
Vivian Wang
Albert Zhou

Thesis submitted in partial fulfillment of the requirements of the Gemstone Program
University of Maryland, 2010

Advisory Committee:
Dr. Mark Kantor, Ph.D., Mentor
Dr. David Cantor, Ph.D., Discussant
Dr. John Cardellina, Ph.D., Discussant
Dr. Thomas Flynn, Ph.D., Discussant
Dr. Barbara Sorkin, Ph.D., Discussant
Dr. Roger Tourangeau, Ph.D., Discussant
Dr. Thomas Wang, Ph.D., Discussant
Acknowledgements

Team IMAC would like to thank:

Dr. Mark Kantor, our mentor, for supporting us throughout the past three years

Dr. Thomas Wang and colleagues, for training and guiding us through our laboratory research at the USDA/ARS

Dr. Thomas Flynn and colleagues, for teaching and advising us with our laboratory research at the FDA/CFSAN

Mr. Michael O'Donnell, Supervisory Mathematical Statistician at the FDA/CFSAN, for helping us analyze our hepatotoxicity data

Ms. Ashley Franklin, our statistician, for helping with the analysis of our survey data

Love/Avon Army of Women, for an overwhelming response to our survey

Dr. James Wallace, Dr. Rebecca Thomas, and the entire Gemstone staff, for keeping us on track through their organization, advice, and encouragement

Dr. David Cantor, Dr. John Cardellina, Dr. Barbara Sorkin, Dr. Roger Tourangeau, our discussants, for graciously giving their time to critique our thesis and presentation

Mr. Bob Garber, our librarian, for helping us navigate library resources

Dr. Katerina Thompson, for helping us apply for and obtain HHMI funding

Dr. John Milner, for assisting us with funding via an NCI/USDA agreement

Funding sources: a grant to University of Maryland from the Howard Hughes Medical Institute Undergraduate Science Education Program, collaborators at FDA/CFSAN and USDA/ARS, NCI/USDA agreement, and the Gemstone Program
# Table of Contents

**ACKNOWLEDGEMENTS**  
**TABLE OF CONTENTS**  
**LIST OF TABLES**  
**LIST OF FIGURES**  
**LIST OF ABBREVIATIONS**  

## 1. INTRODUCTION  
1.1 CANCER AND CAM  
1.2 NEED FOR RELIABLE INFORMATION  
1.3 CHOOSING BLACK COHOSH  
1.4 STUDY OBJECTIVES  
1.5 STUDY DESIGN  
1.6 EXPECTED RESULTS  

## 2. LITERATURE REVIEW  
2.1 BREAST CANCER AND INTEGRATIVE MEDICINE  
  2.1.1 Brief Overview of Breast Cancer and Treatments  
  2.1.2 Prevalence of CAM Use  
  2.1.3 Perceptions of Integrative Medicine: Information and Communication  
2.2 BREAST CANCER AND HDS  
  2.2.1 Definition of HDS  
  2.2.2 Patterns of HDS Use  
  2.2.3 Concerns about the Safety of HDS  
2.3 BREAST CANCER AND BLACK COHOSH  
  2.3.1 Prevalence and Patterns of Black Cohosh Use  
  2.3.2 Efficacy of Black Cohosh: Previous Studies  
  2.3.3 Black Cohosh and SERMs  
  2.3.4 Hepatotoxicity of Black Cohosh  

## 3. METHODS  
3.1 SURVEY: HDS USES AND PERCEPTIONS  
  3.1.1 Questionnaire Design  
  3.1.2 Pilot Testing  
  3.1.3 Subject Recruitment  
  3.1.4 Questionnaire Completion  
  3.1.5 Statistical Analysis  
3.2 IN VITRO STUDY: EFFECTS OF ACTEIN ON BREAST CANCER CELL PROLIFERATION  
  3.2.1 Materials  
  3.2.2 MCF-7 Cell Culture  
  3.2.3 SRB Assay for Cell Proliferation  
  3.2.4 Gene Expression Analysis  
  3.2.5 Statistical Analysis  
3.3 IN VITRO STUDY: HEPATOTOXICITY OF ACTEIN IN LIVER CELLS  
  3.3.1 Materials  
  3.3.2 HepG2/C3A Cell Culture  
  3.3.3 DCFDA Assay for Oxidative Stress  
  3.3.4 EROD Assay for Cytochrome P4501A1 Activity  
  3.3.5 Nile Red Assay for Steatosis
5.1 HDS USES AND PERCEPTIONS

5.1.1 Patterns of HDS Use Among Breast Cancer Survivors

5.1.1.1 Most Common Supplements Used Are Soy, Green Tea, Flaxseed, Ginger and Curcumin

5.1.1.2 Patients Consult Internet More Often Than Healthcare Practitioners

5.1.1.3 Family, Friends, Doctors, and Religion Not Influential in HDS Use

5.1.1.4 Demographic Limitations of Sample

5.1.1.5 Black Cohosh Was Used For Menopausal Symptoms

5.1.1.6 Hypothesis About Survivor HDS Use Confirmed

5.1.2 Perspectives on HDS

5.1.2.1 Reported Reasons for Using HDS

5.1.2.2 Beginning or Ending HDS Use

5.1.3 Survey Limited To Online Respondents and Fixed Questions

5.1.4 Contributions to Existing Literature

5.1.5 Other Survey Limitations

5.1.5.1 Ambiguity with Medical Purposes vs. Other Reasons

5.1.5.2 Advertising Bias

5.1.5.3 Problems with Open-ended Questions

5.1.5.4 Source of Sample Population

5.1.5.5 Increased Access to the Internet

5.2 Effects of Actein on Breast Cancer Cell Proliferation

5.2.1 SERMs and Actein Inhibit MCF-7 Cell Proliferation

5.2.2 Actein Alters the Expression of Breast Cancer- and Drug Metabolism-Related Genes

5.3 Hepatotoxicity of Actein in Liver Cells

5.3.1 Hepatotoxicity of Actein Is Concentration Dependent in HepG2/C3A Cells

5.3.2 Interactions Between Actein and SERMs Are Weak and Few

5.4 Conclusions

5.5 Future Studies

APPENDICES

APPENDIX A. Survey

APPENDIX B. Lack of Hepatotoxicity of Tamoxifen and Lack of Interactions Between Actein and SERMs

APPENDIX C. Howard Hughes Medical Institute Grant Application

APPENDIX D. Institutional Review Board (IRB) Approval

APPENDIX E. IRB Addendum

APPENDIX F. IRB Renewal

GLOSSARY

WORKS CITED
List of Tables

Table 1. Demographic variables and HDS use among breast cancer survivors. ......46
Table 2. Cluster analysis of demographic characteristics........................................48
Table 3. Cluster analysis of HDS use.................................................................52
Table 4. Use of HDS before and after breast cancer diagnoses. .........................53
Table 5. Most frequent reasons for using HDS..................................................55
Table 6. Reasons for using HDS..........................................................................57
Table 7. Most frequent side effects treated by HDS. ........................................60
Table 8. Perceived reliability of HDS sources of information.............................67
Table 9. Individual effects of actein, tamoxifen, and raloxifene on hepatotoxicity indicators.................................................................80
Table 10. Combination effects of actein and tamoxifen on hepatotoxicity indicators. .........................................................................................99
Table 11. Combination effects of actein and raloxifene on hepatotoxicity indicators. .........................................................................................104
List of Figures

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Estrogen-receptor pathway in a cell</td>
<td>6</td>
</tr>
<tr>
<td>2</td>
<td>Chemical structure of actein</td>
<td>23</td>
</tr>
<tr>
<td>3</td>
<td>Use of HDS for medicinal and other purposes</td>
<td>49</td>
</tr>
<tr>
<td>4</td>
<td>Relative use of each individual HDS for medicinal and other purposes</td>
<td>50</td>
</tr>
<tr>
<td>5</td>
<td>Use of HDS to treat and/or prevent breast cancer recurrence</td>
<td>54</td>
</tr>
<tr>
<td>6</td>
<td>Correlation of HDS and reasons for use</td>
<td>58</td>
</tr>
<tr>
<td>7</td>
<td>Perceived efficacy of individual HDS</td>
<td>62</td>
</tr>
<tr>
<td>8</td>
<td>Frequency of use of HDS sources of information</td>
<td>63</td>
</tr>
<tr>
<td>9</td>
<td>Perceived reliability of HDS sources of information</td>
<td>65</td>
</tr>
<tr>
<td>10</td>
<td>Influence of various factors on decision to use or not use HDS</td>
<td>68</td>
</tr>
<tr>
<td>11</td>
<td>Concern regarding safety and labeling of HDS</td>
<td>69</td>
</tr>
<tr>
<td>12</td>
<td>Actein, tamoxifen, and raloxifene inhibit MCF-7 cell proliferation</td>
<td>71</td>
</tr>
<tr>
<td>13</td>
<td>Actein, tamoxifen, and raloxifene inhibit estradiol-induced proliferation of MCF-7 cells</td>
<td>72</td>
</tr>
<tr>
<td>14</td>
<td>Actein does not interact with tamoxifen or raloxifene in inhibition of MCF-7 cell proliferation</td>
<td>74</td>
</tr>
<tr>
<td>15</td>
<td>Actein does not interact with tamoxifen or raloxifene in inhibition of estradiol-induced proliferation of MCF-7 cells</td>
<td>75</td>
</tr>
<tr>
<td>16</td>
<td>Actein induces CDKN1A expression at high concentration</td>
<td>76</td>
</tr>
<tr>
<td>17</td>
<td>Actein inhibits the induction of TFF1 by estradiol</td>
<td>77</td>
</tr>
<tr>
<td>18</td>
<td>Actein induces CYP1A1 expression</td>
<td>78</td>
</tr>
<tr>
<td>19</td>
<td>Actein induces VEGFA expression</td>
<td>79</td>
</tr>
<tr>
<td>20</td>
<td>Actein decreases the amount of ROS at 0.76 µM</td>
<td>81</td>
</tr>
<tr>
<td>21</td>
<td>Actein increased CYP1A1 activity</td>
<td>82</td>
</tr>
<tr>
<td>22</td>
<td>Actein does not affect intracellular neutral lipid accumulation</td>
<td>83</td>
</tr>
<tr>
<td>23</td>
<td>Actein does not affect membrane phospholipid accumulation</td>
<td>83</td>
</tr>
<tr>
<td>24</td>
<td>Actein does not affect mitochondrial membrane potential or p-glycoprotein activity</td>
<td>84</td>
</tr>
<tr>
<td>25</td>
<td>Actein does not affect cell viability</td>
<td>85</td>
</tr>
<tr>
<td>26</td>
<td>Actein does not affect albumin production</td>
<td>86</td>
</tr>
<tr>
<td>27</td>
<td>Tamoxifen increases the amount of ROS</td>
<td>87</td>
</tr>
<tr>
<td>28</td>
<td>Tamoxifen increases CYP1A1 activity</td>
<td>88</td>
</tr>
<tr>
<td>29</td>
<td>Tamoxifen increases intracellular neutral lipid accumulation</td>
<td>89</td>
</tr>
<tr>
<td>30</td>
<td>Tamoxifen decreases p-glycoprotein activity at low concentrations</td>
<td>90</td>
</tr>
<tr>
<td>31</td>
<td>100 µM Tamoxifen decreases cell viability</td>
<td>91</td>
</tr>
<tr>
<td>32</td>
<td>100 µM Tamoxifen decreases albumin production</td>
<td>92</td>
</tr>
<tr>
<td>33</td>
<td>Raloxifene decreases the amount of ROS</td>
<td>93</td>
</tr>
<tr>
<td>34</td>
<td>Raloxifene increases CYP1A1 activity</td>
<td>94</td>
</tr>
<tr>
<td>35</td>
<td>Raloxifene increases intracellular neutral lipid accumulation</td>
<td>95</td>
</tr>
<tr>
<td>36</td>
<td>Raloxifene increases membrane phospholipid accumulation</td>
<td>95</td>
</tr>
<tr>
<td>37</td>
<td>100 µM Raloxifene increases p-glycoprotein activity</td>
<td>96</td>
</tr>
<tr>
<td>38</td>
<td>Raloxifene decreases cell viability</td>
<td>97</td>
</tr>
<tr>
<td>39</td>
<td>Raloxifene increases albumin production</td>
<td>98</td>
</tr>
</tbody>
</table>
Figure 40. Actein and tamoxifen interact to affect CYP1A1 activity. ......................... 100
Figure 41. Actein and tamoxifen have a synergistic effect on membrane phospholipid accumulation at high concentration. ................................................................. 101
Figure 42. Actein and tamoxifen have a synergistic effect on cell viability at high concentration. ........................................................................................................... 103
Figure 43. Actein and raloxifene affect membrane phospholipids accumulation. 106
Figure 44. Actein and raloxifene interact to affect cell viability. ......................... 107
Figure 45. Proposed mechanism of action for actein in MCF-7 cells. .............. 126
Figure 46. Tamoxifen does not affect membrane phospholipid accumulation. 159
Figure 47. Actein and tamoxifen do not have an interaction effect on oxidative stress. ........................................................................................................... 159
Figure 48. Actein and tamoxifen do not have an interaction effect on intracellular neutral lipid accumulation. ................................................................. 160
Figure 49. Actein and tamoxifen do not have an interaction effect on R123 retention. ................................................................. 160
Figure 50. Actein and tamoxifen do not have an interaction effect on albumin production. ................................................................................................. 161
Figure 51. Actein and raloxifene do not have an interaction effect on oxidative stress. ........................................................................................................... 161
Figure 52. Actein and raloxifene do not have an interaction effect CYP1A1 activity. ......................................................................................... 162
Figure 53. Actein and raloxifene do not have an interaction effect on intracellular neutral lipid accumulation. ................................................................. 162
Figure 54. Actein and raloxifene do not have an interaction effect on R123 retention. ........................................................................................................... 163
Figure 55. Actein and raloxifene do not have an interaction effect on albumin production. ................................................................................................. 163
List of Abbreviations

ANOVA: analysis of variance
ATCC: American Type Culture Collection
CAM: complementary and alternative medicine
CDKN1A: cyclin-dependent kinase inhibitor 1A
cDNA: complementary deoxyribonucleic acid
CSS: charcoal/dextran-stripped fetal bovine serum
CYP1A1: cytochrome P450, family 1, subfamily A, polypeptide 1
DCF: dichlorofluorescein
DCFDA: dihydrodichlorofluorescein
DEPC: diethylpyrocarbonate
DMSO: dimethylsulfoxide
E₂: 17β-estradiol
EDTA: ethylenediaminetetraacetic acid
ER-: estrogen receptor negative
ER+: estrogen receptor positive
EROD: 7-ethoxyresorufin
FBS: fetal bovine serum
GAPDH: glyceraldehyde 3-phosphate dehydrogenase
HBSS: Hanks’ balanced salt solution
HDS: herbal dietary supplements
HPLC: high performance liquid chromatography
IM: integrative medicine
IRB: Institutional Review Board

M: moles/liter

MCF-7: Michigan Cancer Foundation-7

mL: milliliter

mRNA: messenger ribonucleic acid

NCCAM: National Center for Complementary and Alternative Medicine

NHIS: National Health Interview Survey

NIH: National Institutes of Health

OD: optical density

PBS: phosphate-buffered saline

p-value: probability value

RNA: ribonucleic acid

ROS: reactive oxygen species

RPMI: Roswell Park Memorial Institute

RT-PCR: real-time polymerase chain reaction

SAS: Statistical Analysis Software

SEM: standard error of the mean

SERM: selective estrogen receptor modulator

SRB: sulforhodamine B

TCA: trichloroacetic acid

TFF1: trefoil factor 1

VEGFA: vascular endothelial growth factor A

µg: microgram
μM: micromole/liter
1. Introduction

1.1 Cancer and CAM

Cancer, a disease characterized by uncontrolled cell growth and division, is the second most common cause of death in the United States and responsible for one-fourth of all deaths. In 2009, an estimated 1,479,350 new cancer cases were diagnosed in the United States (American Cancer Society, 2009). Of these, 192,370 were breast cancer cases diagnosed in females. Tragically, 40,170 women died from breast cancer in 2009. Cancer not only has a devastating emotional impact on its victims and their families, but also has a significant economic impact. The National Institutes of Health (NIH) estimated that about $228.1 billion were spent on cancer-related costs in 2008 (American Cancer Society, 2009). Certain risk factors such as family history, race, and other demographic factors may increase the likelihood of cancer diagnosis and affect the outcome, but in general, cancer can affect anyone regardless of age, lifestyle, gender, or ethnicity.

Radiation and chemotherapy, common conventional cancer treatments, often kill or damage healthy cells in addition to cancerous cells. These treatments also produce many side effects, including hair loss, vomiting, nausea, fever, immunodeficiency, and fatigue (Smith & Toonen, 2007). Even if a patient’s cancer can be overcome through treatment, his/her quality of life is usually significantly decreased. Activities once taken for granted - eating, taking a walk outside, or working a full-time job - often have to be curtailed.
The drawbacks of conventional treatments cause many cancer patients to seek complementary and alternative therapies. Complementary and alternative medicine (CAM) is becoming increasingly popular, especially among those with life-threatening illnesses such as cancer (Boon, Olatunde, & Zick, 2007; D. M. Eisenberg et al., 1998). According to the NIH National Center for CAM (NCCAM), CAM is “a group of diverse medical and health care systems, practices, and products that are not generally considered part of conventional medicine” (National Center for Complementary and Alternative Medicine, 2010). CAM can include a variety of therapies, from prayer and meditation to food, vitamins, and herbal supplements. Specifically, alternative medicine is used in place of conventional treatments, while complementary medicine is used in conjunction with conventional treatments. When complementary medicine use is based on scientific evidence and overseen by a physician or practitioner, it is often known as Integrative Medicine (IM) (National Center for Complementary and Alternative Medicine, 2010). Increasing the use of IM is a goal of CAM researchers since physicians need adequate and accurate information to fully advise their patients on incorporating non-standard treatments into their regimes.

1.2 Need for Reliable Information

A study conducted in 2004 found that over half of the cancer patients surveyed began to use one or more CAM modalities following cancer diagnosis (Vapiwala, Mick, Hampshire, Metz, & Denittis, 2006). However, many patients do not discuss their CAM use with their doctors. In fact, less than one-third of the
patients said that their healthcare providers were their primary source of information about CAM (Vapiwala, et al., 2006).

Physicians are often wary of recommending CAM for several reasons. First, most forms of CAM have not been clinically proven safe and effective, leaving physicians without the information necessary to make recommendations. Second, many health care providers have received little education and training in this area (Tovey & Broom, 2007). Some cancer patients choose not to discuss CAM use with health care providers, either because they believe discussing CAM is unimportant or because they expect that the health professional will disapprove. Instead, patients often turn to friends, family, the Internet, or vitamin/herbal store employees to learn more about CAM, despite the fact that these may not be reliable sources of information (Vapiwala, et al., 2006).

Failure to discuss CAM use with a physician, as well as a lack of reliable information regarding CAM, could be extremely harmful to the patient. When CAM is used, unexpected interactions may occur between CAM and conventional treatments, resulting in unpleasant or even dangerous side effects. For example, St. John’s Wort is an herbal dietary supplement that can decrease the effectiveness of some cancer treatments, such as the drug irinotecan, by increasing the rate at which anticancer drugs are metabolized (Lumpkin & Alpert, 2000). Symptoms of harmful interactions may not be recognized, especially if the healthcare provider is unaware of the patient’s CAM use, resulting in adverse physiological effects. Additionally, since metabolism varies among individuals, use of a form of CAM that may be effective for one cancer patient may actually be detrimental for someone else.
Despite the general lack of reliable information available regarding many CAM therapies, the use of CAM is widespread. In particular, herbal dietary supplements (HDS) are used by many breast cancer survivors. Although various definitions of HDS exist, we defined HDS as “a product that contains a plant or a plant part that is used for medicinal or therapeutic properties.” Such products “are meant to be taken by mouth as fresh or dried products or in tablet, capsule, powder, softgel, gelcap, or liquid form” (See Appendix A). For our study, we chose to focus on one particular HDS, black cohosh, because reliable and conclusive information about this particular supplement is lacking and the literature is contradictory.

1.3 Choosing Black Cohosh

Black cohosh (Actaea racemosa) is a perennial herb in the buttercup family that is also known as black baneberry, black bugbane, and black snakeroot. It is native to the central and eastern United States and Canada and considered endangered in Illinois and Massachusetts (United States Department of Agriculture, 2010).

Black cohosh is used for many reasons, and there are various ways that it is ingested. The stems and roots of black cohosh can be used to make a tea infusion. Alternatively, black cohosh can be taken in the form of capsules, pills containing solid extracts, or liquid extracts known as tinctures (National Center for Complementary and Alternative Medicine, 2010). Historically, black cohosh was used by Native Americans for arthritis, rheumatism, and female conditions such as menstrual irregularity, painful menstruation, and pain after childbirth (Foster, 2009). Today, black cohosh is commonly used by women to alleviate symptoms of
menopause. The German Commission E Monographs authorize the labeling of black cohosh for premenstrual discomfort, absence of menstruation, and menopausal symptoms (Blumenthal, 1998).

We selected black cohosh for further study because it is commonly used by women specifically for menopausal symptoms, which breast cancer survivors frequently experience (Burstein & Winer, 2000). Also, breast cancer survivors have reported using black cohosh to alleviate side effects from chemotherapy and other conventional cancer therapies (Jacobson et al., 2001).

Furthermore, some black cohosh extracts contain bioactive compounds that are a potential cause for concern. The chemical structure of black cohosh extracts includes several components identified as triterpene glycosides, compounds similar in structure to estrogens (Farnsworth, et al., 2008). Estrogens are a family of female hormones, the most prevalent ones being estradiol and estrone. Only tissues that express estrogen receptors (ERs), proteins inside cells that bind to the estrogen molecule, are responsive to estrogenic ligands. Compounds that are similar in structure to estrogen can also bind to these estrogen receptors, and are considered estrogenic. There are two main types of ERs: ERα, found mostly in the uterus, breast, ovaries, and hypothalamus, and ERβ, found mostly in other tissues including lungs, heart, brain, bone, and kidney. Upon binding estrogen or estrogenic compounds, the estrogen receptors activate a series of events as shown in Figure 1. One of the events triggered by the binding of estrogenic ligands to ERα is the transcription of genes whose products promote cell growth and division (National Cancer Institute, 2005).
Figure 1. Estrogen-receptor pathway in a cell.
Estradiol or an estrogenic compound binds to the estrogen receptor expressed in a cell. The estrogen-receptor complex initiates a series of events in the cell. This results in certain genes being expressed and transcribed into mRNA. The mRNA strands are then translated into proteins involved in cell growth and division (National Cancer Institute, 2005).

Normally, the ERα pathway is important for preparing a female for sexual reproduction, but it can also increase the risk for breast cancer since it promotes cell proliferation in the breast. Random mutations in the DNA of breast cells could be amplified by increased cell proliferation, since each time a cell divides, DNA is duplicated and could incorporate defects with each successive duplication. Some spontaneous mutations may be harmless, but others could alter a critical gene in a way that leads to the development of cancer.

Breast cancer tumors are classified as estrogen-receptor positive or estrogen-receptor negative based on whether they express estrogen receptors. The growth of estrogen receptor-positive breast cancer tumors is promoted by estrogen or estrogenic compounds (National Cancer Institute, 2005).
Therefore, the structural similarity between some compounds in black cohosh extracts and estrogens is cause for concern, because black cohosh extracts could possibly promote the growth of estrogen-receptor positive tumor cells by activating estrogen-related pathways. This concern is especially important for women who are also taking other supplements or medications that affect hormonal pathways, including the highly prescribed chemotherapy drugs tamoxifen and raloxifene.

Several research groups have investigated whether or not various black cohosh extracts, and specific compounds contained in the extracts, are estrogenic. However, variations in methodology and compounds tested have resulted in a somewhat contradictory body of evidence, as described in the following chapter (Boon, et al., 2007; Shen, et al., 2002). These extracts and compounds have been shown to have both proliferative (growth and division-inducing, i.e., cancer-promoting) and antiproliferative effects in various systems, which will also be discussed in the literature review. Furthermore, several reports of liver damage and liver failure have been associated with black cohosh use, necessitating investigation of the herb’s safety and possible liver toxicity (Mahady, et al., 2008).

Because of the prevalent use of black cohosh among female breast cancer survivors and the controversy surrounding its safety and breast cancer-related effects, we selected black cohosh as an herbal dietary supplement that warrants further study.

1.4 Study Objectives

Our research objectives were to investigate the use and perceptions of HDS, particularly black cohosh, among a group of female breast cancer survivors and to test
the efficacy and safety of black cohosh on human breast cancer cells and human liver cells, respectively. Specifically, we proposed the following questions to guide our research:

Which HDS do female breast cancer survivors use most often, and in what combinations? What are their reasons for using these HDS? Do they use black cohosh directly to treat cancer? How effective do they consider these HDS in cancer prevention, cancer treatment, or in alleviating the side effects of conventional cancer therapy? What types of resources do breast cancer survivors consult to obtain information about HDS, and how reliable do they feel these resources are? How do the sources of information consulted affect HDS use? Are breast cancer survivors concerned about the safety and labeling of HDS? Are there correlations between the use and perceptions of HDS? What are the biological effects of black cohosh in vitro? Is black cohosh safe according to in vitro studies? Are female breast cancer survivors’ uses and perceptions of HDS in alignment with in vitro data on the efficacy and safety of black cohosh?

1.5 Study Design

In order to accomplish our objective, we utilized a mixed methods approach that combined survey and laboratory research. The survey portion of our research was designed to study the use and perceptions of HDS by female breast cancer survivors. The survey provided evidence regarding types of supplements used, patterns of use, reasons for use, perceived efficacy of supplements, sources of HDS information, perceived reliability of sources of information, and concerns regarding
safety and labeling. By analyzing the survey results, we gained knowledge of the general beliefs and practices of breast cancer survivors regarding HDS.

In the laboratory portion of our research, we studied the biological effects and safety of black cohosh. In one aspect of our laboratory research, we investigated the effects of actein, a bioactive compound from black cohosh, on human breast cancer cell proliferation (cell growth). High levels of cell proliferation and particularly estradiol-induced cell proliferation, cell growth as a result of the steroid estradiol, are characteristics of breast cancer that promote tumor growth and metastasis, and decreased cell proliferation would be beneficial. In the second aspect of our laboratory research, we assessed the safety of actein by determining its potential hepatotoxicity through various assays on human liver cells.

Based on the findings of our survey and laboratory research, we examined the degree to which breast cancer survivors’ uses and perceptions of HDS, including black cohosh, were in alignment with the demonstrated biological effects of this particular HDS as shown in our study and in the literature.

1.6 Expected Results

Since the HDS we focused on in our survey were chosen based on their reported use in the literature, we expected that they would be widely used by many participants. We anticipated that more popular and better known HDS would be used more frequently and considered more reliable. We also expected that the reasons for using HDS would vary widely among our sample subjects. We predicted that subjects would trust a wide variety of sources, even though some of these sources
may not be considered scientifically reputable or reliable. Lastly, we hypothesized that more highly educated, higher-income women would be more likely to use HDS than other demographic groups as was the case in published studies (see Literature Review).

Based on previous studies, actein was expected to inhibit breast cancer cell proliferation and estradiol-induced cell proliferation. We expected that actein, along with tamoxifen and raloxifene, two anti-cancer drugs currently used in conventional treatment, would have a synergistic inhibitory effect. This means that the combination of actein and chemotherapeutic drugs would inhibit cell proliferation more than simply combining the effects of actein and either drug in isolation.

Regarding the safety of black cohosh as a dietary supplement, we hypothesized that black cohosh is a safe supplement, because there are no previous reports that conclusively linked taking black cohosh supplements regularly to liver damage or other significant side effects. Specifically we predicted that actein, at normal physiological concentrations, would not be toxic to liver cells in vitro because in the literature actein has not been demonstrated to exhibit hepatotoxicity.
2. Literature Review

2.1 Breast Cancer and Integrative Medicine

2.1.1 Brief Overview of Breast Cancer and Treatments

Other than skin cancers, breast cancer is the most commonly diagnosed cancer among women (American Cancer Society, 2009). In 2000, women in the United States had a 12.6% lifetime risk of developing breast cancer and a 3.6% risk of death from breast cancer (Burstein & Winer, 2000). According to the American Cancer Society, there were an estimated 192,370 new cases of breast cancer in women in 2009 (American Cancer Society, 2009). Despite this high annual incidence, the American Cancer Society reported that there had actually been a 2.2% decrease in female breast cancer rates every year between 1999 and 2005 (American Cancer Society, 2009). Furthermore, from the 1960s to 2009, the five-year survival rate for female breast cancer patients improved from 63% to 89% (American Cancer Society, 2009). As of 2005, there were an estimated 2,477,850 female breast cancer survivors alive (American Cancer Society, 2009). Despite the advances that have been made in the fight against breast cancer, many patients are still affected by this disease each year. As a result, more research is needed on the etiology of breast cancer and its treatment.

Chemotherapy is used to treat many different types of cancers, including breast cancer. Since cancer is characterized by unregulated cell division, most chemotherapy agents work by targeting quickly dividing cells throughout the entire
body. Unfortunately, this also targets healthy cells throughout the body that grow and divide rapidly, including cells in the lining of the mouth and intestines, bone marrow, and hair follicles. Radiation therapy, which uses radiation to kill cancer cells in a specific area of the body, can also damage nearby healthy cells (American Cancer Society, 2010). Resulting side effects from radiation often include hair loss, nausea, vomiting, fever, immunodeficiency, and fatigue (Smith & Toonen, 2007). Even when a patient’s cancerous tumors can be destroyed through treatment, the patient’s quality of life is decreased by the side effects of this treatment. Therefore, many patients seek complementary and alternative treatments in order to alleviate these side effects, increase immune system function, augment overall health, and sometimes even to replace conventional therapy (Balneaves, Weeks, & Seely, 2008).

In addition to the side effects associated with general chemotherapy and radiation therapy, breast cancer patients frequently experience amenorrhea, premature menopause, and menopausal symptoms such as hot flashes (Burstein & Winer, 2000). They also may experience changes in the urogenital epithelium due to endocrine-targeted therapies and the action of chemotherapy on the ovaries (Burstein & Winer, 2000; Ganz, 2005). Hormone replacement therapy with estrogen alone or together with progestin has been found to be effective in relieving menopausal symptoms (National Institutes of Health, 2005). However, estrogen stimulates the growth and development of some types of breast cancer, and there is some evidence that hormone use is related to breast cancer recurrence and mortality (Deniz et al., 2007). Therefore, many women are turning to CAM as an alternative to hormone therapy in
order to reduce side effects of breast cancer treatment and menopause (Antoine, Liebens, Carly, Pastijn, & Rozenberg, 2007).

2.1.2 Prevalence of CAM Use

CAM is becoming increasingly popular among the general population, especially among those with life-threatening illnesses such as cancer (Eisenberg, et al., 1998; Vapiwala, et al., 2006). The 1999 National Health Interview Survey (NHIS) found that 29% of adults in the United States used at least one CAM modality (Ni, Simile, & Hardy, 2002). The 2002 National Health Interview Survey (NHIS) found that 75% of adults in the United States had used CAM and that 18.9% of all people surveyed used natural products (Davis et al., 2008; D.M. Eisenberg, Davis, & S.L., 2004). Barnes, et al., analyzed the CAM supplement usage data from the NHIS in 2002 and again in 2007 to measure CAM trends among adults and children in the United States. The results in both years were similar, but the use of CAM (excluding prayer) increased from 36% in 2002 to 40% in 2007 (Barnes, Powell-Griner, McFann, & Nahin, 2002).

Frequent use of CAM was also specifically reported among cancer patients. Of outpatients in a large cancer center in the southern United States, 69% reported using some form of CAM (Richardson, Sanders, Palmer, Greisinger, & Singletary, 2000). A study conducted in 2004 found that over half of the cancer patients surveyed began to use one or more CAM modalities following cancer diagnosis (Vapiwala, et al., 2006). More often than not, cancer patients used a combination of CAM modalities to treat their symptoms (Hök, Wachtler, Falkenberg, & Tishelman,
Studies of cancer survivors suggest that those who had used at least one CAM modality were more likely to have survived cancer for extended periods of time, received chemotherapy or radiation therapy, been patients in clinical trials, been women, and had relatively high household incomes (Goldstein et al., 2005; Lerner & Kennedy, 1992; Sparber, 2000; Vapiwala, et al., 2006).

CAM use is widespread among breast cancer survivors as well. In a survey of San Francisco Bay area breast cancer patients including four different ethnic groups, 50% reported using some form of CAM (Lee, Lin, Wrensch, Adler, & Eisenberg, 2000). Breast cancer patients reported increased use of complementary treatments after cancer diagnosis and studies suggest that CAM use is greater among breast cancer patients who are younger, have higher levels of education or income, have a history of chemotherapy, and have attended a support group (Adler, 1999; H. Boon et al., 2000; Lengacher et al., 2006; Matthews, Sellergren, Huo, List, & Fleming, 2007).

One common type of CAM used by breast cancer patients is dietary supplements, such as HDS or vitamins and minerals. A 2002 study showed that 73% of patients with advanced-stage breast cancer used some form of CAM, with HDS being the most common modality (Shen, et al., 2002). In a study comparing 2005 survey data to 1998 data, breast cancer patients reported using a variety of complementary treatments, most commonly an herbal supplement or high-dosage vitamin. The use of these therapies significantly increased from 66.7% in 1998 to 81.9% in 2005, with 41% of breast cancer patients reporting that they used CAM in treating their breast cancer (Boon, et al., 2007). In a 2002 survey of 115 breast cancer
survivors at a large oncology center, 69% used CAM and 39% specifically used herbs or medicinal herbal teas (Matthews, et al., 2007).

The NHIS examined reasons for use of CAM and prevalence of CAM use among certain demographic groups. Of the 149,271 participants who reported using CAM, 657 reported using CAM modalities for menopause. Although a small portion of the total survey respondents, there are a number of individuals who use CAM specifically to treat menopause symptoms. Furthermore, the 2002 study found CAM use more likely among higher-educated women, and a majority of CAM users were likely to try CAM because they believed it would be helpful when combined with conventional medical treatments (Barnes, Powell-Griner, McFann, & Nahin, 2002). In both 2002 and 2007, CAM use was positively correlated with the number of medical conditions or visits to doctors, suggesting that those with illnesses were more likely to use CAM (Barnes, Bloom, & Nahin, 2008).

However, all of the estimates on the prevalence of CAM use are cast in doubt when the inconsistencies in the definition of CAM are considered. A 2006 study of 334 breast cancer patients arrived at two disparate estimates of CAM usage: a “conservative” figure (19.5%) and a “liberal” figure (79.9%). The intent of this study was to expose the differences in CAM definitions among researchers (Balneaves, Bottorff, Hislop, & Herman, 2006). Eisenberg, et al., recommended differentiating between the “alternative” and the “complementary” portions of CAM in order to more precisely discriminate survey results (Eisenberg, et al., 2004). Our study attempts to differentiate the two terms by asking survey participants whether they have ever undergone conventional cancer treatments. Those who have undergone
conventional treatments while using CAM are considered to have used complementary medicine. Those who solely used non-conventional treatments are considered to have used alternative medicine. More research is necessary to determine the specific complementary and alternative therapies that women are using and their reasons for use, within the confines of a discrete definition of CAM. Our study specifically investigates one type of CAM, HDS, under narrowly defined criteria.

2.1.3 Perceptions of Integrative Medicine: Information and Communication

A female breast cancer survivor’s choice to use or not use complementary or alternative therapies is affected by many factors, including her physician’s knowledge about and attitude towards CAM modalities, her sources of information, and her personal opinions and background. The prevalence of CAM use among cancer patients underscores the need for further research on how patients decide to use such therapies, how they communicate with their healthcare providers about the therapies, and how safe and effective these therapies are.

The lack of clear distinctions between CAM and non-CAM therapies and the definitions of CAM modalities make reproducible testing on safety and efficacy difficult (Adams & Jewell, 2007). Much of the existing knowledge about CAM is based on cultural beliefs, rather than on scientific evidence, indicating the need for further studies (Adams & Jewell, 2007). Additionally, individual genetic and behavioral differences make the safety and efficacy of CAM patient-specific, which serves as an additional challenge to clinical analysis (Tomlinson, Hu, & Lee, 2008).
The lack of adequate research funds and barriers in recruiting patients for studies further impede conducting rigorous, peer-reviewed investigations. This overall lack of available scientific data hinders physicians from suggesting CAM therapies to patients (Adams & Jewell, 2007).

Physicians are often wary of recommending many CAM modalities, including HDS, due to their perception of limited empirical evidence of the safety and efficacy of CAM (Tovey & Broom, 2007). One survey investigated how confident health care professionals were about their knowledge of HDS, and how well healthcare professionals communicated with their patients regarding HDS. The authors found that healthcare professionals need more training about HDS, in order to establish consistent communication about HDS with patients (Kemper, Gardiner, Gobble, & Woods, 2006). A survey of internists at Mayo Clinic in Rochester, MN found that 76% had never referred a patient to a CAM practitioner, yet 57% thought incorporating CAM would positively affect patient satisfaction (Wahner-Roedler, et al., 2006). A 2004 European study found that oncology professionals were less skeptical about the use of CAM when it was used in conjunction with conventional therapy, as opposed to it being used solely as an alternative therapy (Richardson, Mâsse, Nanny, & Sanders, 2004). A major concern among many physicians is that using alternative medicine during the earlier stages of cancer is unwise at a time when conventional therapy could be more effective in eradicating the tumor. Delaying the use of conventional medical treatment could have irreparable consequences and result in more harm to the body (Adams & Jewell, 2007).
Despite the risks of CAM, including HDS, and the lack of information available about safety and drug interactions, many people do not discuss their use of CAM with their healthcare providers. In a survey of people over the age of 50 in the United States, only 31% of CAM users said that they had discussed CAM with their physician. Also, 22% reported that friends or family were their primary source of information about CAM, while only 12% said that their physician was their primary source (American Association of Retired Persons, 2007). In a survey of cancer patients and physicians, 61% of patients reported that they did not discuss CAM use with their physician, most frequently because the doctor did not ask about CAM use. Additionally, only 52% of the physicians said that they had referred patients to sources of information on CAM (Roberts, et al., 2005). Clearly, more efforts need to be made to encourage patient-physician communication and to adequately educate both patients and physicians about CAM, including HDS. While our survey does not specifically explore patient-physician communication, it does include several questions intended to gauge the influence of physicians on their patients’ decisions to use HDS, and the extent that female breast cancer survivors turn to their physicians for information regarding HDS.

2.2 Breast Cancer and HDS

2.2.1 Definition of HDS

Our study addressed the need for more detailed information about breast cancer survivors’ use of HDS. CAM, including herbal supplements, is commonly used by women who have been diagnosed with breast cancer (Shen, et al., 2002). For
the purposes of our study, we used the NCCAM definition of HDS: a product containing a plant or plant part used for medicinal or therapeutic properties, taken by mouth as fresh or dried product or in tablet, capsule, powder, softgel, gelcap, or liquid form (National Center for Complementary and Alternative Medicine, 2010).

2.2.2 Patterns of HDS Use

In order of retail sales, the top-selling herbal supplements in 2002 were: garlic, ginkgo, echinacea, soy, saw palmetto, ginseng, St. John’s wort, black cohosh, cranberry, valerian, milk thistle, evening primrose, kava kava, bilberry, grape seed, yohimbe, green tea, ginger, Pycnogenol®, and aloe vera (Blumenthal, 2003). According to the 2002 NHIS, the most commonly used non-vitamin, non-mineral natural products in the U.S. in order of prevalence are: echinacea, ginseng, ginkgo biloba, garlic supplements, glucosamine, St. John’s wort, peppermint, fish oils/omega fatty acids, ginger supplements, soy supplements, ragweed/chamomile, bee pollen or royal jelly, kava kava, valerian, and saw palmetto (Barnes, Powell-Griner, McFann, & Nahin, 2002). Among individuals diagnosed with breast cancer, the most commonly used HDS include green tea, flax seeds/flaxseed oil, soy supplements/isoflavone extracts, and mistletoe (Boon, et al., 2007; Fasching, et al., 2007).

Supplements frequently used to relieve menopausal symptoms include soy supplements/isoflavone extracts, red clover supplements/isoflavone extracts, and black cohosh (Nelson, et al., 2006). Chamomile, dong quai root, wild yam cream, evening primrose oil, Korean red ginseng, ginseng, kava extract, St. John’s wort, and
vitex agnus castus oil have also been studied for their estrogenic or menopause-related effects (Geller, Studee, & Chandra, 2005; Srivastava & Gupta, 2007). Other HDS with potential anti-cancer effects include curcumin from turmeric; resveratrol from red grapes, peanuts and berries; diallyl sulfide and S-allyl cysteine from onions; allicin from garlic; lycopene from tomato; capsaicin from red chili; diosgenin from fenugreek; 6-gingerol from ginger; ellagic acid from pomegranate and other plants; ursolic acid from apple, pears, prunes; silymarin from milk thistle; anethol from anise, camphor, and fennel; and eugenol from cloves (Aggarwal & Shishodia, 2006).

2.2.3 Concerns about the Safety of HDS

There was and remains a very real concern about the safety of taking an HDS. A study published in Breast Cancer Research notes that many breast cancer patients look to HDS as a way to evade the painful and damaging symptoms of most standard cancer treatments, despite the fact that there are only a very limited number of scientific trials to establish the safety and efficacy of these supplements (Cassidy, 2003). This author further pointed out that serious health risks can occur when patients take HDS, as these supplements can have harmful interactions with the patient’s regimen of standard treatments. The supplement itself may be harmful at a high dose, and patients often do not tell their doctors that they are taking supplements. This study recommends additional scientific trials to establish a greater knowledge base about the safety and efficacy of HDS, as well as to address improving communication between doctors and patients with respect to HDS and their possible risks (Cassidy, 2003).
There are few studies which compare the uses of HDS between women with female-specific cancer to those in other diagnostic groups. This is an important issue to address because of potential interactions between HDS and conventional treatments used for female-specific cancers (Eschiti, 2007).

2.3 Breast Cancer and Black Cohosh

2.3.1 Prevalence and Patterns of Black Cohosh Use

The patterns and prevalence of black cohosh use are not well documented. While some studies report usage rates of 10% among women, other studies with larger sample sizes report a figure of 2% (Kam, Dennehy, & Tsourounis, 2002; Kelly, et al., 2005). The Canadian black cohosh monograph indicates that the typical black cohosh dose is 40-200 mg of dried root or rhizome per day (Health Canada, 2007; Mahady, et al., 2008). According to a survey of 1296 women aged 45-65 in Sydney, Australia, black cohosh is used primarily by perimenopausal women to relieve hot flashes and is considered to be one of the most effective herbal supplements (van der Sluijs, Bensoussan, Liyanage, & Shah, 2007).

In another survey of 100 self-identified peri- and post-menopausal women at a women’s health conference, 48 reported using herbal supplements to treat their symptoms (32 used herbal supplements alone while 16 used herbal supplements in combination with hormone replacement therapy). Black cohosh was the third most commonly-used supplement, taken by 10% of the participants (Kam, et al., 2002). A much larger survey of 3,853 postmenopausal women over 50 years of age interviewed in the Sloan Survey found only 2% used black cohosh and this usage was
independent of age, race, and education (Kelly, et al., 2005). There is a dearth of demographic data regarding people who use specific dietary supplements such as black cohosh, since most studies report total HDS use. Moreover, there is very little literature addressing factors that influence people’s decisions to use specific supplements, including black cohosh.

2.3.2 Efficacy of Black Cohosh: Previous Studies

Black cohosh is a good candidate for further investigation because the literature on the efficacy of black cohosh is controversial and inconclusive. Black cohosh, although widely used as a menopausal remedy among breast cancer survivors and women without breast cancer, has not been shown to have significant effects on menopause-related symptoms in clinical trials (Antoine, et al., 2007; Kronenberg & Fugh-Berman, 2002). Additionally, some clinical studies have not been able to confirm that black cohosh has any protective role in cancer prevention (Walji, Boon, Guns, Oneschuk, & Younus, 2007).

Nonetheless, in vitro experiments have been able to show that several black cohosh extracts and compounds are cytotoxic and reduce proliferation of human breast cancer cells (Al-Akoum, Dodin, & Akoum, 2007; Einbond, Shimizu et al., 2008; Einbond et al., 2004; Einbond, Su, Wu, Friedman, Wang, Jiang et al., 2007; Einbond, Su, Wu, Friedman, Wang, Ramirez et al., 2007; Einbond, Wen-Cai et al., 2008; Gaube, Wolf, Pusch, Kroll, & Hamburger, 2007; Hostanska, Nisslein, Freudenstein, Reichling, & Saller, 2004a, 2004b). Interpreting results from extracts as opposed to individual active compounds is usually more difficult because extracts
are made of many active compounds and thus, it is hard to distinguish between the effects of different active compounds. As a result, studies have been done to determine the active compounds in black cohosh.

In a 2004 study investigating black cohosh’s active ingredients, black cohosh rhizomes were extracted with methanol/water and three fractions of hexane, ethyl acetate, or water were created by solvent-solvent partitioning. The fraction displaying highest potency in inhibition of breast cancer cell proliferation was further analyzed and a triterpene glycoside called actein (Figure 2) was found to be the most potent compound. Actein not only inhibited MCF-7 breast cancer cell proliferation, but also caused cell cycle arrest in G1, the stage in which cells typically grow the most (Einbond, et al., 2004). Since then, actein has become one of the most studied active ingredients in black cohosh.

![Figure 2. Chemical structure of actein.](image)

Figure 2. Chemical structure of actein.
Actein is one of several compounds that have been isolated from *Actaea racemosa* rhizomes and extracts. The structure was determined by spectroscopic methods (Mercier & Balansard, 1935; Watanabe, Mimaki, Sakagami, & Sashida, 2002).

Whether black cohosh contains phytoestrogens or has estrogen-like effects is controversial. When a compound is estrogenic, it behaves like estrogen and can increase cell proliferation in cells with estrogen receptors; that is, an estrogenic compound can induce estrogen-responsive breast cancer growth. Although previous studies did not observe binding between estrogen receptors and a black cohosh
extract, estrogenic effects were reported in *in vivo*, *ex vivo*, and *in vitro* assays (Bolle, Mastrangelo, Perrone, & Evandri, 2007; Liu, Yu, Huo, Lu, & Chen, 2001). For instance, according to a study done by Liu, et al., when black cohosh extracts were tested on MCF-7 human breast cancer cells, estrogen receptor levels increased and the doubling time of MCF-7 cells decreased. If the doubling time decreases, then the cells are able to grow faster. These results suggest that the black cohosh extract used was estrogenic, mimicking the behavior of estrogen and increasing breast cancer cell proliferation.

Other studies have concluded that black cohosh does not have estrogenic effects *in vitro or in vivo* (Davis, et al., 2008; Lupu, et al., 2003; Ruhlen, et al., 2007). For instance, Davis’ study using a MMTV-neu mouse tumor model, which is used to study the pathways of mammary tumor development, found that black cohosh did not lead to an increase in estradiol levels and did not promote tumor formation, suggesting that black cohosh does not have estrogenic impacts on mammary cells (Davis, et al., 2008). Possible explanations for contradictions in reported estrogenic effects are the use of different models, parts of the plant or extracts, sources of the plant material, or doses or concentrations used. Overall, the efficacy of black cohosh in treating menopausal symptoms and affecting breast cancer cell proliferation is inconclusive. While studies have generally shown black cohosh to be anti-proliferative, these studies have not been able to conclusively determine whether black cohosh is estrogenic.
2.3.3 Black Cohosh and SERMs

Since most breast cancer patients take conventional anti-cancer drugs, investigating any effect black cohosh may have on the actions of these anti-cancer drugs is important. Some typical anti-cancer drugs are selective estrogen receptor modulators (SERMs), which are frequently prescribed to patients with estrogen receptor positive (ER+) breast cancer in order to reduce estrogen-induced proliferation of the cancerous breast tissue. SERMs will bind to estrogen receptors in specific tissues and will act as agonists or antagonists. An agonist will mimic the response of bound estrogen; an antagonist will block or inhibit the response (Fabian & Kimler, 2005; Lewis & Jordan, 2005). Since ER+ breast cancer cells have ERα proteins that bind estrogen and stimulate cell growth, ERα antagonists can prevent ER+ breast cancer growth (Fabian & Kimler, 2005).

Two typical SERMs used to treat breast cancer are tamoxifen and raloxifene. Both tamoxifen and raloxifene predominately act as ER antagonists in breast tissue. Tamoxifen has long been established as an effective treatment for prevention and for early and late stages of breast cancer, having now been used for over three decades (Cuzick, et al., 2003; Fabian & Kimler, 2005). However, tamoxifen has also been shown to increase the rate of endometrial cancer, since it acts differently on ERβ than on ERα, and resistance to the drug can develop in some patients (Fisher et al., 1998; Vogel et al., 2006). More recently, raloxifene, a second generation SERM, has been developed, and shown to be as effective in preventing and treating breast cancer as tamoxifen (Vogel, et al., 2006). Raloxifene has been shown clinically to decrease the
risk of invasive breast cancer in postmenopausal women with osteoporosis (Cummings, et al., 1999).

For ER+ breast cancer patients or survivors, understanding the interactions between black cohosh and SERMs is crucial. Investigations that focus on potential interactions when black cohosh is used in combination with SERMs should consider four possible outcomes: a synergistic effect, an additive effect, an antagonistic effect, or no effect. In other words, black cohosh may work together with SERMs to reduce cancer cell growth to an extent greater than the sum of their two effects, black cohosh may work together with SERMS to reduce cancer cell growth to an extent equal to the sum of their effects, it may work against SERMs and reduce the amount that SERMs inhibit breast cancer cell proliferation, or it may not have any effect on SERM function. Al-Akoum found a synergistic effect in vitro for tamoxifen and black cohosh on inhibition of proliferation of ER+ MCF-7 breast cancer cells at increasing concentrations of tamoxifen (Al-Akoum, et al., 2007). Similarly, Einbond found a synergistic effect between actein and different classes of chemotherapy agents on the inhibition of estrogen receptor negative (ER-) MDA-MB-453 human breast cancer cell proliferation (Einbond et al., 2006).

2.3.4 Hepatotoxicity of Black Cohosh

While determining the efficacy of black cohosh in treating breast cancer is important, so too is assessing its safety. Previous investigators have examined the effect of black cohosh on liver function as an indicator of how safe it is to consume, since the liver is chiefly responsible for breaking down and removing harmful
substances from the body (Gurley, et al., 2006; Lude, et al., 2007). Exposure of the liver to certain herbs, drugs, chemicals or metabolic by-products that adversely affect liver function results in hepatotoxicity.

In addition to controversy regarding its estrogenic effects, black cohosh’s hepatotoxic effects are also debated. Previous *in vitro* and *in vivo* hepatotoxicity assays of black cohosh have provided some evidence that black cohosh may have adverse effects in the liver. One study using a 60% ethanolic black cohosh extract found that at media concentrations \( \geq 75 \, \mu g/mL \), cytotoxicity in HepG2 liver cells becomes apparent; at \( \geq 10 \, \mu g/mL \) mitochondrial \( \beta \)-oxidation is impaired; at \( \geq 100 \, \mu g/mL \) mitochondrial membrane potential is decreased; and at \( \geq 300 \, \mu g/mL \) oxidative phosphorylation is impaired (Lude, et al., 2007). However, these HepG2 cells were treated when less than 100% confluent, and thus the results may not be applicable to the adult liver, since confluent cells better mimic the conditions of an actual liver (Flynn & Ferguson, 2008; Lude, et al., 2007). Confluence refers to cells that have formed a complete monolayer over the culture surface. In this study, steatosis was also observed in rat livers after rats were given more than 500 \( \mu g/kg \) body weight of black cohosh extract (Lude, et al., 2007). Another study found that a black cohosh extract containing 6 triterpene glycosides had an IC\(_{50}\) value (half maximal inhibitory concentration, i.e., the concentration necessary for 50% inhibition) of 0.027 mg/mL for inhibition of CYP3A4, though the isolated compounds were not as effective in inhibiting CYP3A4 (Tsukamoto, Aburatani, & Ohta, 2005). CYP3A4 is a cytochrome P450 enzyme involved in drug metabolism in the liver. If black cohosh
extracts inhibit the activity of this enzyme, as suggested by the study, then the extracts may change the way the liver is able to process other drugs.

There have also been several case reports of human liver malfunction attributed to black cohosh use. In two case reports, the patients were admitted to a hospital and found to have normal levels of albumin and bilirubin, but hepatotoxicity was indicated by increased levels of gamma glutamyl transpeptidase which returned to normal after the patient discontinued use of black cohosh (Joy, Joy, & Duane, 2008). In another case report, a woman stopped taking her daily Remifemin® tablet (a black cohosh alternative to hormone replacement therapy) after experiencing darkened urine on day six, and was later admitted to a hospital for lethargy, jaundice, and incoherence. After undergoing a liver transplant, her liver was found to have significant hepatic necrosis and weighed only 398 g, whereas a normal female liver weighs 1400 g (National Institutes of Health, 2004). In 2007, one woman began to experience irregular liver function test results two weeks after taking black cohosh root supplements. After two months, she was hospitalized and found to have hepatitis, fibrosis, and low liver cell recovery, but after a liver transplant her symptoms vanished and have since not reoccurred (Dunbar & Solga, 2007).

While there have been various case reports suggesting that black cohosh is toxic to the liver, confounding factors in these case studies limits the ability to draw definitive conclusions. Some of the study design problems seen in case studies reporting black cohosh as a probable cause of liver failure or hepatitis include use of multiple HDS, concurrent alcohol consumption, and/or a lack of data on the type of extract or dosage (Borrelli & Ernst, 2008). A causality assessment based on an
updated diagnostic algorithm from the Council for International Organizations of Medical Sciences showed that out of 42 case reports of severe hepatotoxicity in menopausal women using black cohosh, none were caused by this HDS (Teschke & Schwarzenboeck, 2009).

In 2004, NIH held a “Workshop on the Safety of Black Cohosh in Clinical Studies,” in which experts reviewed 51 adverse event reports from the FDA, World Health Organization (WHO), Australian Adverse Drug Reactions Advisory Committee, German Federal Institute for Drugs and Medical Devices, and Committee on Safety of Medicines in the U.K. The workshop consensus was that although there was no decisive evidence of adverse hepatic effects, continued monitoring was warranted (National Institutes of Health, 2004). In 2007, a follow-up workshop was held by the Office of Dietary Supplements of NIH. This second workshop generated five recommendations: 1) increase adherence to nomenclature guidelines among healthcare professionals when reporting adverse events associated with herbal medicines, 2) increase communication between regulatory and monitoring agencies regarding adverse event report-related data and analyses, 3) encourage research in monitoring herbal product related-adverse event reports, 4) conduct research on clinical benefit and safety of black cohosh, its chemistry, and the mechanism of action of black cohosh extracts and their chemical constituents, and 5) monitor liver function before, during, and after any clinical trials of black cohosh products (Betz, et al., 2009).

In addition to hepatotoxicity, the potential interaction of black cohosh with other drugs is an important consideration when investigating the effects of black
cohosh on liver function. P-glycoprotein is responsible for pumping foreign substances out of a cell. If a substance is present that changes the activity of p-glycoprotein, that substance can change the length of time that other substances, including conventional drugs, remain in cells and in the body. In one study where eight subjects took 40 mg of black cohosh supplement for 14 days, their p-glycoprotein activities measured before and after this period were not found to be significantly different, showing that black cohosh does not affect levels of this liver function-related protein in humans (Gurley, et al., 2006).

Another concern with respect to black cohosh and liver function is the possible under reporting of any hepatotoxicity after its use. The Dietary Supplements Information Expert Committee (DSI-EC) of the US Pharmacopeial Convention found that poison control centers collect more adverse event reports (AER) than FDA MedWatch, the main organization that handles AER, and that there is insufficient national and international communication about these reports and coordination in detecting health risks (Gardiner et al., 2008). Thus, side effects of black cohosh may not be adequately reported. Concerns about under reporting adverse effects associated with dietary supplements led the DSI-EC to recommend additional research on the safety of HDS (Gardiner, et al., 2008). The US Pharmacopeia’s DSI-EC recently decided that black cohosh products should be labeled with the following: "Discontinue use and consult a healthcare practitioner if you have a liver disorder or develop symptoms of liver trouble, such as abdominal pain, dark urine, or jaundice” (Mahady, et al., 2008). In summary, there is conflicting and inconclusive information on the hepatotoxicity of black cohosh. Further, few studies have addressed adverse
effects in the liver from potential interactions between black cohosh and conventional chemotherapeutic drugs.
3. Methods

3.1 Survey: HDS Uses and Perceptions

3.1.1 Questionnaire Design

The questionnaire consisted of mostly forced-choice questions that addressed participants’ attitudes and behaviors with respect to HDS (See Appendix A). Initially, subjects were asked what their current stage of breast cancer was, whether they had ever received conventional cancer treatments, whether they had received conventional cancer treatments within the past 12 months, and whether they had ever used an HDS.

Those who reported ever using an HDS were then asked whether black cohosh, chamomile, curcumin, evening primrose, flaxseed, green tea, ginger, ginseng, kava kava, milk thistle, red clover, saw palmetto, soy, St. John’s wort, and valerian were used for medicinal or other purposes. They were also asked whether each supplement was used before and/or after their cancer diagnosis. Open-ended questions regarding general reasons for using each supplement, as well as side effects of conventional cancer treatment that were treated with each supplement, were included. Subjects were then asked to rate the effectiveness at treating side effects of conventional therapy for each HDS they used. There were also opportunities for additional comments.

Participants were next asked which sources of information were influential in their decisions to use or not use supplements, and were asked to rate how reliable they
considered their sources of information. Possible sources of information included websites found through internet databases and search engines, government websites, information provided by companies or company websites, breast cancer support groups or other cancer related non-profit organizations, cancer related health organizations, popular magazines or newspapers, academic journals, friends, family members, physicians and other health care providers, and practitioners specializing in CAM. These categories were determined at the discretion of the Gemstone team members and not based directly on the literature. Finally, subjects were asked to rate how concerned they were regarding safety and labeling of supplements.

The exact number of questions completed depended on whether or not subjects reported using HDS, and how many they used. Participants were asked from 7 to 71 questions; for each supplement a participant had used, further questions were asked regarding the specifics of its use. The survey also included demographic questions—including race, religion, income, education level, and age—for describing the sample and for analysis purposes. The survey was approved by the University of Maryland Institutional Review Board (IRB) to ensure that our survey met ethical standards with respect to research on human subjects. An online version of the survey with programmed skip patterns was created using the website SurveyMonkey.com.

3.1.2 Pilot Testing

The survey was pilot tested through a variety of informal channels before being administered to the target sample. The first pilot test sample included
University of Maryland GEMS100 and GEMS202 students during the academic school year 2008-2009. Pilot test procedures for this sample involved administering a paper version of the survey, asking participants to fill out a feedback form (See Appendix B), and conducting a cognitive interview with the participants.

The second pilot test sample included staff and patients at the Georgetown Lombardi Cancer Center (Washington, DC). Pilot test procedures for this sample involved administering the survey in an interview format. Afterwards, the survey questions were discussed and feedback was received. In some cases, participants were asked to participate in mock scenarios to more adequately test the survey’s skip-pattern features. All pilot test participants were volunteers, and the completed surveys were destroyed.

3.1.3 Subject Recruitment

All subjects were recruited via an advertisement on the breast cancer support website BreastCancer.net or via an e-mail blast from Love/Avon Army of Women. BreastCancer.net is an online support group and educational site that provides information to breast cancer patients. Love/Avon Army of Women is a partnership between the Dr. Susan Love Research Foundation and the Avon Foundation for women. This non-profit agency works to eradicate breast cancer and to improve the quality of women’s health through research, education, and advocacy.

For subjects recruited through BreastCancer.net, potential subjects who viewed the advertisement clicked on the link and were directed to the team website (http://teams.gemstone.umd.edu/classof2010/imac/). From here, subjects followed a
link to complete our online survey on SurveyMonkey.com. For subjects recruited from Love/Avon Army of Women, registered volunteers of Love/Avon Army of Women received an e-mail blast that contained the direct link to our survey. In order to proceed with the survey, subjects had to agree that they met the criterion of being breast cancer survivors over the age of 18, and also had to complete the consent form.

3.1.4 Questionnaire Completion

Considering only the subjects who met the criterion of being a female cancer survivor over the age of 18, 1106 subjects began the questionnaire and 1072 subjects completed the entire questionnaire. Of the 1106 subjects, approximately 98 subjects were recruited from BreastCancer.net and 1008 subjects were recruited from Love/Avon Army of Women. The exact number of subjects from each sample population could not be determined, but the number was estimated based on the date that each survey was taken. The survey was first advertised on BreastCancer.net, and the Love/Avon Army of Women e-mail blast was not sent out until later in the data collection process. Thus, the surveys completed on earlier dates were from BreastCancer.net and those completed later on were from Love/Avon Army of Women. All surveys took participants about 20 minutes to complete and were completed entirely online at SurveyMonkey.com.
3.1.5 Statistical Analysis

Raw data from the survey were downloaded directly into a Microsoft Excel spreadsheet and fixed-answer questions were pre-coded by SurveyMonkey.com as part of the services provided. Open-ended questions were coded by hand by members of the IMAC team. Data analyses were performed using both Excel and Statistical Analysis Software (SAS) System for Windows v.9.1 (SAS Institute, Cary, NC). Microsoft Excel was used to determine basic descriptive data and SAS was used to complete statistical tests, including basic descriptive statistics, chi-square tests, analysis of variance, and correspondence analysis. All p-values were \( < 0.05 \) unless noted.

Basic descriptive statistics, including mean, median, mode, and standard deviation, were used to analyze the number of supplements used by each subject.

A Chi-square goodness of fit test was used to determine which HDS were used most and least frequently, which HDS were used more to treat conventional cancer therapy symptoms, which sources of information subjects were more likely to consult, and which information sources were considered more reliable.

A Chi-square test of association was used to determine whether each specific HDS was used more for medicinal or other purposes, whether and how HDS use in general changed before and after cancer diagnosis, whether and how use of each specific HDS changed before and after cancer diagnosis, whether there was a difference between perceived efficacies of supplements, whether or not sources of information were considered equally reliable, whether sources used are associated
with use of specific HDS, and whether there was an association between age (pre vs. post menopause) and starting or stopping black cohosh use.

A one-way ANOVA was used to determine whether HDS in general were used more for medicinal or other purposes. A multi-way ANOVA test was used to determine whether there was an association between the sources consulted and the number of HDS used.

Correspondence analysis was used to determine which supplements were perceived as more effective, and whether certain HDS were taken together. Cluster analysis was used to determine whether specific demographic clusters were more likely to use HDS in general. Lastly, logistic regression was used to determine whether specific demographic clusters were more likely to use certain HDS.

3.2 In vitro Study: Effects of Actein on Breast Cancer Cell Proliferation

3.2.1 Materials

Actein was purchased from Planta Analytica (Danbury, CT; lot number PA VI-13.A); purity was over 95% by high performance liquid chromatography (HPLC). Tamoxifen, raloxifene hydrochloride, trichloroacetic acid (TCA), sulforhodamine B (SRB), 17β-estradiol, and dimethylsulfoxide (DMSO) were purchased from Sigma-Aldrich (St. Louis, MO). Fetal bovine serum (FBS), charcoal stripped FBS (CSS), 0.25% trypsin-EDTA, phosphate-buffered saline (PBS), and RPMI-1640 medium were obtained from Invitrogen (Carlsbad, CA). Solutions of tamoxifen, raloxifene, estradiol, and actein were prepared at 1 x 10^{-2} M concentration in DMSO. Estradiol
was diluted to a final concentration of 1 nM in medium. Tamoxifen and raloxifene were diluted in RPMI-1640 medium to final concentrations of 0.1, 1, 5, and 10 µM. Actein was diluted to test concentrations of 0.1, 1, 5, 10, and 25 µM.

3.2.2 MCF-7 Cell Culture

ER+ human breast cancer MCF-7 cells were obtained from the ATCC (Rockville, MD) and were cultured in RPMI-1640 medium with phenol red and 2 mM L-glutamine, 5% FBS, and antibiotics (100 units/mL penicillin and 100 µg/mL streptomycin, BioSource International, Camarillo, CA). The cells were cultured as monolayers at 37°C and 5% CO₂. Cells were split every 7 days in a 1:5 ratio, and experiments were performed with cells from passages 7-17. For some experiments, medium was replaced with RPMI-1640 medium without phenol red with 2 mM L-glutamine, 5% CSS, and antibiotics one week prior to the experiment to remove exogenous estrogens.

3.2.3 SRB Assay for Cell Proliferation

MCF-7 cells were seeded (2.5 × 10⁴ cells/well) in 24-well Costar plates (Corning, NY). Following a 24-hour incubation, medium was replaced with DMSO control or test compounds dissolved in DMSO. Treatment medium was replaced every 24 hours for 96 to 120 hours. Cell growth was analyzed using the SRB assay described by Skehan, et. al, and Rubinstein, et. al (1990; 1990). Treated cells were fixed with 200 µL/well of 10% TCA at 4°C and incubated at 4°C for 1 hour. Cells
were then washed 5 times with water and left to air dry. The fixed cells were stained for 20 minutes with 0.4% SRB in 1% acetic acid, and then washed five times with 1% acetic acid and left to air dry. Bound dye was solubilized with 10 mM unbuffered Tris base at pH 10.5 for 5 minutes on a gyratory shaker. Aliquots were transferred to a 96-well microtiter plate, and absorbance was read by a plate reader at 490 nm. If necessary, dilutions were performed on all samples to ensure that readings were below 1.8 optical density (OD) units. For cell proliferation assays, data were expressed as mean ± SEM. Control and treated cells were compared using one-way ANOVA.

3.2.4 Gene Expression Analysis

MCF-7 cells were seeded (2.5 × 10⁵ cells/well) in 6-well Costar plates (Corning, NY). Following a 24-hour incubation, medium was replaced with DMSO control or test compounds dissolved in DMSO. Treatment medium was replaced every 24 hours for 48 hours. Treated cells were washed with PBS, and total RNA was isolated and quantified with Trizol (Invitrogen) and spectrophotometric analysis according to the manufacturer's instructions. To synthesize cDNA, 1 µg of total RNA was reverse-transcribed with the AffinityScript Multiple Temperature cDNA Synthesis Kit as described in the provided First-Strand cDNA Synthesis Protocol (Stratagene, La Jolla, CA). cDNA was diluted to a total volume of 200 µL in DEPC-treated water.

mRNA levels of genes of interest were determined by real-time PCR (RT-PCR) using the synthesized cDNA. Primers and probes for glyceraldehyde-3-
phosphate dehydrogenase (GAPDH); vascular endothelial growth factor A (VEGFA); trefoil factor 1 (TFF1); cytochrome P450, family 1, subfamily A, polypeptide 1 (CYP1A1); and cyclin-dependent kinase inhibitor 1A (p21, Cip1, CDKN1A) were obtained as TaqMan Gene Expression Assays (Applied Biosystems; Foster City, CA). Each 20 µL of PCR reaction mix contained 4.3 µL cDNA, 10 µL TaqMan Fast Universal Master Mix (2x, Applied Biosystems), and 1 µL TaqMan Gene Expression Assay mixture. RT-PCR was performed with an ABI-PRISM 7000 Sequence Detector (Applied Biosystems). PCR conditions were 50°C for 2 minutes, denaturation at 95°C for 10 minutes, followed by 46 cycles at 95°C for 15 seconds each, and 60°C for 1 minute. Each sample was assayed in triplicate in a 96-well optical plate. Ratios between experimental and control target gene expression were calculated after normalization of expression values to the housekeeping gene GAPDH.

3.2.5 Statistical Analysis

All treatment experiments were repeated at least twice. Cell proliferation experiments were performed in quadruplicate and gene expression assays in triplicate. For cell proliferation and gene expression assays, data were expressed as mean ± SEM. Control and treated cells were compared using one-way ANOVA followed by a multiple comparison post-test. Probability values less than or equal to 0.05 were considered significant. Statistical analysis was performed with GraphPad Prism.
3.3 *In vitro* Study: Hepatotoxicity of Actein in Liver Cells

3.3.1 Materials

Actein was obtained from Planta Analytica (Danbury, CT; lot number PA VI-13.A); purity was over 95% by HPLC. Tamoxifen and raloxifene hydrochloride were purchased from Sigma-Aldrich (St. Louis, MO). HepG2/C3A cells were obtained from ATCC (Manassas, VA) and media components were obtained from Invitrogen (Grand Island, NY), except for CSS which was received from Atlanta Biologicals (Lawrenceville, GA). Reagents for the endpoint assays were obtained from Sigma-Aldrich (St. Louis, MO), with the exception of the assay reagents for the albumin assay, which were obtained from Bethyl Laboratories (Montgomery, TX).

A BMG PolarStar™ Plate Reading Spectrofluorometer was used for all endpoint assays except for the albumin assay, for which an ELISA plate reader was used. For the test compounds, solutions of 0, 0.76, 1.55, 3.10, 6.25, 12.50, 25.00, 50.00, and 100.00 µM of actein, raloxifene, and tamoxifen in culture media and <0.5% DMSO were used to determine concentrations where significant levels of hepatotoxicity occurred. For the combination studies, test compound solutions of 90 µM tamoxifen with 0, 10, 30, and 100 µM actein, 50 µM raloxifene with 0, 10, 30, and 100 µM actein, 30 µM actein with 0, 3, 10, and 30 µM raloxifene, and 30 µM actein with 0, 3, 10, and 30 µM tamoxifen were used.
3.3.2 HepG2/C3A Cell Culture

Cyropreserved HepG2/C3A cells were thawed and plated on the inner 60 wells of 96-well plates in a culture medium consisting of 2 mM GlutaMAX™, 1% ITS, 1% MEM-NEAA, 10 mM HEPES, 10% CDS-FBS, 10 nM triiodothyronine, 20 nM dexamethasone, 1 mg/mL albumin, 9.4 µg/mL linoleic acid, 9.4 µg/mL oleic acid, 2 nM testosterone, 2 nM progesterone, and 0.4 nM estradiol in phenol-red free DMEM, to an average density of $2.0 \times 10^5$ viable cells/mL. The cells were incubated at 37°C and 5% CO₂. Culture medium was changed every 48-72 hours until cells reached confluence on culture day 10.

3.3.3 DCFDA Assay for Oxidative Stress

On day 10, cells were treated with the non-fluorescent dye dihydrodichlorofluorescein diacetate (DCFDA) for 30 minutes at 37°C and 0% CO₂, and then treated with the test compound. After a 45-48 hour incubation at 37°C and 5% CO₂, the fluorescent product dichlorofluorescein (DCF), which resulted from any oxidation of DCFDA by reactive oxygen species (ROS), was measured as pmol of DCF formed/45-48 hrs/µg DNA with the spectrofluorometer (Yerushalmi, Dahl, Devereaux, Gumpricht, & Sokol, 2001).

3.3.4 EROD Assay for Cytochrome P450IA1 Activity

After completion of the DCFDA Assay, on day 12 cells were washed with Hanks’ Balanced Salt Solution (HBSS) and treated with 7-ethoxyresorufin (EROD).
The amount of fluorescent resorufin product of CYP1A1 dealkylation of EROD was measured every 3 minutes for 1 hour to determine the pmol of resorufin formed/min/µg DNA (Donato, Gomez-Lechon, & Castell, 1993; Lubinski, Flint, & Durham, 1994). After this assay, cells were washed with HBSS and stored at -80°C until assayed for double-stranded DNA.

3.3.5 Nile Red Assay for Steatosis

On day 10 the test compounds were added, and the plate was incubated at 37°C in 5% CO₂ for 45-48 hours. 50 µL of media were removed from each well and stored at -80°C on a separate 96-well plate to be saved for the albumin assay. Cells were washed with HBSS, incubated with Nile Red for 15 minutes at 37°C and 5% CO₂, and the fluorescence of Nile Red in the phospholipid and neutral lipid areas of the cells was determined in fluorescence units/µg DNA (McMillian, et al., 2001). After this assay, cells were washed with HBSS and stored at -80°C until assayed for double-stranded DNA.

3.3.6 Total Double-Stranded DNA Assay for Cell Death

The plates were thawed at room temperature, refrozen at -80°C with dH₂O, thawed at room temperature, treated with non-fluorescent Hoeschst 33258 (H33258), protected from exposure to light and incubated at room temperature for 30 minutes. The fluorescence of H33258 bound to double-stranded DNA was measured in µg/0.1 mL with a spectrofluorometer (Rago, Mitchen, & Wilding, 1990).
3.3.7 Quantification of Albumin Secretion to Measure Cell Viability

The plates with the 50 µL of media per well were thawed so that 5 µL from each well could be used to determine ng of albumin/well via the Bethyl Laboratories human albumin ELISA method.

3.3.8 R123 Assay for Mitochondrial Membrane Depolarization

On day 10 the test compounds were added to the cells and incubated for 2.5 hours at 37°C in 5% CO₂. Rhodamine 123 was added and incubated with the cells for 30 minutes at 37°C in 5% CO₂. Cells were washed with HBSS and incubated with the test compound for 45-48 hours at 37°C and 5% CO₂. The fluorescence of rhodamine 123 was read on day 12 to determine pmol of rhodamine retained/45-48 hours (Rat, Korwin-Zmijowska, Warnet, & Adolphe, 1994). After this assay, cells were washed with HBSS and stored at -80°C until assayed for double-stranded DNA.

3.3.9 Statistical Analysis

The data were tested for normal distribution using the Shapiro-Wilk Test. One-way ANOVA, followed by the Fisher LSD post-test was used to analyze the effects of actein, tamoxifen, and raloxifene on the HepG2/C3A cells as measured by the endpoint assays. Two-way ANOVA was used for the interaction studies. Statistical analysis was performed with SAS 9.1.
4. Results

4.1 HDS Uses and Perceptions

4.1.1 Demography of Sample

Of the subjects that completed the demographic portion of the survey, the majority were \( \geq 51 \) years of age (Table 1). Only about one-third of the participants were between the ages of 25 and 51, and only one participant was under the age of 25. The vast majority (94%) of participants reported themselves as white/Caucasian. Regarding their religious beliefs, approximately two-thirds were of Christian religion.

The average participant reported having both a high level of education completed and a high household income. Approximately two-thirds of participants reported an annual household income of at least $60,000, and nearly 40% of all participants reported an annual household income of over $100,000. Regarding their highest education completed, approximately 30% had completed a Bachelor’s degree and 40% had completed an advanced or professional degree.

Over half of the survey participants reported that their cancer was currently in remission. Of participants whose cancer was not in remission, the majority reported that their cancer was in one of the less advanced stages, including Stage 0, Stage 1, Stage 2A, and Stage 2B. The vast majority of participants (1077, 97.6%) had been treated with conventional cancer treatments, and approximately one third (354, 32.1%) had been treated with conventional cancer treatments within the last 12 months (data not shown).
Table 1. Demographic variables and HDS use among breast cancer survivors.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Total</th>
<th>Used HDS</th>
<th>Did Not Use HDS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>% of total^b</td>
<td>n</td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18-25</td>
<td>1</td>
<td>0.1%</td>
<td>1</td>
</tr>
<tr>
<td>36-50</td>
<td>301</td>
<td>28.0%</td>
<td>268</td>
</tr>
<tr>
<td>51-65</td>
<td>301</td>
<td>55.9%</td>
<td>531</td>
</tr>
<tr>
<td>65+</td>
<td>142</td>
<td>13.2%</td>
<td>120</td>
</tr>
<tr>
<td><strong>Race</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>1011</td>
<td>94.0%</td>
<td>882</td>
</tr>
<tr>
<td>Black</td>
<td>19</td>
<td>1.8%</td>
<td>16</td>
</tr>
<tr>
<td>Asian</td>
<td>16</td>
<td>1.5%</td>
<td>17</td>
</tr>
<tr>
<td>Hispanic</td>
<td>6</td>
<td>0.6%</td>
<td>5</td>
</tr>
<tr>
<td>Other</td>
<td>23</td>
<td>2.1%</td>
<td>22</td>
</tr>
<tr>
<td><strong>Education^c</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade</td>
<td>2</td>
<td>0.2%</td>
<td>1</td>
</tr>
<tr>
<td>High</td>
<td>63</td>
<td>5.9%</td>
<td>42</td>
</tr>
<tr>
<td>Some College</td>
<td>181</td>
<td>16.8%</td>
<td>161</td>
</tr>
<tr>
<td>Associate’s</td>
<td>88</td>
<td>8.2%</td>
<td>80</td>
</tr>
<tr>
<td>Bachelors</td>
<td>312</td>
<td>29.0%</td>
<td>283</td>
</tr>
<tr>
<td>Advanced</td>
<td>416</td>
<td>38.7%</td>
<td>364</td>
</tr>
<tr>
<td>Other</td>
<td>13</td>
<td>1.2%</td>
<td>11</td>
</tr>
<tr>
<td><strong>Income^d</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;$20,000</td>
<td>39</td>
<td>3.6%</td>
<td>35</td>
</tr>
<tr>
<td>$20,000-39,999</td>
<td>105</td>
<td>9.8%</td>
<td>89</td>
</tr>
<tr>
<td>$40,000-59,999</td>
<td>116</td>
<td>10.8%</td>
<td>97</td>
</tr>
<tr>
<td>$60,000-99,999</td>
<td>303</td>
<td>28.2%</td>
<td>268</td>
</tr>
<tr>
<td>&gt;$100,000</td>
<td>408</td>
<td>38.0%</td>
<td>364</td>
</tr>
<tr>
<td>Don’t Know^e</td>
<td>104</td>
<td>9.7%</td>
<td>89</td>
</tr>
<tr>
<td><strong>Religion</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Christian</td>
<td>714</td>
<td>66.6%</td>
<td>625</td>
</tr>
<tr>
<td>Jewish</td>
<td>102</td>
<td>9.5%</td>
<td>84</td>
</tr>
<tr>
<td>Muslim</td>
<td>1</td>
<td>0.1%</td>
<td>1</td>
</tr>
<tr>
<td>Hindu</td>
<td>1</td>
<td>0.1%</td>
<td>1</td>
</tr>
<tr>
<td>Buddhist</td>
<td>12</td>
<td>1.1%</td>
<td>12</td>
</tr>
<tr>
<td>Deist/Agnostic</td>
<td>40</td>
<td>3.7%</td>
<td>32</td>
</tr>
<tr>
<td>Atheist</td>
<td>26</td>
<td>2.4%</td>
<td>20</td>
</tr>
<tr>
<td>None</td>
<td>111</td>
<td>10.4%</td>
<td>101</td>
</tr>
<tr>
<td>Other</td>
<td>65</td>
<td>6.1%</td>
<td>63</td>
</tr>
<tr>
<td><strong>Cancer Stage</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Remission</td>
<td>555</td>
<td>50.3%</td>
<td>499</td>
</tr>
<tr>
<td>Stage 0</td>
<td>107</td>
<td>9.7%</td>
<td>82</td>
</tr>
<tr>
<td>Stage 2 A</td>
<td>78</td>
<td>7.1%</td>
<td>68</td>
</tr>
<tr>
<td>Stage 2 B</td>
<td>65</td>
<td>5.9%</td>
<td>60</td>
</tr>
<tr>
<td>Stage 3 A</td>
<td>24</td>
<td>2.2%</td>
<td>20</td>
</tr>
<tr>
<td>Stage 3 B</td>
<td>18</td>
<td>1.6%</td>
<td>17</td>
</tr>
<tr>
<td>Stage 3 C</td>
<td>6</td>
<td>0.5%</td>
<td>4</td>
</tr>
<tr>
<td>Stage IV</td>
<td>31</td>
<td>2.8%</td>
<td>30</td>
</tr>
<tr>
<td>Other/Don’t Know^e</td>
<td>56</td>
<td>5.1%</td>
<td>45</td>
</tr>
</tbody>
</table>

^apercentage of all subjects that reported themselves as a member of each demographic group.

^bpercentage within each demographic group that used or did not use HDS, respectively.

^chighest level of education attained by the subject.

^dtotal household income for the past year.

^e“Don’t Know” includes those who did not know or did not wish to report their answer.
We performed a cluster analysis to group subjects with similar characteristics into categories. Cluster analysis confirmed the homogeneity of the sample—that is, mostly older, high income, and highly educated participants. Age, income, and education were used to group participants into three clusters or groups in which combinations of age, income, and education levels occur together. Race was excluded from this analysis because it cannot be ranked ordinally. Cubic clustering criterion determined the number of clusters (3) and an iterative process determined the mean of each cluster. Then, each participant was assigned to the cluster with the closest mean.

Cluster 1 is older, highly educated and high income (n=392); Cluster 2 is older, less educated, and middle income (n=344); and Cluster 3 is younger, highly educated, and high income (n=224). Cluster 1 consists of individuals that are best described as 51-65 years of age, with incomes greater than $100,000, and advanced degrees. Cluster 2 generally describes individuals who are also 51-65 years of age, but of incomes between $40,000-50,000 and with Associate’s degrees. Cluster 3 best describes individuals between 36-50 years of age, with incomes exceeding $100,000 and advanced degrees (Table 2).

The overlap in these clusters shows that most of the sample is either older or a high earner with advanced education (if not both) as shown by the nearly 400 participants who fall in Cluster 1.
Table 2. Cluster analysis of demographic characteristics.

<table>
<thead>
<tr>
<th>Cluster</th>
<th>n</th>
<th>Description</th>
<th>Age</th>
<th>Income</th>
<th>Education</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>392</td>
<td>Older, high income, high education</td>
<td>51 – 65</td>
<td>&gt; $100,000</td>
<td>Advanced Degree</td>
</tr>
<tr>
<td>2</td>
<td>344</td>
<td>Older, middle income, less education</td>
<td>51 – 65</td>
<td>$40,000-50,000</td>
<td>Associate’s Degree</td>
</tr>
<tr>
<td>3</td>
<td>224</td>
<td>Younger, high income, high education</td>
<td>36 – 50</td>
<td>&gt; $100,000</td>
<td>Advanced Degree</td>
</tr>
</tbody>
</table>

*a* characterizes the cluster relative to all participants.  
*b* represent the average for each cluster.

4.1.2 Frequency of Supplement Use

965 (87.6%) subjects reported using herbal dietary supplements. The frequency of herbal dietary supplement use within each demographic group is displayed in Table 1. Including only those subjects who reported using at least one HDS, each subject used an average of about 6 HDS (mean=5.70, median=6, mode=6, standard deviation=2.49). When including both those who did and did not use HDS, an average of 5 HDS were used by each subject (mean=4.97, median=5, mode=6, standard deviation=3.00).

The most commonly used supplements for non-medicinal purposes were soy, green tea, chamomile, ginger, and flaxseed, respectively (Figure 3). Each of these was used by at least one-third of all survey subjects, while soy was used by over half of participants (50.3%). Less than one-fourth of subjects reported using each of the remaining supplements for non-medicinal purposes, including 41 subjects (4.3%) who reported using black cohosh for non-medicinal purposes.

The most commonly used supplements for medicinal purposes were flaxseed, green tea, ginger, and curcumin/turmeric, respectively. At least one fourth of participants reported using each of these supplements for medicinal purposes, while
flaxseed was used by 43.8% of all participants who responded to the question regarding flaxseed use. Less than one fourth of participants reported using each of the remaining supplements for medicinal purposes, with 191 subjects (19.8%) using black cohosh for medicinal purposes (Figure 3).

![Use of Herbal Dietary Supplements by HDS](image)

**Figure 3. Use of HDS for medicinal and other purposes.**
Use for medicinal purposes includes only instances when an HDS is taken with the specific intent to treat a medical condition or symptom, or to improve health. Use for other purposes includes uses in cooking and for other non-medically related reasons. Total is the sum of medicinal and other use.

It is important to note that subjects were forced to report their supplement use as being either for medicinal or non-medicinal purposes, and were not able to report using supplements for both purposes. To determine the overall use of HDS irrespective of purpose, HDS use was analyzed using a chi-square goodness of fit test. According to this analysis, the most frequently used HDS for both purposes were
flaxseed, green tea, curcumin, and ginger. The least frequently used HDS were saw palmetto, red clover, and kava kava (Figure 3).

![Use of HDS for Medicinal and Other Purposes as Percentage of Total Users Per HDS](image)

**Figure 4.** Relative use of each individual HDS for medicinal and other purposes.
Use for medicinal purposes includes only instances when an HDS is taken with the specific intent to treat a medical condition or symptom, or to improve health. Use for other purposes includes uses in cooking and for other non-medically related reasons.

While supplements were used almost equally for medicinal and non-medicinal purposes overall, there were differences in how individual supplements were used. Most supplements were used more often for one purpose than the other. For example, black cohosh, curcumin, evening primrose, flaxseed, milk thistle, red clover, St. John’s wort, and valerian were used significantly more for medicinal purposes as indicated by a chi-square test of association. Conversely, chamomile, ginger, ginseng, green tea, saw palmetto, and soy were used significantly more for non-
medicinal (other) purposes. Kava kava was used about equally for medicinal and other purposes (Figure 4).

The demographic clusters previously obtained were examined with HDS use via chi square analysis of variance to determine whether the number of HDS used differed between the clusters. In fact, there is no significant difference between the number of HDS used and demographic cluster membership. To determine whether cluster membership can predict the use of specific HDS, a logistic regression was estimated for each supplement where HDS use is the dependent variable and cluster membership is the explanatory variable. Cluster membership appears to only the affect the use of milk thistle (p=0.0298) and soy (p=0.0042), where Cluster 1 is less likely to than Clusters 2 and 3 to use milk thistle and Cluster 3 is less likely than Clusters 1 and 2 to use soy. Because Cluster 1 shares characteristics with the other clusters, the interpretation of the milk thistle regression is unclear. Yet because Cluster 3 is younger than Clusters 1 and 2, it can be speculated that older women are more likely to use soy.

Cluster analysis was also used to determine whether or not there were common patterns of HDS use, with certain groups of HDS frequently used together (Table 3). Survey respondents clustered into 4 groups when considering the simultaneous use of all HDS, based on two criteria: the number of HDS used relative to the overall average, and the type of HDS used relative to the overall frequencies of use. Thus the number of supplements used can be greater than, equal to, or less than average; while the kinds of supplements used can be common or rare. Cluster 1 used an average number of HDS (μ=6.2), and used mostly common supplements, with
increased use of curcumin, chamomile, ginger, ginseng and soy compared to other clusters. Cluster 2 used a high number of HDS \((µ=9.1)\), and used both common and rare HDS, with increased use of all HDS. Cluster 3 used a minimal number of HDS \((µ=2.8)\) and used mostly common known HDS, with the highest use of flaxseed and green tea. Cluster 4 used an average number of HDS \((µ=5.4)\), but used both common and rare HDS, with increased use of black cohosh, milk thistle, and evening primrose (rare HDS) as well as soy, chamomile, ginger, green tea and flaxseed (common HDS) compared to other clusters. The clusters are roughly equal in size, while Cluster 1 contains a plurality of survey participants.

**Table 3. Cluster analysis of HDS use.**

<table>
<thead>
<tr>
<th>Cluster</th>
<th>n</th>
<th>Number of HDSa ((µ))</th>
<th>Increased Use of Common HDSb</th>
<th>Increased Use of Rare HDSb</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>305</td>
<td>Average (6.2)</td>
<td>Curcumin, Chamomile, Ginger, Ginseng, Soy</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>152</td>
<td>High (9.1)</td>
<td>All</td>
<td>All</td>
</tr>
<tr>
<td>3</td>
<td>220</td>
<td>Minimal (2.8)</td>
<td>Flaxseed, Green Tea</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>191</td>
<td>Average (5.4)</td>
<td>Soy, Chamomile, Ginger, Green, Tea, Flaxseed</td>
<td>Black Cohosh, Milk Thistle, Evening Primrose</td>
</tr>
</tbody>
</table>

acharacterizes HDS use as “minimal,” “average,” or “high” relative to all survey participants.

b“Common” and “Rare” HDS are inferred from the chi square test of association used to determine which supplements are used most frequently. “Increased Use” is relative to all survey participants.

In addition to examining patterns of HDS use in general, our survey also examined HDS use prior to and after breast cancer diagnoses. If subjects answered “yes” to using a particular supplement, they were then prompted to disclose whether they used the supplement before their diagnosis and/or after their diagnosis. The aim of this analysis was to determine whether HDS use changed after cancer was diagnosed. In order to assess the change, a pair-wise comparison was conducted by coding the responses as a positive change (began HDS use after diagnosis), a negative change (discontinued use after diagnosis), or no change. On average, the change ratio was 44.7% positive change, 28.6% negative change, and 29.4% no change,
suggesting that subjects were more likely to begin taking HDS after they were diagnosed with breast cancer.

However, the changes in use varied among the different supplements. We used chi-square tests of association to determine if a specific HDS was more likely to undergo a change in use after cancer diagnosis and whether the change was more likely to be positive or negative. We found that the use of black cohosh, curcumin, evening primrose, flaxseed, ginseng, kava kava, milk thistle, red clover, St. John's wort, saw palmetto, and soy was more likely to change after cancer diagnosis than the use of chamomile, ginger, green tea or valerian. Use of chamomile, curcumin, flaxseed, ginger, green tea and milk thistle increased after cancer diagnosis while the use of kava kava and St. John's wort decreased after diagnosis. The use of black cohosh, evening primrose, ginseng, red clover, saw palmetto, soy, and valerian both increased and decreased after cancer diagnosis, indicating that approximately equal numbers of survey respondents either started or stopped taking these supplements after being diagnosed with breast cancer (Table 4).

Table 4. Use of HDS before and after breast cancer diagnoses.

<table>
<thead>
<tr>
<th>Mostly Increased Use After Diagnoses(^a)</th>
<th>Mostly Decreased Use After Diagnoses(^b)</th>
<th>Both Increased and Decreased Use(^c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chamomile</td>
<td>Kava Kava</td>
<td>Black Cohosh</td>
</tr>
<tr>
<td>Curcumin</td>
<td>St. John’s Wort</td>
<td>Evening Primrose</td>
</tr>
<tr>
<td>Flaxseed</td>
<td></td>
<td>Ginseng</td>
</tr>
<tr>
<td>Ginger</td>
<td></td>
<td>Red Clover</td>
</tr>
<tr>
<td>Green Tea</td>
<td></td>
<td>Saw Palmetto</td>
</tr>
<tr>
<td>Milk Thistle</td>
<td></td>
<td>Soy</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Valerian</td>
</tr>
</tbody>
</table>

\(^a\)most likely to have their use begin after breast cancer diagnoses.
\(^b\)most likely to have their use end after breast cancer diagnoses.
\(^c\)had their use start and stop almost equally after diagnosis.
4.1.3 Reasons for Supplement Use

Subjects were asked open-ended questions regarding specific reasons for why they used HDS. Thirteen of the 15 supplements were reported by at least one subject as being used to directly treat cancer or prevent its recurrence. The only supplements that were not reported as being used to treat or prevent cancer were St. John’s wort and valerian. Most notably, 125 subjects reported using green tea, 112 subjects reported using curcumin, 100 subjects reported using flaxseed, and 25 subjects reported using soy to directly treat or prevent cancer recurrence (Figure 5).

![Use of HDS to Treat or Prevent Breast Cancer Recurrence](image)

**Figure 5. Use of HDS to treat and/or prevent breast cancer recurrence.**

This chart displays the number of respondents who reported using each HDS to specifically treat and/or prevent the recurrence of their breast cancer as reported in open-ended questions addressing reasons for use.

Survey respondents indicated several other reasons for using HDS, shown in Table 5. Table 5 shows the most frequently cited reasons for using HDS, per each
specific type of HDS. “Most frequent reasons” are those cited by at least 10 percent of respondents who answered the questions about reasons for use.

**Table 5. Most frequent reasons for using HDS.**

<table>
<thead>
<tr>
<th>HDS</th>
<th>Total Respondents Who Cited Reasons</th>
<th>Most Frequent Reasons for Use</th>
<th>Number of Respondents</th>
<th>Percentage of Users</th>
</tr>
</thead>
<tbody>
<tr>
<td>Black Cohosh</td>
<td>188</td>
<td>Hot Flashes, Menopause</td>
<td>124</td>
<td>66.0 %</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>42</td>
<td>22.3 %</td>
</tr>
<tr>
<td>Chamomile</td>
<td>163</td>
<td>Anxiety, Insomnia, Nausea</td>
<td>81</td>
<td>49.7 %</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>71</td>
<td>43.6 %</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>33</td>
<td>20.2 %</td>
</tr>
<tr>
<td>Curcumin</td>
<td>243</td>
<td>Cancer Treatment, Pain/Inflammation</td>
<td>112</td>
<td>46.1 %</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>87</td>
<td>35.8 %</td>
</tr>
<tr>
<td>Evening Primrose</td>
<td>192</td>
<td>Hot Flashes, Menopause</td>
<td>53</td>
<td>27.6 %</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>30</td>
<td>15.6 %</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>25</td>
<td>13.0 %</td>
</tr>
<tr>
<td>Flaxseed</td>
<td>420</td>
<td>Cancer Treatment, Omega 3, Cholesterol, Overall Health, Digestion</td>
<td>100</td>
<td>23.8 %</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>77</td>
<td>18.3 %</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>73</td>
<td>17.4 %</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>55</td>
<td>13.1 %</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>45</td>
<td>10.7 %</td>
</tr>
<tr>
<td>Ginger</td>
<td>281</td>
<td>Nausea</td>
<td>213</td>
<td>75.8 %</td>
</tr>
<tr>
<td>Ginseng</td>
<td>140</td>
<td>Fatigue, Cognitive Function</td>
<td>60</td>
<td>42.9 %</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>30</td>
<td>21.4 %</td>
</tr>
<tr>
<td>Green Tea</td>
<td>417</td>
<td>Antioxidants, Cancer Treatment, Overall Health</td>
<td>157</td>
<td>37.6 %</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>125</td>
<td>30.0 %</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>53</td>
<td>12.7 %</td>
</tr>
<tr>
<td>Kava Kava</td>
<td>49</td>
<td>Anxiety, Insomnia</td>
<td>24</td>
<td>49.0 %</td>
</tr>
<tr>
<td>Milk Thistle</td>
<td>150</td>
<td>Liver Function</td>
<td>113</td>
<td>75.3 %</td>
</tr>
<tr>
<td>Red Clover</td>
<td>58</td>
<td>Hot Flashes, Menopause</td>
<td>11</td>
<td>19.0 %</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>9</td>
<td>15.5 %</td>
</tr>
<tr>
<td>Saw Palmetto</td>
<td>13</td>
<td>Overall Health</td>
<td>3</td>
<td>23.1 %</td>
</tr>
<tr>
<td>Soy</td>
<td>161</td>
<td>Hot Flashes, Diet, Menopause</td>
<td>45</td>
<td>28.0 %</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>29</td>
<td>18.0 %</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>27</td>
<td>16.8 %</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>25</td>
<td>15.5 %</td>
</tr>
<tr>
<td>St. John’s Wort</td>
<td>149</td>
<td>Depression/Mood, Anxiety</td>
<td>114</td>
<td>76.5 %</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>22</td>
<td>14.8 %</td>
</tr>
<tr>
<td>Valerian</td>
<td>180</td>
<td>Insomnia, Anxiety</td>
<td>136</td>
<td>75.6 %</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>37</td>
<td>20.6 %</td>
</tr>
</tbody>
</table>

*a ratio of the “Number of Respondents” over the “Total Respondents who Cited Reasons” for that supplement.

It is important to note that questions regarding specific reasons for use were open-ended, which may have introduced bias according to how the questions were interpreted. After the survey was administered, similar responses to open-ended questions were aggregated into discrete categories, such as digestive problems or
immune support, to compare the reasons for use for a particular HDS. The categories were further aggregated in order to compare reasons for use among all HDS. A concise list of categories is necessary to determine whether there are actual differences in the reasons for using different supplements, and reasons cited by only a few respondents would skew the distribution. Further, several respondents cited multiple reasons for using a particular HDS. For the first round of coding, each reason a subject gave was individually counted, but the second round of coding eliminated this “double-counting” by assigning a new variable denoting that a particular subject gave multiple reasons for taking an HDS.

The reasons for using HDS were ultimately condensed from the original 30+ categories into 8 broad categories (Table 6). Each of these broad categories includes more specific but still relatively similar reasons for use.
**Table 6. Reasons for using HDS.**

<table>
<thead>
<tr>
<th>Broad Category*</th>
<th>Specific Reasons for Use</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart (HRT)</td>
<td>Heart health</td>
</tr>
<tr>
<td></td>
<td>Cholesterol</td>
</tr>
<tr>
<td></td>
<td>Blood Pressure</td>
</tr>
<tr>
<td>Psychiatric (PSYCH)</td>
<td>Mood</td>
</tr>
<tr>
<td></td>
<td>Cognition</td>
</tr>
<tr>
<td></td>
<td>Depression</td>
</tr>
<tr>
<td></td>
<td>Anxiety</td>
</tr>
<tr>
<td></td>
<td>Insomnia</td>
</tr>
<tr>
<td>Estrogen (EST)</td>
<td>Estrogen</td>
</tr>
<tr>
<td></td>
<td>PMS</td>
</tr>
<tr>
<td></td>
<td>Menopause</td>
</tr>
<tr>
<td></td>
<td>Hot Flashes</td>
</tr>
<tr>
<td>Cancer Treatment (CAN)</td>
<td>Cancer treatment</td>
</tr>
<tr>
<td></td>
<td>Prevention of recurrence</td>
</tr>
<tr>
<td>Diet (DIET)</td>
<td>Diet</td>
</tr>
<tr>
<td></td>
<td>Omega fatty acids</td>
</tr>
<tr>
<td></td>
<td>Antioxidants</td>
</tr>
<tr>
<td></td>
<td>Fiber</td>
</tr>
<tr>
<td>General Health (HLTH)</td>
<td>General health and well-being</td>
</tr>
<tr>
<td></td>
<td>Immune support</td>
</tr>
<tr>
<td>Misc. bodily functions (BODY)</td>
<td>Digestion</td>
</tr>
<tr>
<td></td>
<td>Liver function</td>
</tr>
<tr>
<td></td>
<td>Bone/skeletal</td>
</tr>
<tr>
<td></td>
<td>Pain/inflammation</td>
</tr>
<tr>
<td>Chemotherapeutic Symptoms (CHEMO)</td>
<td>Nausea</td>
</tr>
<tr>
<td></td>
<td>Fatigue</td>
</tr>
<tr>
<td></td>
<td>Weight control</td>
</tr>
<tr>
<td></td>
<td>Cell damage</td>
</tr>
<tr>
<td></td>
<td>Hair damage</td>
</tr>
<tr>
<td></td>
<td>Acne</td>
</tr>
<tr>
<td></td>
<td>Eye problems</td>
</tr>
</tbody>
</table>

*Each broad category includes specific reasons for use.

In order to further analyze which supplements were used for which reasons, we used a correspondence analysis. Figure 6 shows the supplements and the reasons for use arranged in four distinct clusters. Correlations between a supplement and its use can be inferred by the clusters of plots. For example, the triangle cluster indicates that chamomile, kava kava, valerian, and St. John’s wort were used primarily for
psychological reasons, such as anxiety and insomnia. Black cohosh, soy, red clover, and evening primrose were used mostly for estrogen related reasons, such as symptoms of menopause (rectangle cluster). As seen in the square cluster, ginger and ginseng were used mostly to treat common side effects of chemotherapy. Flaxseed, saw palmetto, curcumin, green tea, and milk thistle were all used for a combination of reasons, including heart health, cancer treatment, improved diet, general health, body functions, and multiple/other reasons (oval cluster, Figure 6).

**Figure 6. Correlation of HDS and reasons for use.**
Correspondence analysis was used to determine correlations between groups of HDS and broad categories of reasons for use. Abbreviations for reasons: CAN, cancer; EST, estrogen related reasons; PSYCH, psychological reasons; CHEMO, side effects of conventional therapies; HRT, heart health; HLTH, general health; DIET, dietary reasons; BODY, digestive, liver, and bone health as well as pain/inflammation; MULTI, multiple reasons; OTH, other reasons. Abbreviations for supplements: BC, black cohosh; EP, evening primrose; SP, saw palmetto; RC, red clover; Flax, flaxseed; Cur, curcumin; MT, milk thistle; GT, green tea; Cham, chamomile; KK, kava kava; SJW, St. John’s wort; Val, valerian.
4.1.4 HDS to Treat Side Effects of Conventional Treatments

After being asked the general reasons for using supplements, subjects were asked whether or not they used HDS to treat side effects of conventional cancer therapy. A chi-square goodness of fit test showed that ginger was by far the most commonly used supplement to treat side effects of conventional cancer treatments, and was the only HDS used more than 50% of the time for this purpose. Other commonly used HDS to treat side effects of conventional therapies were chamomile, black cohosh, green tea, flaxseed, and milk thistle. HDS used least often to treat side effects of conventional cancer therapies were saw palmetto, kava kava, red clover, St. John's wort, and soy.

Those who reported using a supplement to treat side effects of conventional therapies were then asked which specific side effects of conventional cancer treatments they had hoped to treat with each supplement. Table 7 shows how many survey participants cited side effects they hoped to treat with each supplement and of those side effects, the most frequently cited.

These results should be interpreted with caution, however, as it appears that the survey questions addressing reasons for using HDS, and the symptoms or side effects of conventional cancer treatments that subjects hoped to treat with HDS, may have been misunderstood. Many respondents who answered both of these open-ended questions provided the same response. For example, a respondent would indicate “inflammation” as both a reason for using curcumin as well as the side effect of conventional therapy she hoped to alleviate by taking the supplement. In addition, several respondents simply listed “cancer treatment” as a side effect of the
conventional cancer therapy, further suggesting that some of the survey questions were ambiguous. Nevertheless, the number of respondents who answered the question regarding symptoms of conventional treatments was about half the number who answered the question about reasons for using HDS. This is consistent with the notion that the number of subjects who used HDS to treat side effects of conventional cancer therapies would be a smaller subset of those who used HDS for any medical purposes.

Table 7. Most frequent side effects treated by HDS.

<table>
<thead>
<tr>
<th>HDS</th>
<th>Total Respondents Who Cited Side Effects</th>
<th>Most Frequent Side Effects</th>
<th>Number of Respondents</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Black Cohosh</td>
<td>63</td>
<td>Hot Flashes</td>
<td>56</td>
<td>88.9%</td>
</tr>
<tr>
<td>Chamomile</td>
<td>63</td>
<td>Anxiety</td>
<td>31</td>
<td>49.2%</td>
</tr>
<tr>
<td>Curcumin</td>
<td>45</td>
<td>Nausea</td>
<td>22</td>
<td>34.9%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Insomnia</td>
<td>20</td>
<td>31.7%</td>
</tr>
<tr>
<td>Evening Primrose</td>
<td>45</td>
<td>Hot Flashes</td>
<td>35</td>
<td>77.8%</td>
</tr>
<tr>
<td>Flaxseed</td>
<td>67</td>
<td>Digestion</td>
<td>19</td>
<td>28.4%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hot Flashes</td>
<td>15</td>
<td>22.4%</td>
</tr>
<tr>
<td>Ginger</td>
<td>173</td>
<td>Nausea</td>
<td>156</td>
<td>90.2%</td>
</tr>
<tr>
<td>Ginseng</td>
<td>43</td>
<td>Cognitive Function</td>
<td>11</td>
<td>25.6%</td>
</tr>
<tr>
<td>Green Tea</td>
<td>78</td>
<td>Immune Support</td>
<td>19</td>
<td>24.4%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fatigue</td>
<td>18</td>
<td>23.1%</td>
</tr>
<tr>
<td>Kava Kava</td>
<td>11</td>
<td>Anxiety</td>
<td>5</td>
<td>45.5%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Insomnia</td>
<td>4</td>
<td>36.4%</td>
</tr>
<tr>
<td>Milk Thistle</td>
<td>30</td>
<td>Liver Function</td>
<td>46</td>
<td>76.7%</td>
</tr>
<tr>
<td>Red Clover</td>
<td>14</td>
<td>Hot Flashes</td>
<td>6</td>
<td>42.9%</td>
</tr>
<tr>
<td>Soy</td>
<td>31</td>
<td>Hot Flashes</td>
<td>22</td>
<td>71.0%</td>
</tr>
<tr>
<td>Saw Palmetto</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>St. John’s Wort</td>
<td>21</td>
<td>Depression/Mood</td>
<td>18</td>
<td>85.7%</td>
</tr>
<tr>
<td>Valerian</td>
<td>59</td>
<td>Insomnia</td>
<td>48</td>
<td>81.4%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Anxiety</td>
<td>13</td>
<td>22.0%</td>
</tr>
</tbody>
</table>

"ratio of “Number of Respondents” over “Total Respondents Who Cited Side Effects” for that supplement.

4.1.5 Perceived Efficacy of HDS

Our survey also asked how effective subjects considered each HDS, and repeated this question for each supplement. Overall, the supplements included in our survey were generally considered effective. Of the 781 total responses to the fifteen
questions regarding effectiveness, the forced-choice responses of “not effective,” “somewhat effective,” “very effective,” and “don’t know” are distributed equally. For statistical analysis, however, “don’t know” responses were excluded because we cannot interpret their meaning and because they skew the results. Some supplements were rated “don’t know” more than other supplements. For example, 62.9% of responses for milk thistle effectiveness were “don’t know,” versus 1.6% of responses for chamomile effectiveness. Each possible response was calculated as a ratio of the total number of responses per supplement. The average ratios across supplements were 18.0% “not effective,” 47.2% “somewhat effective,” and 34.8% “very effective.”

A chi-square test of association indicated that there were significant differences of perceived effectiveness among individual HDS. Ginger, green tea, milk thistle, and curcumin were considered very effective. Ginseng, valerian, flaxseed, red clover, and chamomile were considered somewhat effective. Black cohosh, soy, St. John’s wort, and evening primrose were considered not effective. Saw palmetto and kava kava were excluded from this analysis because they received too few responses (Figure 7).
Figure 7. Perceived efficacy of individual HDS.
This chart shows the relative percentage of respondents that reported each supplement as “not effective,” “somewhat effective,” and “very effective.” Saw palmetto and Kava kava were excluded due to low reported use. “Don’t know” responses were also excluded.

4.1.6 Sources of Information

Survey participants were asked to rate how often they consulted various sources of information, and were provided with the following choices: websites found through search engines, government websites, companies, non-profit organizations, cancer related health organizations, popular magazines and newspapers, academic journals, friends, family, health care providers, and complementary and alternative medicine practitioners. Survey participants could indicate that they consulted the source never, rarely, sometimes, often, or indicate that they were unsure or did not know in this forced-choice question.
Most (n=986) respondents reported having sought information on herbal dietary supplements. Subjects were also more likely to consult some sources of information more than others. A chi-square goodness of fit test determined that subjects were more likely to consult websites found through search engines, non-profit organizations and support groups, and health care practitioners. They were less likely to consult for-profit companies, primary journal articles, or family members (Figure 8).

**Figure 8. Frequency of use of HDS sources of information.**
The frequency that each source of information was consulted by survey participants. Websites includes any websites found through databases and search engines, such as Yahoo and Google. GovtWeb includes only government sponsored websites, such as the NIH website. Companies includes any information obtained from for profit companies, such as GNC. NonProfit includes any information from non-profit organizations, such as Susan G. Komen. CRHOs includes information obtained from cancer related health organizations, such as the American Cancer Society. MagsNews includes popular magazines or newspapers, such as Time or the Washington Post. Journals includes academic journals, such as The Journal of the American Medical Association. HCP includes any regular health care provider, such as nurses, oncologists, and general physicians. Practitioner includes only practitioners that specialize in CAM. Don’t know and unsure answers are not included in the chart.
4.1.7 Reliability of Information Sources

Subjects were also asked to rate how reliable or trustworthy they consider each of the aforementioned sources of information regarding HDS. Here, the response choices were “not reliable,” “somewhat reliable,” and “very reliable,” along with “I don’t know what this is” and “don’t know/not sure” options. In general, survey participants felt that their sources of information about HDS were either somewhat or very reliable. A chi-square goodness of fit test indicated that cancer-related health organizations, health care practitioners, academic journals, non-profit organizations, government websites, and HDS practitioners were considered more reliable than for-profit companies, family, friends, or magazines/newspapers. The most frequently used source of information—websites found through search engines—was generally viewed favorably, as 708 respondents indicated this source was “somewhat reliable,” 120 indicated “very reliable,” and 95 reported “not reliable” (Figure 9).
Figure 9. Perceived reliability of HDS sources of information.
The perceived reliability of each source of information that was consulted by survey participants. Websites includes any websites found through databases and search engines, such as Yahoo and Google. GovtWeb includes only government sponsored websites, such as the NIH website. Companies includes any information obtained from for profit companies, such as GNC. NonProfit includes any information from non-profit organizations, such as Susan G. Komen. CRHOs includes information obtained from cancer related health organizations, such as the American Cancer Society. MagsNews includes popular magazines or newspapers, such as Time or the Washington Post. Journals includes academic journals, such as The Journal of the American Medical Association. HCP includes any regular health care provider, such as nurses, oncologists, and general physicians. Practitioner includes only practitioners that specialize in CAM. Don’t know and unsure answers are not included in the chart.

4.1.8 Decision Influences

In order to determine correlations between sources of information used and the decision to use or not use HDS, a multi-way ANOVA test was used. Respondents who answered I don’t know or unsure for at least one information source were excluded from this analysis. We found that journals (p=0.0369), websites found through search engines (p=0.0249), government websites (p=0.0449) and CAM
practitioners (p<0.0001) were correlated with the number of HDS used. Journals, websites, and CAM practitioners were associated with increased HDS use while government websites was associated with decreased HDS use.

A further analysis using chi-square tests of association was performed to determine whether correlations existed between sources of information and the use of specific HDS. With respect to black cohosh, people who consulted health care providers or CAM practitioners were more likely to use black cohosh than people who did not consult health care providers or CAM practitioners (p=0.0405 and p=0.0493 respectively).

Table 8 shows the correspondence of sources of information with specific HDS use. For each of the following sources of information, increased or decreased use of the HDS listed was correlated with the source. It is interesting to note that milk thistle and curcumin, which were both considered “very effective” in terms of efficacy, followed the same use pattern. Also, academic journals and CAM practitioners were associated with increased use of several supplements. Only government websites, cancer related health organizations, healthcare providers, and family were associated with decreased use of at least one supplement.
<table>
<thead>
<tr>
<th>Source Consulted</th>
<th>More Likely to Use&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Less Likely to Use&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Websites</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Government Websites</td>
<td>Ginger (p=0.0214)</td>
<td>Red Clover (p=0.0287)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Kava Kava (p=0.0305)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Saw Palmetto (p=0.0057)</td>
</tr>
<tr>
<td>For-profit Companies</td>
<td>Ginseng (0.0009)</td>
<td></td>
</tr>
<tr>
<td>Non-profit Organizations</td>
<td>Green Tea (p=0.0033)</td>
<td>Red Clover (p=0.0180)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Valerian (p=0.0052)</td>
</tr>
<tr>
<td>Cancer Related Health Organizations</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Magazines and Newspapers</td>
<td>Flaxseed (p=0.0278)</td>
<td></td>
</tr>
<tr>
<td>Academic Journals</td>
<td>Red clover (p=0.0348)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Curcumin (p=0.0003)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Flaxseed (p=0.0236)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Milk thistle (p&lt;0.0001)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ginger (p=0.0163)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ginseng (p=0.0380)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Green Tea (p=0.0140)</td>
<td></td>
</tr>
<tr>
<td>Friends</td>
<td>Soy (p=0.0119)</td>
<td></td>
</tr>
<tr>
<td>Family</td>
<td>Ginseng (p=0.0138)</td>
<td>Red Clover (p=0.0269)</td>
</tr>
<tr>
<td>Healthcare Providers</td>
<td>Chamomile (p=0.0282)</td>
<td>Curcumin (p=0.0034)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Milk Thistle (p=0.0041)</td>
</tr>
<tr>
<td>CAM Practitioners</td>
<td>Red Clover (p&lt;0.0001)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Curcumin (p&lt;0.0001)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Chamomile (p&lt;0.0001)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Flaxseed (p=0.0001)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Milk Thistle (p&lt;0.0001)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ginger (p=0.0008)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Evening Primrose (p&lt;0.0001)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ginseng (p&lt;0.0001)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Kava Kava (p=0.0005)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Saint John's Wort (p&lt;0.0001)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Valerian (p&lt;0.0001)</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> For each of the information sources listed, use of the source was correlated with increased (“more likely to use”) or decreased (“less likely to use”) use of the HDS.

Survey participants were asked to rate the amount of influence that their family, friends, physician, and religion had on their decision to use an herbal dietary supplement. All four categories were rated overwhelmingly as being not influential,
but physicians received the greatest number of responses (n=334) as being very influential (Figure 10).

Figure 10. Influence of various factors on decision to use or not use HDS. The extent to which survey participants felt that family, friends, physicians, and religion influenced their decision to use or not use HDS. Responses of Don’t Know/Unsure are not included.

4.1.9 Safety Issues: Labeling and Testing

Survey respondents were generally concerned about the issue of proper labeling and safety testing of herbal dietary supplements. Close to half reported being “very concerned” and half reported being “somewhat concerned” about each of these issues. Only a small percentage of respondents reported not being concerned about either safety or labeling issues (Figure 11).
Figure 11. Concern regarding safety and labeling of HDS.
Participants were asked to rank their level of concern regarding adequate safety testing of HDS and proper labeling of HDS. Proper labeling includes insuring that the label indicates the correct concentration of supplement as well as the purity.
4.2 Effects of SERMs and Actein on Breast Cancer Cells

4.2.1 Individual Effects of Actein and SERMs on MCF-7 Cell Proliferation

Tamoxifen and raloxifene are both selective estrogen receptor modulators (SERMs) commonly prescribed to reduce the risk of developing breast cancer, to help treat existing breast cancer, and to help reduce the risk of breast cancer recurrence. Although the effects of tamoxifen and raloxifene on breast cancer cell proliferation are established, we measured the proliferation of MCF-7 human breast cancer cells treated with tamoxifen or raloxifene alone to establish a baseline for our combination studies (Coezy, Borgna, & Rochefort, 1982; Levenson, et al., 2002). Tamoxifen significantly inhibited MCF-7 cell proliferation at 5.0 µM, and raloxifene significantly inhibited MCF-7 cell proliferation at 0.1 µM (Figure 12).

Actein significantly inhibited MCF-7 cell proliferation at a concentration of 5.0 µM (3.3 µg/mL). According to the NCI’s Developmental Therapeutics Program database, the half maximal inhibitory concentrations (IC\textsubscript{50}) for tamoxifen and raloxifene in MCF-7 cells are 1.6 µM and 0.1 µM, respectively (Collins). These values were smaller than the concentrations we observed, which were between 5 and 10 µM for raloxifene, about 10 µM for tamoxifen, and slightly above 10 µM for actein. The difference is likely due to differences in culture conditions or length and frequency of treatment.
Figure 12. Actein, tamoxifen, and raloxifene inhibit MCF-7 cell proliferation. Actein and tamoxifen significantly inhibit the proliferation of MCF-7 cells at 5.0 μM; raloxifene significantly inhibits proliferation at 0.1 μM. Cells were maintained in medium with FBS and treated for 72 hours with actein, tamoxifen, or raloxifene in medium at the indicated concentrations. Relative number of viable cells, shown as mean ± SEM and normalized by DMSO control, was determined by SRB assay.

4.2.2 Individual Effects of Actein and SERMs on Estrogen-Induced MCF-7 Cell Proliferation

Actein significantly inhibited estradiol-induced cell proliferation at 10.0 μM and thus may have an anti-estrogenic effect (Figure 13). Before this experiment, MCF-7 cells were maintained in phenol red-free media with CSS to remove exogenous estrogens. Additionally, unlike cells cultured in media with FBS, actein alone did not significantly inhibit proliferation of cells cultured with phenol red-free media with CSS. Cells were also treated with tamoxifen or raloxifene with and without estradiol to observe their anti-estrogenic effects. Tamoxifen inhibited estradiol-induced growth significantly at 10.0 μM, and raloxifene at 1.0 μM (Figure
13). For tamoxifen and raloxifene, the observed IC$_{50}$ was between 1 and 10 µM. For actein, the observed IC$_{50}$ was greater than 10 µM.

![Inhibition of Estradiol-Induced MCF-7 Cell Proliferation](image)

**Figure 13.** Actein, tamoxifen, and raloxifene inhibit estradiol-induced proliferation of MCF-7 cells.

Actein and tamoxifen significantly inhibit estradiol-induced proliferation of MCF-7 cells at 10.0 µM; raloxifene significantly inhibits estradiol-induced proliferation at 1.0 µM. Cells were maintained in medium with CSS and treated for 72 hours with actein, tamoxifen, or raloxifene at the indicated concentrations and 1.0 nM E2 in medium. Relative number of viable cells, shown as mean ± SEM and normalized by the DMSO control, was determined by SRB assay.

### 4.2.5 Combined Effect of SERMs and Actein on MCF-7 Cell Proliferation

Since most breast cancer survivors who use HDS use them in addition to their prescribed drug regimen, we were interested in how treating cells with a combination of actein and the commonly prescribed SERM drugs tamoxifen and raloxifene would affect cell proliferation. Using the combination index method of Chou and Talalay, Einbond’s group had determined that combinations of the anthracycline doxorubicin (17 nM), taxane paclitaxel (1 nM), or antimetabolite 5-fluorouracil (15 nM) and
actein (0.3 µM, 1.5 µM, or 3 µM, respectively) exhibited synergistic inhibition of MDA-MB-453 breast cancer cell proliferation (Chou & Talalay, 1984).

We were particularly interested in whether a synergistic effect would be observed in combination with SERMs, as they act by binding to estrogen receptors in breast cells in a way that prevents the stimulation of the cell proliferation pathway by estrogen in some tissues. Although black cohosh extracts and the isolated triterpene glycosides have been shown to not interact with estrogen receptors, their (anti-)estrogenic properties are still unclear (Burdette, et al., 2003; Jiang, et al., 2008; Liu, et al., 2001; Mahady, 2003).

Having confirmed the growth inhibitory and anti-estrogenic effects of actein, tamoxifen, and raloxifene alone, we began our study of the combined effects of actein and each of the SERMs. For these experiments, we used concentrations of the SERMs near their IC$_{50}$ as reported by NCI’s Developmental Therapeutics Program database. This concentration was also close to the lowest concentration at which significant inhibition was observed in our experiments. Initially, cells were treated for 72 hours as in the previous cell proliferation experiments. No significant differences were observed between the SERMs alone with estradiol and the combination of the SERMs and actein with estradiol. Thus, treatments were extended to 120 hours and a broader range of concentrations was used.
Figure 14. Actein does not interact with tamoxifen or raloxifene in inhibition of MCF-7 cell proliferation.

Actein does not significantly affect the inhibition of MCF-7 cell proliferation by tamoxifen or raloxifene. Cells were maintained in medium with CSS and treated for 120 hours with actein at the indicated concentrations, 1.0 µM tamoxifen or 0.1 µM raloxifene, and 1.0 nM E₂ in medium. Relative number of viable cells, shown as mean ± SEM, was determined by SRB assay.

No synergistic growth inhibitory effect was observed when cells were treated with a combination of a SERM and actein in the presence or absence of estradiol (Figure 14-15). Since synergism was not indicated, we did not perform follow-up experiments with a complete range of concentrations of both the drugs and actein in order to determine the combination index for each drug and actein. Additionally, in contradiction to our previous results, actein significantly stimulated cell proliferation in both 120 hours experiments, suggesting that the length of the treatment affected whether a proliferative (and antagonistic, when combined with the SERMs) or anti-proliferative effect was observed.
**Figure 15.** Actein does not interact with tamoxifen or raloxifene in inhibition of estradiol-induced proliferation of MCF-7 cells.

Actein does not significantly affect the inhibition of MCF-7 cell proliferation by tamoxifen or raloxifene. Cells were maintained in medium with CSS and treated for 120 hours with actein at the indicated concentrations, 1.0 µM tamoxifen or 0.1 µM raloxifene, and 1.0 nM E$_2$ in medium. Relative number of viable cells, shown as mean ± SEM, was determined by SRB assay.

### 4.2.6 Effect of Actein on Gene Expression in MCF-7 Cells

Cell proliferation experiments provide only rudimentary information about the physiological effects of a compound. In order to gain insight into the mechanism of action of actein, we used RT-PCR to measure the expression of four genes of interest in MCF-7 cells treated with a range of 0-25 µM actein with and without 1 nM estradiol. First, we quantified $CDKN1A$ expression (Figure 16). $CDKN1A$ encodes cyclin-dependent kinase inhibitor 1A (p21, Cip1), a protein that arrests the cell cycle by inhibiting the activity of cyclin-dependent kinase 2 or 4 complexes. $CDKN1A$ is typically induced in response to stress stimuli such as DNA damage (Bokoch, 2003). In our study, $CDKN1A$ was significantly induced only at the highest concentration of
actein in both the presence and absence of estradiol, at a 1.9 and 2.2-fold change relative to the housekeeping gene GAPDH, respectively.

**Figure 16.** Actein induces *CDKN1A* expression at high concentration.

*CDKN1A*, which encodes a protein that arrests the cell cycle in response to DNA damage, is significantly induced in MCF-7 cells by treatment with 25 µM actein. Cells were maintained in medium with CSS and treated for 48 hours with actein at the indicated concentrations and 1.0 nM E2 in medium, RNA was isolated, cDNA was synthesized, and relative level of *CDKN1A* transcription, shown as mean ± SEM, was determined by RT-PCR with appropriate primer and probe.

To further investigate the controversial (anti-)estrogenic effects of actein, the expression of *TFF1*, an oncogenic gene that is classically induced by estradiol, was measured (Perry, Kannan, Grandison, Mitchell, & Lobie, 2008). As expected, *TFF1* was induced (4.8-fold) in the presence of estradiol. Actein was able to block the induction of *TFF1* by estradiol with an IC₅₀ between 1 and 10 µM, confirming the anti-estrogenic effect observed in the cell proliferation studies (Figure 17). This is the first report of actein’s anti-estrogenic effect at the level of transcription. Actein alone did not significantly induce *TFF1* at any of the tested concentrations.
Figure 17. Actein inhibits the induction of *TFF1* by estradiol.

The induction of *TFF1*, a classically estradiol-induced gene, by estradiol treatment, is significantly repressed by treatment with 10 µM actein. Cells were maintained in medium with CSS and treated for 48 hours with actein at the indicated concentrations and 1.0 nM E2 in medium, RNA was isolated, cDNA was synthesized, and relative level of *TFF1* transcription, shown as mean ± SEM, was determined by RT-PCR with appropriate primer and probe.

Because breast cancer survivors frequently combine dietary herbal supplements with their drug regimen, we were interested in how actein affects the expression of xenobiotic metabolizing enzymes. These enzymes metabolize foreign chemicals that are not normally produced or found in the organism. *CYP1A1* encodes one such enzyme, a cytochrome P450 isozyme also known as aryl hydrocarbon hydroxylase, that oxidizes drugs and other foreign compounds. Gaube reported an induction of *CYP1A1* of about 20-fold in MCF-7 cells treated with 15 µg/mL black cohosh extract, and a slight induction in cells treated with 20 µM actein (Gaube, et al., 2007). We suspected that culture conditions would greatly influence the expression of *CYP1A1*, so we conducted the experiment with two different types of culture media, RPMI-1640 with 5% FBS and phenol red-free RPMI-1640 with 5%
CDS. Actein significantly induced CYP1A1 under both culture conditions at 1.0 µM, but to a much greater extent in FBS-supplemented media than in CDS-supplemented media (Figure 18). Interestingly, the combination of actein treatment with estradiol blocked the induction of CYP1A1 by actein. In the presence of estradiol, CYP1A1 was only significantly induced by the highest concentration actein treatment.

![Effect of Actein and Culture Conditions on CYP1A1](image)

**Figure 18. Actein induces CYP1A1 expression**

CYP1A1, which encodes a protein that is involved in the oxidation of xenobiotics, steroids, and lipids, is significantly induced in MCF-7 cells by treatment with 1.0 µM actein -E2 and with 25 µM actein +E2. Cells were maintained in medium with A (CSS) or B (FBS) and treated for 48 hours with actein at the indicated concentrations and 1.0 nM E2 in medium, RNA was isolated, cDNA was synthesized, and relative level of CYP1A1 transcription, shown as mean ± SEM, was determined by RT-PCR with appropriate primer and probe.

Gaube measured 4-fold induction of VEGFA when MCF-7 cells were treated with 15 µg/mL black cohosh extract and slightly less induction when they were treated with 20 µM actein (Gaube, et al., 2007). VEGFA encodes a vascular endothelial growth factor, which promotes angiogenesis, vasculogenesis, and endothelial cell growth, processes that are essential to tumor development. VEGFA has been reported to be estradiol-induced in human endometrial cells, but we did not
find it to be estradiol-sensitive in the MCF-7 cells (Mueller, et al., 2000). VEGFA was significantly induced in both culture media at the highest concentration actein treatment and the level of induction was similar in the presence and absence of estradiol (Figure 19).

**Figure 19. Actein induces VEGFA expression.**

VEGFA, which encodes a protein that promotes angiogenesis, vasculogenesis and endothelial cell growth, is significantly induced in MCF-7 cells by treatment with 25 µM actein. Cells were maintained in medium with A (CSS) or B (FBS) and treated for 48 hours with actein at the indicated concentrations and 1.0 nM E₂ in medium, RNA was isolated, cDNA was synthesized, and relative level of VEGFA transcription, shown as mean ± SEM, was determined by RT-PCR with appropriate primer and probe.

4.3 Hepatotoxicity of SERMs and Actein on Liver Cells

4.3.1 Hepatotoxicity of Actein Alone, Tamoxifen Alone, and Raloxifene Alone on Liver Cells

In general, actein was not found to be toxic to the HepG2/C3A liver cells, and the SERMS tamoxifen and raloxifene were largely found to be toxic to this cell line.
only at relatively high concentrations. The individual results for the various endpoint
assays used to test for different modes of hepatotoxicity are summarized in Table 9.

<table>
<thead>
<tr>
<th>Hepatotoxicity Indicator</th>
<th>Actein</th>
<th>Tamoxifen</th>
<th>Raloxifene</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell Viability</td>
<td>0</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Reactive Oxygen Species</td>
<td>0</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>CYP1A1 Activity</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Neutral Lipid Content</td>
<td>0</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Phospholipid Content</td>
<td>0</td>
<td>0</td>
<td>+/-++</td>
</tr>
<tr>
<td>p-Glycoprotein</td>
<td>0</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Mitochondrial Membrane</td>
<td>0</td>
<td>0</td>
<td>++</td>
</tr>
<tr>
<td>Mitochondrial Membrane</td>
<td>0</td>
<td>0</td>
<td>++</td>
</tr>
<tr>
<td>Mitochondrial Membrane</td>
<td>0</td>
<td>0</td>
<td>++</td>
</tr>
<tr>
<td>Mitochondrial Membrane</td>
<td>0</td>
<td>0</td>
<td>++</td>
</tr>
<tr>
<td>Albumin</td>
<td>0</td>
<td>++</td>
<td>+</td>
</tr>
</tbody>
</table>

0 indicates no change compared to control at any concentrations tests, + indicates an effect compared to control at moderate concentrations, and ++ indicates an effect compared to control at high concentrations, as determined by Fisher LSD tests in SAS 9.1.

### 4.3.1.1 Effect of Actein on Oxidative Stress

We found a significant decrease in the amount of oxidative stress between the control group and the HepG2/C3A liver cells treated with 0.76 µM actein as shown in Figure 20. This was somewhat expected as one study showed that black cohosh extracts were able to scavenge ROS to protect cells from DNA damage in S80 breast cancer cells treated with quinines (Burdette, et al., 2002). However, our results are not pharmacologically relevant as there was only a slight decrease at 0.76 M that did not reach a 50% reduction in ROS.
Figure 20. Actein decreases the amount of ROS at 0.76 µM.
Actein significantly decreases the amount of ROS in HepG2/C3A liver cells at 0.76 µM. Confluent cells were treated with 0, 0.76, 1.55, 3.1, 6.25, 12.5, 25, 50, and 100 µM actein for 48 hours, and ROS was measured with the DCFDA assay. Data were normalized as a percentage of the mean value of the 0 µM control, and expressed as mean ± SEM; *p<0.05 Fisher LSD Test.

4.3.1.2 Effect of Actein on CYP1A1 Activity

We found that actein caused a significant increase in the amount CYP1A1 activity compared to the control groups when the HepG2/C3A liver cells were treated with 6.25 and 100 µM, but a significant decrease at 0.76 µM, as shown in Figure 21. The increases in CYP1A1 activity were not entirely expected, as the literature generally indicates that black cohosh extracts inhibits CYP450 activity (Tsukamoto, et al., 2005).
**Figure 21.** Actein increased CYP1A1 activity.  
Levels of CYP1A1 activity changed at varying concentrations of actein in HepG2/C3A liver cells. Confluent cells were treated with 0, 0.76, 1.55, 3.1, 6.25, 12.5, 25, 50, and 100 µM actein for 48 hours, and CYP1A1 activity was measured with the EROD assay. Data were normalized as a percentage of the mean value of the 0 µM control, and expressed as mean ± SEM; *p<0.05, ***p<0.001 Fisher LSD Test.

4.3.1.3 Effect of Actein on Neutral Lipid and Phospholipid Retention

There were no significant differences in the amount of neutral lipid or phospholipid accumulation between the control groups and the HepG2/C3A liver cells treated with any of the concentrations of actein, as shown in Figure 22 and Figure 23, respectively. This contradicted the results reported in Lude, et al., of observed steatosis in the livers of rats treated with black cohosh extract (Lude, et al., 2007). However, this contradiction may suggest that compounds other than actein in the black cohosh extract can cause steatosis, which would then imply the need for further studies.
Figure 22. Actein does not affect intracellular neutral lipid accumulation. Confluent HepG2/C3A liver cells were treated with 0, 0.76, 1.55, 3.1, 6.25, 12.5, 25, 50, and 100 µM actein for 48 hours, and lipid accumulation was measured with the Nile Red assay. Data were normalized as a percentage of the mean value of the 0 µM control, and expressed as mean ± SEM.

Figure 23. Actein does not affect membrane phospholipid accumulation. Confluent HepG2/C3A liver cells were treated with 0, 0.76, 1.55, 3.1, 6.25, 12.5, 25, 50, and 100 µM actein for 48 hours, and lipid accumulation was measured with the Nile Red assay. Data were normalized as a percentage of the mean value of the 0 µM control, and expressed as mean ± SEM.
4.3.1.4 Effect of Actein on Mitochondrial Membrane Depolarization and p-Glycoprotein Activity

We found no significant differences in the extent to which mitochondrial membrane depolarization occurred between the control group and the HepG2/C3A cells treated with different concentrations of actein, and actein was not shown to affect p-glycoprotein activity, as shown in Figure 24. Our results did not confirm the results found by Lude’s group that black cohosh decreases the mitochondrial membrane potential, but our results for p-glycoprotein activity agreed with the results of Gurley, et al. (2007; 2006).

![Figure 24. Actein does not affect mitochondrial membrane potential or p-glycoprotein activity.](image)

Actein does not affect the mitochondrial membrane potential or p-glycoprotein activity in HepG2/C3A cells. Mitochondrial membrane potential and p-glycoprotein activity at 0, 0.76, 1.55, 3.1, 6.25, 12.5, 25, 50, and 100 µM actein were measured with the R123 assay. Data were normalized as a percentage of the mean value of the 0 µM control, and expressed as mean ± SEM.

4.3.1.5 Effect of Actein on Cell Viability

We found no significant differences in the amount of double-stranded DNA
between the control groups and the HepG2/C3A liver cells treated with actein at any of the concentrations tested, as shown in Figure 25.

**Figure 25. Actein does not affect cell viability.**
Actein does not affect HepG2/C3A cell viability. Confluent cells were treated with 0, 0.76, 1.55, 3.1, 6.25, 12.5, 25, 50, and 100 µM actein for 48 hours, and cell viability was measured with the DNA assay. Data were normalized as a percentage of the mean value of the 0 µM control, and expressed as mean ± SEM.

### 4.3.1.6 Effect of Actein on Albumin Production

The albumin assay on the HepG2/C3A liver cells treated with varying concentrations of actein displayed a decrease in albumin levels starting at 25 µM. However, there were no statistically significant differences in albumin production at any of the concentrations of actein tested, as shown in Figure 26. The lack of a change in albumin levels supports similar findings in case reports (Joy, et al., 2008).
Figure 26. Actein does not affect albumin production.

Actein does not affect albumin production by HepG2/C3A cells. Confluent cells were treated with 0, 0.76, 1.55, 3.1, 6.25, 12.5, 25, 50, and 100 µM actein for 48 hours, and 50 uL of media were taken from each well to use in the albumin ELISA. Data were normalized as a percentage of the mean value of the 0 µM control, and expressed as mean ± SEM.

4.3.2 Hepatotoxicity of Tamoxifen

4.3.2.1 Effect of Tamoxifen on Oxidative Stress

When determining the toxic concentrations of tamoxifen on the HepG2/C3A liver cells used, we found a significant increase in the amount of oxidative stress between the control group and the HepG2/C3A liver cells treated with 12.5 and 100 µM tamoxifen, as shown in Figure 27.
**Figure 27. Tamoxifen increases the amount of ROS.**

Reactive oxygen species increase in HepG2/C3A cells treated with 12.5 and 100 µM tamoxifen. Confluent cells were treated with 0, 0.76, 1.55, 3.1, 6.25, 12.5, 25, 50, and 100 µM tamoxifen for 48 hours, and ROS was measured with the DCFDA assay. Data were normalized as a percentage of the mean value of the 0 µM control, and expressed as mean ± SEM; ***p<0.001 Fisher LSD Test.

**4.3.2.2 Effect of Tamoxifen on CYP1A1 Activity**

We found that tamoxifen caused significant increases in the amount of CYP1A1 activity compared to the control groups in HepG2/C3A cells starting at 50 µM tamoxifen, as shown in Figure 28.
Figure 28. Tamoxifen increases CYP1A1 activity.
Levels of CYP1A1 activity increased at high concentrations of tamoxifen in HepG2/C3A liver cells. Confluent cells were treated with 0, 0.76, 1.55, 3.1, 6.25, 12.5, 25, 50, and 100 μM tamoxifen for 48 hours, and CYP1A1 activity was measured with the EROD assay. Data were normalized as a percentage of the mean value of the 0 μM control, and expressed as mean ± SEM; *p<0.05, **p<0.01 Fisher LSD Test.

4.3.2.3 Effect of Tamoxifen on Neutral Lipid and Phospholipid Retention

There was a significant increase in the amount of neutral lipid accumulation in the membranes of HepG2/C3A cells when treated with 50 and 100 μM tamoxifen, as shown in Figure 29. We found no significant differences in the amount of phospholipids retained in the cell membrane between the control group and any of the concentrations of tamoxifen tested, as shown in Figure 46 in Appendix B.
Figure 29. Tamoxifen increases intracellular neutral lipid accumulation.
Tamoxifen significantly increases neutral lipid accumulation in HepG2/C3A cells starting at 50 µM. Confluent cells were treated with 0, 0.76, 1.55, 3.1, 6.25, 12.5, 25, 50, and 100 µM tamoxifen for 48 hours, and lipid accumulation was measured with the Nile Red assay. Data were normalized as a percentage of the mean value of the 0 µM control, and expressed as mean ± SEM; *p<0.05, ***p<0.001 Fisher LSD Test.

4.3.2.4 Effect of Tamoxifen on Mitochondrial Membrane Depolarization and p-Glycoprotein Activity

The significant increase in R123 retention in the HepG2/C3A cells at 0.76 and 1.55 µM tamoxifen indicated that p-glycoprotein activity was inhibited at these low concentrations, as shown in Figure 30. Mitochondrial membrane depolarization was not seen as there was no significant decrease in R123 retained (Figure 30).
Figure 30. Tamoxifen decreases p-glycoprotein activity at low concentrations.
Tamoxifen decreases p-glycoprotein activity at 0.76 and 1.55 µM in HepG2/C3A cells. Mitochondrial membrane potential and p-glycoprotein activity at 0, 0.76, 1.55, 3.1, 6.25, 12.5, 25, 50, and 100 µM tamoxifen were measured with the R123 assay. Data were normalized as a percentage of the mean value of the 0 µM control, and expressed as mean ± SEM; ***p<0.001 Fisher LSD Test.

4.3.2.5 Effect of Tamoxifen on Cell Viability

A significant decrease in the number of cells with double-stranded DNA was seen when HepG2/C3A cells were treated with 100 µM tamoxifen, as shown in Figure 31. Fortunately, 100 µM tamoxifen is beyond the concentration of tamoxifen that would normally be in the body, so this is not a problem for patients using tamoxifen.
Figure 31. 100 µM Tamoxifen decreases cell viability. Tamoxifen decreases HepG2/C3A cell viability at 100 µM. Confluent cells were treated with 0, 0.76, 1.55, 3.1, 6.25, 12.5, 25, 50, and 100 µM tamoxifen for 48 hours, and cell viability was measured with the DNA assay. Data were normalized as a percentage of the mean value of the 0 µM control, and expressed as mean ± SEM; ***p<0.001 Fisher LSD Test.

4.3.2.6 Effect of Tamoxifen on Albumin Production

We additionally found a significant decrease in the amount of albumin produced by the HepG2/C3A cells at 100.00 µM tamoxifen, as shown in Figure 32.
Figure 32. 100 µM Tamoxifen decreases albumin production.
Tamoxifen decreases albumin production by HepG2/C3A cells. Confluent cells were treated with 0, 0.76, 1.55, 3.1, 6.25, 12.5, 25, 50, and 100 µM tamoxifen for 48 hours, and 50 µL of media were taken from the inner 60 wells of 96-well plates to use in the albumin ELISA. Data were normalized as a percentage of the mean value of the 0 µM control, and expressed as mean ± SEM; **p<0.01 Fisher LSD Test.

4.3.3 Hepatotoxicity of Raloxifene

4.3.3.1 Effect of Raloxifene on Oxidative Stress

We found a significant decrease in the amount of reactive oxygen species present when the HepG2/C3A liver cells were treated with 6.25, 25 and 50 µM raloxifene, as shown in Figure 33.
Figure 33. Raloxifene decreases the amount of ROS.
ROS decrease in HepG2/C3A cells treated with 6.25, 50 and 100 µM raloxifene. Confluent cells were treated with 0, 0.76, 1.55, 3.1, 6.25, 12.5, 25, 50, and 100 µM raloxifene for 48 hours, and ROS was measured with the DCFDA assay. Data were normalized as a percentage of the mean value of the 0 µM control, and expressed as mean ± SEM; *p<0.05, **p<0.01 Fisher LSD Test.

4.3.3.2 Effect of Raloxifene on CYP1A1 Activity

We found that raloxifene caused a significant increase in the amount CYP1A1 activity compared to the control groups when the HepG2/C3A liver cells were treated with the 12.5 and 100 µM raloxifene, as shown in Figure 34.
Figure 34. Raloxifene increases CYP1A1 activity.
Levels of CYP1A1 activity increased at several concentrations of raloxifene in HepG2/C3A liver cells. Confluent cells were treated with 0, 0.76, 1.55, 3.1, 6.25, 12.5, 25, 50, and 100 µM raloxifene for 48 hours, and CYP1A1 activity was measured with the EROD assay. Data were normalized as a percentage of the mean value of the 0 µM control, and expressed as mean ± SEM; **p<0.01, ***p<0.001 Fisher LSD Test.

4.3.3.3 Effect of Raloxifene on Neutral Lipid and Phospholipid Retention

There were significant increases in neutral lipid accumulation between the control group and the HepG2/C3A cells starting at 25 µM raloxifene, as shown in Figure 35. The trend for phospholipid retention by HepG2/C3A cells treated with varying concentrations of raloxifene was less clear. We found significant increases in the amount of phospholipids retained in the cell membrane at 1.55, 3.1, 12.50, 25.00, and 100.00 µM raloxifene, but not at 6.5 or 50 µM raloxifene, as shown in Figure 36.
Figure 25. Raloxifene increases intracellular neutral lipid accumulation.
Raloxifene significantly increases neutral lipid accumulation in HepG2/C3A cells starting at 25 µM. Confluent cells were treated with 0, 0.76, 1.55, 3.1, 6.25, 12.5, 25, 50, and 100 µM raloxifene for 48 hours, and lipid accumulation was measured with the Nile Red assay. Data were normalized as a percentage of the mean value of the 0 µM control, and expressed as mean ± SEM; *p<0.05, ***p<0.001 Fisher LSD Test.

Figure 36. Raloxifene increases membrane phospholipid accumulation.
Raloxifene increases phospholipid accumulation in HepG2/C3A cells at various concentrations ranging from 1.55 to 100 µM. Confluent cells were treated with 0, 0.76, 1.55, 3.1, 6.25, 12.5, 25, 50, and 100 µM raloxifene for 48 hours, and phospholipid accumulation was measured with the Nile Red assay. Data were normalized as a percentage of the mean value of the 0 µM control, and expressed as mean ± SEM; *p<0.05, ***p<0.001 Fisher LSD Test.
4.3.3.4 Effect of Raloxifene on Mitochondrial Membrane Depolarization and p-Glycoprotein Activity

We found that less R123 was retained in HepG2/C3A cells treated with 100 µM raloxifene compared to the control, as shown in Figure 37. This indicates either that mitochondrial membrane depolarization and/or an increase in p-glycoprotein activity occurred at 100 µM, a high dose.

**Figure 37.** 100 µM Raloxifene increases p-glycoprotein activity. Raloxifene increases p-glycoprotein activity at 100 µM in HepG2/C3A cells. Mitochondrial membrane potential and p-glycoprotein activity at 0, 0.76, 1.55, 3.1, 6.25, 12.5, 25, 50, and 100 µM raloxifene were measured with the R123 assay. Data were normalized as a percentage of the mean value of the 0 µM control, and expressed as mean ± SEM; ***p<0.001 Fisher LSD Test.

4.3.3.5 Effect of Raloxifene on Cell Viability

Significant decreases in the amount of double-stranded DNA in HepG2/C3A cells treated with raloxifene were seen at 50 and 100 µM raloxifene, as shown in Figure 38. This suggests that raloxifene begins to affect cell viability starting at 50
μM, though the decrease in cell viability was not pharmacologically significant.

Figure 38. Raloxifene decreases cell viability. Raloxifene decreases HepG2/C3A cell viability starting at 50 μM. Confluent cells were treated with 0, 0.76, 1.55, 3.1, 6.25, 12.5, 25, 50, and 100 μM raloxifene for 48 hours, and cell viability was measured with the DNA assay. Data were normalized as a percentage of the mean value of the 0 μM control, and expressed as mean ± SEM; **p<0.01, ***p<0.001 Fisher LSD Test.

4.3.3.6 Effect of Raloxifene on Albumin Production

The HepG2/C3A cells seemed to increase their albumin production when treated with 1.55 and 3.1 μM raloxifene, as shown in Figure 39.
Figure 39. Raloxifene increases albumin production.
Raloxifene increases albumin production by HepG2/C3A cells at 1.55 and 3.1 µM. Confluent cells were treated with 0, 0.76, 1.55, 3.1, 6.25, 12.5, 25, 50, and 100 µM raloxifene for 48 hours, and 50 µL of media were taken from the inner 60 wells of 96-well plates to use in the albumin ELISA. Data were normalized as a percentage of the mean value of the 0 µM control, and expressed as mean ± SEM; *p<0.05 Fisher LSD Test.

4.3.4 Interactions Between Actein and Tamoxifen

After investigating the effects that actein, tamoxifen, and raloxifene alone had on the HepG2/C3A cells, we investigated the effects of actein with tamoxifen on these cells. This was an important aspect of our study, because breast cancer patients who use or may consider using black cohosh to treat their cancer may also be taking a SERM like tamoxifen concurrently. While actein alone generally did not exhibit toxic effects on the HepG2/C3A cells, we were interested in whether or not actein and tamoxifen would interact to magnify or even minimize some of the toxic effects seen in the HepG2/C3A cells treated with tamoxifen alone. A two-way ANOVA in SAS 9.1 was run for each of the endpoint assays using data from confluent cells treated
with 30 µM actein plus increasing concentrations of tamoxifen and from confluent cells treated with 90 µM tamoxifen plus increasing concentrations of actein. Table 5 summarizes which modes of hepatotoxicity were affected by an interaction between actein and tamoxifen.

<table>
<thead>
<tr>
<th>Mode of Hepatotoxicity</th>
<th>Interaction</th>
<th>Type</th>
<th>p-value&lt;sup&gt;g&lt;/sup&gt;</th>
<th>Corresponding Figure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxidative Stress&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Absent</td>
<td>N/A</td>
<td>0.2930</td>
<td>47</td>
</tr>
<tr>
<td>CYP1A1 Activity&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Present</td>
<td>Indeterminable</td>
<td>&lt; 0.0001</td>
<td>40</td>
</tr>
<tr>
<td>Neutral lipid Steatosis&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Absent</td>
<td>N/A</td>
<td>0.8319</td>
<td>48</td>
</tr>
<tr>
<td>Phospholipid Steatosis&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Present</td>
<td>Synergistic</td>
<td>0.0038</td>
<td>41</td>
</tr>
<tr>
<td>Mitochondrial Membrane Depolarization&lt;sup&gt;d&lt;/sup&gt;</td>
<td>Absent</td>
<td>N/A</td>
<td>0.5617</td>
<td>49</td>
</tr>
<tr>
<td>p-Glycoprotein Activity&lt;sup&gt;d&lt;/sup&gt;</td>
<td>Absent</td>
<td>N/A</td>
<td>0.5617</td>
<td>49</td>
</tr>
<tr>
<td>Cell Viability&lt;sup&gt;e&lt;/sup&gt;</td>
<td>Present</td>
<td>Synergistic</td>
<td>&lt;0.0001</td>
<td>42</td>
</tr>
<tr>
<td>Albumin Production&lt;sup&gt;f&lt;/sup&gt;</td>
<td>Indeterminable&lt;sup&gt;h&lt;/sup&gt;</td>
<td>N/A</td>
<td>N/A</td>
<td>50</td>
</tr>
</tbody>
</table>

<sup>a</sup>measured with DCFDA assay, <sup>b</sup>measured with EROD assay, <sup>c</sup>measured with Nile Red assay, <sup>d</sup>measured with R123 assay, <sup>e</sup>measured with DNA assay, <sup>f</sup>measured with ELISA. <sup>g</sup>p-values were obtained from two-way ANOVA run in SAS 9.1. <sup>h</sup>albumin ELISA was incomplete.

### 4.3.4.1 Effect of Tamoxifen with Actein on Oxidative Stress

When we tested for oxidative stress in HepG2/C3A cells treated with both actein and tamoxifen, we did not find any interaction effect between actein and tamoxifen after running a two-way ANOVA on the raw data. Figure 47 in Appendix B demonstrates the absence of an interaction using data from the HepG2/C3A cells treated with tamoxifen alone and the HepG2/C3A cells treated with 30 µM actein plus varying concentrations of tamoxifen.
4.3.4.2 Effect of Tamoxifen with Actein on CYP1A1 Activity

The two-way ANOVA indicated that tamoxifen and actein interacted to affect CYP1A1 activity in HepG2/C3A cells. The data from the HepG2/C3A cells treated with tamoxifen alone and from the HepG2/C3A cells treated with 30 µM actein plus varying concentrations of tamoxifen were used to depict this interaction, as shown in Figure 44. Whether or not the interaction was synergistic or antagonistic could not be definitively concluded, as there was no clear shift in the dose-response curve (Figure 40).

![Graph showing CYP1A1 activity with Tamoxifen and Actein treatment](image)

**Figure 40. Actein and tamoxifen interact to affect CYP1A1 activity.**
Confluent HepG2/C3A cells were treated with the indicated concentrations of actein and tamoxifen for 48 hours, and CYP1A1 activity was measured with the EROD assay. Data were normalized as a percentage of the mean value of the 0 µM control, and expressed as mean ± SEM.

4.3.4.3 Effect of Tamoxifen with Actein on Neutral Lipid and Phospholipid Retention

When we tested for neutral lipid steatosis in HepG2/C3A cells treated with
both actein and tamoxifen, we did not find any interaction effects after running a two-way ANOVA on the raw data. Figure 48 in Appendix B demonstrates the absence of an interaction using data from the HepG2/C3A cells treated with tamoxifen alone and the HepG2/C3A cells treated with 30 μM actein plus varying concentrations of tamoxifen. However, there did seem to be a synergistic effect between actein and tamoxifen on phospholipid accumulation in the HepG2/C3A cells starting at 30 μM actein and approximately 30 μM tamoxifen, as shown in Figure 41.

![Effect of Tamoxifen with 30 μM Actein on Phospholipid Accumulation in HepG2/C3A Cells](image)

**Figure 41.** Actein and tamoxifen have a synergistic effect on membrane phospholipid accumulation at high concentration. Confluent HepG2/C3A cells were treated with the indicated concentrations of actein and tamoxifen for 48 hours, and lipid accumulation was measured with the Nile Red assay. Data were normalized as a percentage of the mean value of the 0 μM control, and expressed as mean ± SEM.

### 4.3.4.4 Effect of Tamoxifen with Actein on Mitochondrial Membrane Depolarization and p-Glycoprotein Activity

When we tested for R123 retention in HepG2/C3A cells treated with both actein and tamoxifen, we did not find any interaction effect between actein and
tamoxifen after running a two-way ANOVA on the raw data, indicating that there
were no combined effects of these two compounds on mitochondrial membrane
depolarization or p-glycoprotein activity. Figure 49 in Appendix B demonstrates the
absence of an interaction using data from the HepG2/C3A cells treated with
tamoxifen alone and the HepG2/C3A cells treated with 30 µM actein plus varying
concentrations of tamoxifen.

4.3.4.5 Effect of Tamoxifen with Actein on Cell Viability

The two-way ANOVA indicated that tamoxifen and actein interacted to affect
HepG2/C3A cell viability. The data from the HepG2/C3A cells treated with
tamoxifen alone and from the HepG2/C3A cells treated with 30 µM actein plus
varying concentrations of tamoxifen were used to depict this interaction, as shown in
Figure 42. This interaction appeared to be synergistic as the dose-response curve for
tamoxifen with 30 µM actein shifted to the left compared to the curve for tamoxifen
with 0 µM actein (Figure 42). These findings are particularly interesting because
actein alone did not cause any changes in cell viability (Figure 25).
**Figure 42.** Actein and tamoxifen have a synergistic effect on cell viability at high concentration. Confluent HepG2/C3A cells were treated with the indicated concentrations of actein and tamoxifen for 48 hours, and cell viability was determined with the DNA assay. Data were normalized as a percentage of the mean value of the 0 μM control, and expressed as mean ± SEM.

### 4.3.4.6 Effect of Tamoxifen with Actein on Albumin Production

This assay was not able to be completed as the plates containing sample media assay from the HepG2/C3A liver cells treated with 90 μM tamoxifen and increasing concentrations of actein were not well-labeled. Thus, any interaction effects between actein and tamoxifen could not be determined by the two-way ANOVA in SAS 9.1. Figure 50 in Appendix B shows albumin production by HepG2/C3A cells treated with 0 and 30 μM actein at increasing concentrations of tamoxifen based solely on data from HepG2/C3A cells treated with 30 μM actein and increasing concentrations of tamoxifen.
4.3.5 Interactions between Actein and Raloxifene

After looking for potential interaction effects between actein and tamoxifen on the various indications of hepatotoxicity using the HepG2/C3A cells, we did the same for actein and raloxifene. A two-way ANOVA in SAS 9.1 was run for each of the endpoint assays using data from confluent cells treated with 30 µM actein plus increasing concentrations of raloxifene and from confluent cells treated with 50 µM raloxifene plus increasing concentrations of actein. Table 11 summarizes which modes of hepatotoxicity were affected by an interaction between actein and raloxifene.

Table 11. Combination effects of actein and raloxifene on hepatotoxicity indicators.

<table>
<thead>
<tr>
<th>Mode of Hepatotoxicity</th>
<th>Interaction</th>
<th>Type</th>
<th>p-value(^g)</th>
<th>Corresponding Figure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxidative Stress(^a)</td>
<td>Absent</td>
<td>N/A</td>
<td>0.2119</td>
<td>51</td>
</tr>
<tr>
<td>CYP1A1 Activity(^b)</td>
<td>Absent</td>
<td>N/A</td>
<td>0.1175</td>
<td>52</td>
</tr>
<tr>
<td>Neutral lipid Steatosis(^c)</td>
<td>Absent</td>
<td>N/A</td>
<td>0.0787</td>
<td>53</td>
</tr>
<tr>
<td>Mitochondrial Membrane Depolarization(^d)</td>
<td>Absent</td>
<td>N/A</td>
<td>0.9153</td>
<td>54</td>
</tr>
<tr>
<td>p-Glycoprotein Activity(^d)</td>
<td>Absent</td>
<td>N/A</td>
<td>0.9153</td>
<td>54</td>
</tr>
<tr>
<td>Cell Viability(^e)</td>
<td>Present</td>
<td>Indeterminable</td>
<td>0.0483</td>
<td>44</td>
</tr>
<tr>
<td>Albumin Production(^f)</td>
<td>Absent</td>
<td>N/A</td>
<td>0.1231</td>
<td>55</td>
</tr>
</tbody>
</table>

\(^a\)measured with DCFDA assay, \(^b\)measured with EROD assay, \(^c\)measured with Nile Red assay, \(^d\)measured with R123 assay, \(^e\)measured with DNA assay, \(^f\)measured with ELISA.

\(^g\)p-values were obtained from two-way ANOVA run in SAS 9.1.

4.3.5.1 Effect of Raloxifene with Actein on Oxidative Stress

When we tested for oxidative stress in HepG2/C3A cells treated with both actein and raloxifene, we did not find any interaction effect after running a two-way ANOVA on the raw data. Figure 51 (Appendix B) demonstrates the absence of an
interaction using data from the HepG2/C3A cells treated with raloxifene alone and
the HepG2/C3A cells treated with 30 µM actein plus varying concentrations of
raloxifene.

4.3.5.2 Effect of Raloxifene with Actein on CYP1A1 Activity

When we tested for CYP1A1 activity in HepG2/C3A cells treated with both
actein and raloxifene, we did not find any interaction effect after running a two-way
ANOVA on the raw data. Figure 52 (Appendix B) demonstrates the absence of an
interaction using data from the HepG2/C3A cells treated with raloxifene alone and
the HepG2/C3A cells treated with 30 µM actein plus varying concentrations of
raloxifene.

4.3.5.3 Effect of Raloxifene with Actein on Neutral Lipid and
Phospholipid Retention

When we tested for neutral lipid steatosis in HepG2/C3A cells treated with
both actein and raloxifene, we did not find any interaction effects after running a two-
way ANOVA on the raw data. Figure 53 in Appendix B demonstrates the absence of
an interaction using data from the HepG2/C3A cells treated with raloxifene alone and
the HepG2/C3A cells treated with 30 µM actein plus varying concentrations of
raloxifene. However, there did seem to be an interaction between actein and
tamoxifen on phospholipid accumulation in the HepG2/C3A cells, as shown in Figure
43.
**Figure 43.** Actein and raloxifene affect membrane phospholipids accumulation. Confluent HepG2/C3A cells were treated with the indicated concentrations of actein and raloxifene for 48 hours, and phospholipid accumulation was measured with the Nile Red assay. Data were normalized as a percentage of the mean value of the 0 µM control, and expressed as mean ± SEM.

### 4.3.5.4 Effect of Raloxifene with Actein on Mitochondrial Membrane Depolarization and p-Glycoprotein Activity

When we tested for R123 retention in HepG2/C3A cells treated with both actein and raloxifene, we did not find any interaction effects after running a two-way ANOVA on the raw data, indicating that there were no combined effects of these two compounds on mitochondrial membrane depolarization or p-glycoprotein activity.

Figure 54 in Appendix B demonstrates the absence of an interaction using data from the HepG2/C3A cells treated with raloxifene alone and the HepG2/C3A cells treated with 30 µM actein plus varying concentrations of raloxifene.
4.3.5.5 Effect of Raloxifene with Actein on Cell Viability

The two-way ANOVA indicated that raloxifene and actein interacted to affect HepG2/C3A cell viability. The data from the HepG2/C3A cells treated with raloxifene alone and from the HepG2/C3A cells treated with 30 µM actein plus varying concentrations of raloxifene was used to depict this interaction, as shown in Figure 44. However, as there was no clear dose-response shift, the type of interaction could not be determined.

![Amount of Double-Stranded DNA in HepG2/C3A Cells Treated with Varying Concentrations of Raloxifene Alone and With 30 _M Actein](image)

**Figure 44. Actein and raloxifene interact to affect cell viability.** Confluent HepG2/C3A cells were treated with the indicated concentrations of actein and raloxifene for 48 hours, and cell viability was determined with the DNA assay. Data were normalized as a percentage of the mean value of the 0 µM control, and expressed as mean ± SEM.

4.3.5.6 Effect of Raloxifene with Actein on Albumin Production

When we tested for albumin production in HepG2/C3A cells treated with both actein and raloxifene, we did not find any interaction effect after running a two-way ANOVA on the raw data. Figure 55 (Appendix B) demonstrates the absence of an
interaction using data from the HepG2/C3A cells treated with raloxifene alone and the HepG2/C3A cells treated with 30 μM actein plus varying concentrations of raloxifene.
5. Discussion

5.1 HDS Uses and Perceptions

5.1.1 Patterns of HDS Use Among Breast Cancer Survivors

5.1.1.1 Most Common Supplements Used Are Soy, Green Tea, Flaxseed, Ginger and Curcumin

Among supplements used for non-medicinal purposes, soy was used the most, followed by green tea, chamomile, ginger, and flaxseed. A reason that such supplements may be used so frequently is that they are found in many common household products such as food, drinks, and soaps.

Among supplements used for medicinal purposes, the five most often used were flaxseed, green tea, ginger, curcumin, and evening primrose. As shown in our literature review, empirical studies have suggested that flaxseed, green tea, and curcumin may have an inhibitory effect on cancer cell proliferation (Aggarwal & Shishodia, 2006). Ginger and evening primrose, although not linked to inhibition of cancer cell growth, have been shown to affect digestion and PMS symptoms, respectively (Geller, et al., 2005; Srivastava & Gupta, 2007).

5.1.1.2 Patients Consult Internet More Often Than Healthcare Practitioners

Survey participants were asked to rate the sources of information about HDS by how often they consulted them. The sources cited most often for information
about HDS were internet websites, followed by non-profit organizations, and finally healthcare practitioners. Although physicians were consulted less often than websites and the literature from non-profit organizations, our results suggest that the survey participants were consulting their physicians at least sometimes.

It is possible that physicians may not be a major source of information about HDS because of the recognized lack of communication between patients and physicians regarding HDS. Physicians often advise patients to cease taking all HDS when beginning a regimen of conventional medicine in order to avoid possible side effects or dangerous interactions (Cassidy, 2003). Our finding that many survey respondents prefer using websites rather than consulting healthcare providers for information about HDS is consistent with previous findings that indicated a level of discomfort among patients about discussing HDS with their physicians (ICR, 2007).

It is also possible that our survey results may have been different if the question about sources of information had been stated differently. Our study asked how often the subjects consulted sources of information. It is possible that websites found through search engines were consulted most often simply because of their ease of access. Therefore, these results may indicate that breast cancer survivors have greater access to the internet than to their health care providers, rather than an actual preference to consult one source more than the other.

Survey participants were asked to rate the reliability of sources of information about HDS as “very reliable,” “somewhat reliable,” or “not reliable.” Although many subjects reported consulting the internet, a large majority of respondents rated internet websites as only “somewhat reliable.” The most reliable sources of information
about HDS were healthcare practitioners, peer-reviewed journals, and non-profit organizations. This would seem to indicate that although survey respondents were consulting internet sources more often, they recognized the limitations of some websites and realized that the content may not always be reputable. This is also consistent with a previous report in the literature, which found that patients put more trust in professional opinion, even though they may consult other informational sources more frequently (Adams & Jewell, 2007).

5.1.1.3 Family, Friends, Doctors, and Religion Not Influential in HDS Use

Survey participants were asked to rate the influence of their religion and various people in their lives, including physicians, friends, and family members, on their decision either to use or not use HDS. Survey respondents overwhelmingly rated all four categories as mostly being “not influential.” The category receiving the most “very influential” responses was physicians. Religion was almost never an influence on the decision to use HDS, with 990 respondents (95.6%) indicating that religion was “not influential” compared to only 13 respondents who reported it was “very influential.”

Our survey results suggest that outside entities such as family, friends, doctors, and/or religion have relatively little influence on breast cancer survivors’ decisions to use HDS. This result is interesting, since one of this study’s hypothesized reasons for taking HDS was the influence of one’s social network and religious beliefs. At least for the respondents in our sample, we found little evidence
supporting the hypothesis that the decision to use HDS is mainly influenced by
cultural beliefs, contrary to a previous report (Adams & Jewell, 2007). However,
since our survey contained only one proxy (i.e., religion) for cultural beliefs, it is
possible that other cultural beliefs not captured by our survey may have influenced
the decision to use HDS.

Also contrary to previous reports in the literature, our results did not indicate
that friends and family were more influential than physicians in the decision to use
HDS (ICR, 2007). It may be difficult to compare our findings to those of other
studies, however, since the participants in our study reported having an unusually
high education level. Nearly 40% of the sample subjects reported attaining a post-
graduate degree, which is a much higher educational level than that of the general
U.S. population. Moreover, the role of physicians in influencing their patients is
unclear, as other authors have reported on the lack of communication between
patients and doctors concerning HDS. Therefore, we can hypothesize that physicians
may be more influential in the decision to use HDS only when patients feel
comfortable talking about HDS and if they believe such lines of communication are
open to them (Kemper, et al., 2006; Roberts, et al., 2005; Wahner-Roedler, et al.,
2006).

5.1.1.4 Demographic Limitations of Sample

The main demographic captured by the survey was that of upper middle class,
late middle-aged, highly educated Caucasian, Christian females. All other
combinations of ethnicity, income, and gender had so few participants that it was not
possible to make meaningful comparisons. Therefore we were not able to fully analyze our results for relationships based on gender, age, race, and income.

Considering that the average survey respondent reported taking multiple HDS modalities, the results seem to agree with previous findings that educated women are more likely to use HDS than less educated people of either gender (Boon, et al., 2000). While we analyzed HDS use among three clusters of participants based on age, income, and education; the only meaningful inference we could make is that older, female breast cancer survivors may be more likely to use soy.

The skewed demographic of our sample could have occurred because participants were self-selected and no attempt was made to recruit a random sample. It is possible that people with higher education and income levels would be more likely to seek information online about their health concerns or about a particular from which they are suffering. In response to seeing the advertisement for our study, participants seeking information about breast cancer were able to click on a link to access the internet-based survey. The types of people who choose to take internet-based surveys could be representative of the participants in our study.

We did not adjust our data or provide a weighted analysis to reflect the current national population of breast cancer survivors. In the U.S., female breast cancer patients are comprised of 82.2% Caucasian women, 8.6% African American women, 5.4% Hispanic/Latina women, 3.5% Asian American/Pacific Islander women, and 0.3% American Indian/Alaska Native women (Smigal, Jemal, Ward, Cokkinides, Smith, Howe, and Thun, 2006). A weighted analysis of the findings may be useful to more widely apply our results to the overall population of breast cancer survivors.
5.1.1.5 Black Cohosh Was Used For Menopausal Symptoms

The HDS black cohosh has been postulated to have anti-proliferative effects on cancer cells (Einbond, et al., 2004). The lab portion of this study supported this claim, demonstrating an anti-proliferative effect of the active ingredient actein on breast cancer cells in vitro. Since a purpose of the survey was to gather information about the use and perceptions of black cohosh among breast cancer patients, it was disappointing that the majority of respondents did not use black cohosh. Of survey respondents, 232 reported using black cohosh for medicinal or other purposes while 732 respondents did not use it at all. Black cohosh was mainly used to treat hot flashes, PMS symptoms, and menopausal symptoms. Most respondents who reported using black cohosh also reported that it was somewhat effective at treating their symptoms.

As our primary research motive was to investigate how a HDS user’s perceptions about a particular supplement compared to the empirical knowledge available for that HDS, this information directly relates to a major purpose of our study. There appear to be contradictions between the published reports existing in the literature, what our laboratory studies found, and what breast cancer survivors report using. Thus, despite several recently published reports and our own study indicating that black cohosh extracts and certain bioactive compounds may have an anti-proliferative effect, black cohosh was used to treat or prevent cancer by only 2 respondents in our sample. Instead, all the respondents who used black cohosh reported using it to treat menopause-related symptoms, the herb’s traditional use.
5.1.1.6 Hypothesis About Survivor HDS Use Confirmed

The results from our survey analysis were consistent with existing literature and our hypotheses. The majority of the women surveyed reported taking HDS, which was expected since female breast cancer patients are among the groups most likely to take HDS. We found that breast cancer survivors answering our survey were most likely to take black cohosh to treat hot flashes and menopausal symptoms. This and other female conditions have been the traditional use of black cohosh for centuries in American Indian medicine. The most frequently used supplements in our study, curcumin and green tea, are also some of the most well-studied and widely reported supplements appearing in the scientific and popular literature.

Survey respondents reported using websites as their most frequently consulted source of information regarding HDS. This also agreed with our hypothesis, given published studies showing that patients are unlikely to go to their primary physician with questions related to HDS and CAM. Important areas for future study, therefore, are to explore why patients are less likely to consult healthcare providers than other information sources, and to gain further insight about communication barriers that may exist between physicians and patients, particularly with respect to HDS and CAM.
5.1.2 Perspectives on HDS

5.1.2.1 Reported Reasons for Using HDS

HDS are used to treat and alleviate a wide variety of symptoms and conditions. The summary below lists the five supplements most commonly used for medicinal purposes by our survey participants, and the three most often cited reasons for using each of them (listed in order of percentage of respondents):

*Flaxseed:* Flaxseed was used primarily for treating cancer, then for general health, and finally for lowering cholesterol and obtaining omega-3 fatty acids.

*Green Tea:* Green tea was used as an antioxidant, to treat cancer, and to promote general health.

*Ginger:* Respondents used ginger to treat nausea, to aid in digestive problems, and for reducing inflammation.

*Curcumin:* Curcumin was used to assist in treating cancer, to reduce inflammation, and as an antioxidant.

*Evening Primrose:* Respondents stated that they used evening primrose to treat hot flashes, to assist with symptoms of menopause (other than hot flashes), and to reduce the effects of PMS.

Clearly, many survey respondents took or have taken an HDS to directly treat their cancer (i.e., affect tumor growth and/or size). Many others reported taking dietary supplements to treat side-effects of cancer treatments and just to enhance their basic health. Many of the reasons for taking a particular supplement, other than to treat breast cancer, are reasons that are traditionally or culturally associated with the
supplement. For example, most people are aware of the time-honored remedy of using ginger ale for an upset stomach; unsurprisingly, ginger was used primarily to treat nausea.

5.1.2.2 Beginning or Ending HDS Use

Survey participants were asked to state whether they used a certain HDS before their diagnosis with breast cancer and if they used it after their diagnosis. A statistical analysis of the responses showed that breast cancer survivors were likely to begin taking an HDS after their diagnosis with breast cancer, and that this supplement was one not previously reported being taken. This suggests that being diagnosed with breast cancer played a vital role in a person’s decision to take HDS.

Our data indicate that breast cancer survivors’ use of HDS before or after their cancer diagnoses varied with different supplements. Particularly intriguing are the results we found for black cohosh and evening primrose, as these supplements showed an approximately equal number of positive and negative change responses. That is, subjects were as likely to start using them as they were to stop using them after their diagnosis. At least for these supplements, breast cancer survivors may be exposed to conflicting information or messages about how the supplements may interact with their cancer treatments. Or, there may exist an alternative explanation of why similar individuals (i.e, breast cancer survivors) make opposite choices when deciding whether to use HDS. In any case, further study is warranted regarding the extent that individuals view HDS as being curative as opposed to having preventative properties.
5.1.3 Survey Limited To Online Respondents and Fixed Questions

There are two main limitations to the survey portion of our study: the sources of respondents and the types of responses given. We used a convenience sample, as all of the 1106 usable surveys submitted came from members of two online breast cancer patient and survivor communities—BreastCancer.net and the Love/Avon Army of Women. From those two sites, over 1000 surveys were collected. However, the vast majority of respondents were upper middle class, late middle-aged Caucasian women, and only 64 people of other ethnicities and/others income brackets participated. This factor limits the applicability of the data, and we cannot extrapolate our data to other population groups.

In the survey, respondents were asked to identify “other” complementary or alternative medicines besides those specifically spoken of already. This question provided a myriad of possibilities for further research; but given that 528 participants answered with many different types of supplements, no significant conclusions can be drawn from those responses. In other words, nearly half of our survey sample used other supplements not already listed in our survey for medicinal purposes. The major conclusion we can draw from this finding is that breast cancer survivors use many different HDS for many different reasons. At the very least, this underscores the need for further research into HDS and breast cancer. A logical next step would be to further investigate the motivations in the decision to use HDS, and to examine predictors for use.
5.1.4 Contributions to Existing Literature

Despite the limitations, one of the strengths of our study was the large sample size. Because we had more than 1,000 subjects, our survey results contribute to the literature by providing information about the use of certain types of integrative medicine in upper middle class, late-middle aged Caucasian women. In general, our data are consistent with the conclusions of many previous studies cited in the literature review.

Although websites were listed as the source of information most often consulted, physicians were also considered to be influential in the decision to use HDS. This suggests that patients view physicians favorably, and that ways of improving doctor-patient communication about HDS use should be a priority for future research. Considering the large variation in the quality of information available on the internet, it is plausible that many individuals may be exposed to information that is not reputable. Therefore, it would be in the best interests of physicians to become knowledgeable about HDS to the extent that they are able to address this issue with their patients. Moreover, although our survey respondents reported that they consulted websites most often to obtain information about HDS, they also indicated that they trusted the information they received from doctors, journals, and non-profit organizations the most.

An important finding from our survey that may warrant further study is the indication that survey respondents were likely to be using an HDS after being diagnosed with breast cancer. Although we cannot conclude that being diagnosed
with breast cancer caused these respondents to being taking an HDS, it may have contributed at least in part to the decision to take HDS.

Nearly half of respondents indicated they took black cohosh before their diagnosis, but not after, while the other half indicated that they did not take black cohosh before diagnosis, but did take it after. Survey respondents also reported that they began taking or continued taking other HDS, such as green tea and curcumin. Decisions about beginning, continuing, or ending the use of certain dietary supplements may have been influenced by information regarding safety and efficacy about these supplements that was available to the respondents. In the case of black cohosh, the available information may have been contradictory: some studies suggest that black cohosh has inhibitory effects on breast cancer cells, others report that black cohosh showed signs of estrogenic effects, and at least one study indicated that black cohosh showed signs of toxicity to liver cells.

5.1.5 Other Survey Limitations

5.1.5.1 Ambiguity with Medical Purposes vs. Other Reasons

The survey participants were asked about their reasons for using the HDS which they reported using. They were also asked to specify whether it was for medicinal purposes or for other purposes. It is possible that this question may have seemed ambiguous and that some survey respondents may have misunderstood what was meant by “medicinal purposes.” The problem of ambiguity may have arisen because the survey was originally intended to be administered in the form of a person to person interview rather than as an online format. In fact, the survey was originally
pilot tested in an interview format with breast cancer patients. We anticipated that if any question lacked clarity or was not properly interpreted by the respondent, the meaning of the question could be readily explained. Although the original intent of the “reason for use” question was to eliminate responses that pertained to using HDS primarily for non-medical (cooking for example) purposes, we recognize that this question may have caused confusion among some subjects.

5.1.5.2 Advertising Bias

The survey respondent providers, BreastCancer.net and Love/Avon Army of Women are web-based, and both websites included advertisements for different commercially available dietary supplements. It is possible that these advertisements may have influenced the survey participants as the survey sample was gathered from both of these websites. Some usage trends found in our results may have been influenced by the advertisements appearing on these websites. However, BreastCancer.net and Love/Avon Army of Women represent a small portion of the total amount of information available to breast cancer survivors, and we assume that the advertisements our subjects may have been exposed to would have minimal impact on the results we obtained.

5.1.5.3 Problems with Open-ended Questions

The questions about the participants’ reasons for using their particular HDS were open-ended and did not include pre-coded responses. Consequently, a variety of
different responses were given. It was necessary to group and condense these
responses into a smaller number of categories in order to manage the data. However,
this process may have resulted in a source of bias introduced by the coders. It also
appears that some respondents were confused about the meaning of the term
“conventional treatment side effects” versus general reasons for using a supplement.
Thus, the responses to some of the open-ended questions lacked clarity and needed to
be interpreted, which also could have been a source of bias and may have affected the
results.

5.1.5.4 Source of Sample Population

The Love/Avon Army of Women organization provided the majority of the
survey respondents, who were recruited through a mass email “blast.” A relatively
small number of responses were obtained from BreastCancer.net. The members of
Love/Avon Army of Women appear to be highly motivated, actively engaged, and
involved in seeking information about breast cancer and in participating in
fundraising for breast cancer research. They also may be more likely to seek out
solutions and support in dealing with their cancer diagnosis. It is impossible to tell
what the effects of this involvement bias might be; however, it is possible that
respondents may have been more likely to use HDS because they were actively
searching for newer treatment options and were more open to ideas through their
active involvement in managing their disease.
5.1.5.5 Increased Access to the Internet

The fact that the survey participants were recruited through internet sources may have influenced the results. Since the survey was administered over the internet, people who took this survey needed to have internet access to begin with and therefore they may have been biased towards using internet informational sources. It is possible that some subjects may have been frequent or habitual internet users.

5.2 Effects of Actein on Breast Cancer Cell Proliferation

5.2.1 SERMs and Actein Inhibit MCF-7 Cell Proliferation

In our cell proliferation experiments, we confirmed that actein has a growth inhibitory effect on MCF-7 human breast cancer cells and furthermore, inhibits estradiol-stimulated MCF-7 cell proliferation. These effects were statistically significant at 5.0 µM and 10.0 µM, respectively. These concentrations are on the order of the published 3.6 µM peak serum level of actein measured in Sprague-Dawley rats (Einbond et al., 2009). However, the concentrations at which their effects were pharmacologically relevant were generally slightly or significantly higher than the concentrations at which the effects were statistically significant.

The SERM drugs tamoxifen and raloxifene, both of which act by inhibiting estradiol-induced proliferation of ER+ breast cancer cells, statistically significantly inhibited estradiol-stimulated MCF-7 cell proliferation at low concentrations. In this respect, treatment with tamoxifen and raloxifene alone acted as a positive control. Treatments with a combination of actein and either tamoxifen or raloxifene did not
reveal a synergistic effect on growth inhibition or on the inhibition of E₂-stimulated proliferation, even when treatment was extended for 120 hours. These results differed from the potentiation of tamoxifen’s effect reported by Al-Akoum, but that study involved powdered black cohosh root rather than pure compound, and the synergism reported was not determined by the accepted combination index method (Al-Akoum, et al., 2007).

The results underscored the importance of culture conditions and the length of treatment. Large differences were observed in the actein-treated cell proliferation experiments when 72 hour treatments were conducted versus 120 hour treatments. Significant differences were also observed in gene expression and cell proliferation when cells were cultured in RPMI-1640 media supplemented with FBS versus phenol red-free RPMI-1640 media supplemented with CDS. The effect of culture media was likely due to the presence of hormonal compounds in RPMI-1640 with FBS that are absent in phenol red-free media with CDS, especially phenol red itself, which has a known estrogenic effect (Berthois, Katzenellenbogen, & Katzenellenbogen, 1986). The origin of the time-related variation was less clear and necessitates further study.

Based on this in vitro model, actein, a bioactive compound in black cohosh, has anti-estrogenic and anti-proliferative effects that warrant additional research of actein as a potential chemopreventative or chemotherapeutic agent. However, the proliferative effect of actein observed when treatments were conducted for 120 hours rather than 72 hours may be cause for concern. This effect may be related to the pharmacokinetic properties of actein.
5.2.2 Actein Alters the Expression of Breast Cancer- and Drug Metabolism-Related Genes

By using RT-PCR to measure the expression of *CDKN1A, CYP1A1, TFF1,* and *VEGFA* in MCF-7 cells treated with a range of actein concentrations, we found that *CDKN1A* and *CYP1A1* were significantly induced only at the highest concentration of actein with or without estradiol. *CDKN1A* and *VEGFA* are both involved in hypoxia-response, one of the pathways in the integrated stress response pathway to which Einbond’s group attributed the anti-proliferative and gene expression effects of actein and black cohosh in breast cancer cells and rats (Einbond, et al., 2009; Einbond, Su, Wu, Friedman, Wang, Ramirez, et al., 2007). *CYP1A1*, a gene encoding a drug-metabolizing enzyme, was more significantly induced in media containing phenol red and FBS than in media with CSS and without phenol red. This effect was not due to the presence of exogenous estrogens, as estradiol treatment actually blocked the induction of *CYP1A1* by actein, showing that estrogen and actein have opposing effects on the regulation of *CYP1A1* expression in this case. The difference in induction between the two types of media may be related to the presence of other exogenous compounds in the FBS-containing media.

Interestingly, actein alone had no effect on the expression of *TFF1*, and actein was able to block estradiol-stimulated induction of *TFF1*. To our knowledge, this is the first direct evidence of actein’s anti-estrogenic activity at the transcriptional level. Overall, our study provided additional evidence that actein is anti-estrogenic in ER+ breast cancer cells, as shown by its inhibition of estradiol-stimulated MCF-7 cell proliferation and of estradiol-stimulated *TFF1* induction at low, physiological
concentrations. Our results are consistent with those of other researchers who have found actein to be anti-estrogenic (Einbond, Su, Wu, Friedman, Wang, Jiang, et al., 2007; Zierau, Bodinet, Kolba, Wulf, & Vollmer, 2002).

Based on the gene expression data obtained in this study, we propose the mechanism of action shown in Figure 59 for actein to explain its anti-proliferative and anti-estrogenic effects. Actein activates the detoxification pathway by increasing expression of CYP1A1 while deactivating the progression of estrogen-responsive breast cancer by blocking the induction of estrogen responsive genes like TFF1 by estradiol. However, at high concentrations that are unlikely to be achieved physiologically, actein also induces VEGFA, which promotes angiogenesis and metastasis.

**Figure 45. Proposed mechanism of action for actein in MCF-7 cells.**
Actein activates the expression of CYP1A1 which aids in the detoxification of carcinogens, blocks the induction of TFF1 by E2 thereby slowing cancer progression, and induces VEGFA only at high concentrations as part of the hypoxia response.

5.3 Hepatotoxicity of Actein in Liver Cells

5.3.1 Hepatotoxicity of Actein Is Concentration Dependent in HepG2/C3A Cells

Previous literature indicates that actein is toxic at high concentrations, however several of our assays showed actein as having no significant effects on
HepG2/C3A cells (Lude, et al., 2007). For instance, even the highest concentration of actein did not have a significant effect on neutral lipid or phospholipid retention, mitochondrial membrane activity and p-glycoprotein activity, cell viability, or albumin production. While we did find actein to affect ROS levels in HepG2/C3A cells, this was at a low (0.76 µM) concentration and the effect was actually inhibitory. This finding suggests that actein may be able to reduce elevated levels of ROS and therefore warrants further study.

One incidence of potential hepatotoxicity we found involved CYP1A1 activity levels. At 100 µM and 6.25 µM actein, the activity of CYP1A1 was significantly increased; however, at a lower concentration of 0.76 µM actein, CYP1A1 activity was significantly reduced. The literature available suggested that actein may have an inhibitory effect on cytochrome P450 activity; however, these studies were performed on CYP34A instead of CYP1A1 (Tsukamoto, et al., 2005). Therefore, the variability in these results stresses the need for further investigation.

### 5.3.2 Interactions Between Actein and SERMs Are Weak and Few

Our initial investigation of the effects of actein alone on HepG2/C3A cells demonstrated a lack of hepatotoxicity of actein; however, further studies were required to examine the effects of interactions between actein and the SERMs tamoxifen and raloxifene. When actein and tamoxifen were combined, we found a synergistic effect with phospholipid accumulation and cell viability. However, we did not find any interaction with mitochondrial membrane depolarization and p-glycoprotein activity, neutral lipid steatosis or oxidative stress. While we did identify
an interaction between actein and tamoxifen with CYP4501A1 activity, the nature of this interaction was unclear due to the ambiguity of the dose response curve. Our two-way ANOVA indicated an interaction between actein and tamoxifen with two intersecting curves representing tamoxifen alone and the other tamoxifen with 30uM actein. After the intersection of the two curves, the nature of the interaction could be identified by the shifting of the respective curves, however our graph did not show the curves to have any significant shifts. Therefore, it was difficult to definitively conclude the nature of the apparent interaction. In hindsight, we conclude that this and other dose response curve ambiguities were the result of poor experimental design. Upon consulting with a statistician after our assays were performed, we found that in order to produce more telling, accurate results, we would have had to use the same set of concentrations for tamoxifen and raloxifene at 0 and 30 µM actein. From this we learned the importance of an early statistician consultation, and we suggest a proper reinvestigation at a future time.

When actein and raloxifene were combined, we found an interaction with phospholipid accumulation and cell viability. The dose response shift was weak and ambiguous, respectively, resulting in an unclear interaction. We did not find an interaction between actein and raloxifene with ROS activity, CYP4501A1 activity, neutral lipid steatosis, mitochondrial membrane depolarization and p-glycoprotein activity, or albumin production. While the interactions discovered between actein and tamoxifen, and actein and raloxifene were few, the only identifiable interactions occurred at very high concentration and were synergistic. Therefore, this information
could serve as a useful catalyst for further exploration into actein’s potential drug interactions.

### 5.4 Conclusions

In order to address the lack of reliable information regarding the use, safety, and efficacy of HDS, Team IMAC applied a mixed methods approach. A survey was used to investigate current patterns of, perceptions regarding, sources of information about, and communication related to HDS use. The survey instrument was developed, pilot tested, and then administered online to members of BreastCancer.net and Love/Avon Army of Women. To add to the amount of reliable safety and efficacy information available regarding HDS and breast cancer, a specific supplement, black cohosh, was selected for further study due to the presence of conflicting published findings.

Our survey found that most breast cancer survivors who responded use HDS, generally in conjunction with conventional therapies. Of the supplements that our respondents reported using specifically to treat breast cancer, curcumin, flaxseed, and green tea were the most common. Interestingly, these supplements are also some of the best characterized in scientific literature. As expected, of the women who reported using black cohosh, nearly all said that they used the supplement to ameliorate hot flashes and other symptoms of menopause. The most commonly consulted source of information about HDS was internet websites, although respondents also reported that they perceived websites as less reliable than health care
practitioners, government websites, academic journals, breast cancer support groups, and cancer related health organizations.

In our *in vitro* studies of actein, a bioactive component of black cohosh, MCF-7 cells were employed as a model for ER+ breast cancer and HepG2/C3A cells were used to model the human liver. This allowed us to examine actein’s effects on both the breast, the target tissue for chemopreventive and chemotherapeutic breast cancer drugs, and the liver, which processes xenobiotics. Based on cell proliferation and gene expression experiments with the MCF-7 cells, actein inhibited cell proliferation, was anti-estrogenic, and did not change the effect of the SERMs tamoxifen and raloxifene. We also observed significant variations in results based on culture conditions and the time course of treatments, which, in addition to the use of various black cohosh preparations and model systems in experiments, could be the source of existing contradictory results in the scientific literature. In the liver cell assays used to determine whether actein may be hepatotoxic, toxicity was only indicated at very high concentrations (>25 µM). In combination with the SERMs, actein actually lowered some of the effects indicative of liver toxicity as compared to treatment with the SERMs alone.

We conclude that HDS use among women who have been diagnosed with breast cancer continues to be widespread. There is a need for further research in order to increase the quantity, quality, and availability of information regarding the safety and efficacy of HDS. Based on our finding that websites are commonly consulted, the Internet may be a good platform through which to provide this information, as well as to encourage women to consult their health care providers.
regarding any HDS use. Furthermore, we conclude that actein, a bioactive component of black cohosh, is anti-proliferative and anti-estrogenic under the conditions of our experiment, and that experimental conditions including culture media and length of treatment may have led to conflicting published results. Clearly, continued research is needed in both areas of our project, in 1) the biological study of supplement effects alone and in combination with commonly used conventional medicine, and in 2) the sociological and psychological study of supplement use and how to ensure that cancer survivors use supplements in a safe and potentially beneficial way.

5.5 Future Studies

Our study had a few important limitations that could be reduced in future studies. Our survey was administered online to subscribers of BreastCancer.net and Love/Avon Army of Women, which meant that we were unable to draw conclusions about the larger population of women affected by breast cancer. This also meant that our sample population was not demographically representative of breast cancer survivors at-large. Since demographic factors may be related to women’s perceptions about HDS, this could have skewed our results. To obtain a more representative, random sample, the survey instrument could be administered at one or several large cancer hospitals. Future studies could also add questions about supplements that women used which were not listed on our survey instrument, such as mushroom extracts, melatonin, dong quai, fish oils, and ocean plant extracts. In order to obtain more information about women’s motivations for using various supplements and
perceptions of those supplements, a more in-depth, qualitative study could be conducted using focus groups or interviews.

One of the main limitations of our in vitro studies was that the cell lines used as model systems for human ER+ breast cancer tissue and liver tissue do not necessarily respond in the same way that the human body would respond. Additionally, our results were obtained with only one chemical component of black cohosh, therefore our results cannot entirely explain effects that may be had with the intact herbal supplement. Now that we have shown actein to be anti-estrogenic, anti-proliferative, and non-hepatotoxic at low concentrations, the use of an animal model could provide additional insight into whether this effect might also be observed in humans. Our laboratory results also suggest future studies into the mechanism by which the observed effects occurred, such as how actein is able to block the induction of TFF1 by estradiol. Because interactions between HDS, conventional therapies, and differences between individual people can cause enormous variance in how safe or effective a given supplement is, more work is needed within the scientific community in developing a coherent, consistent methodology for measuring interactions, given these many variables.
Appendices

Appendix A. Survey

Breast Cancer and Herbal Dietary Supplement Use

1. Consent Form

Project Title: Herbal Dietary Supplement Use Among Breast Cancer Survivors

Why is this research being done?

This is a research project being conducted by Gemstone Team Integrative Medicine and Cancer (JIMAC) at the University of Maryland, College Park. We are inviting you to participate in this research project because you are a female breast cancer survivor over the age of 18, or you are breast cancer free member of our control group over the age of 18. The purpose of this research project is to investigate the uses and perceptions of herbal dietary supplements among a group of female breast cancer survivors. In a separate study, we will test the effects and safety of the most commonly used herbal dietary supplements in a laboratory.

What will I be asked to do?

The procedures involve completing a short online survey from the link on our website. The survey will take approximately 20 minutes to complete, and will only be completed once. The questions will relate to the use of herbal dietary supplements during and after cancer treatment. They will include reasons for use, sources of information about the reliability of herbal dietary supplements, duration of use, perceived efficacy, and perceived safety. We will also ask you some basic demographic questions, such as your race and education level, so that we can describe the group of participants when we report the results of this study. Your participation in this survey will place you in a raffle drawing to win a fifty dollar cash prize, unless you decide not to participate in the raffle. You will be asked whether or not you wish to enter the raffle following completion of the survey.

What about confidentiality?

Your personal information will be kept confidential. To help protect your confidentiality, your surveys will be stored in a secure file on a password protected computer. The answers from your survey form will only be accessible to members of the Gemstone team, the team mentor Dr. Mark Kantor, and a University of Maryland statistician. Your survey will have an identification number and will not be linked to your name in any way. If you decide to participate in the raffle drawing, we will keep your name and telephone number in a separate list so that we may contact you if you are selected to receive a prize in the drawing. After the drawing is over that list will be destroyed. If we write a report or article about this research project, your identity will not be revealed. The information you share with us will be summarized along with all of the study participants’ responses. Your information may be shared with representatives of the University of Maryland, College Park or governmental authorities if you or someone else is in danger or if we are required to do so by law.

What are the risks of this research?

There may be some risks from participating in this research study. There are both psychological and emotional risks that could result from participation in the questionnaire. Since cancer is a very sensitive topic for those who have been personally affected by it, respondents may undergo emotional discomfort and psychological distress as they recall their experiences. In some cases completing the questionnaire may cause past feelings of depression, hopelessness, and fear to reoccur.

What are the benefits of this research?

This research is not designed to help you personally, but the results may help the investigators learn more about dietary supplements that help relieve some of the side effects of chemotherapy or other medical treatments. The benefits to you may include feeling a sense of satisfaction and gratification in knowing that your contribution may help others in the same situation. We hope that, in the future, other people might benefit from this study through improved understanding of the benefits and safety of herbal dietary supplement use during cancer treatment.
Breast Cancer and Herbal Dietary Supplement Use

Do I have to be in this research? May I stop participating at any time?

Your participation in this research is completely voluntary. You may choose not to take part at all. If you decide to participate in this research, you may stop participating at any time. If you decide not to participate in this study or if you stop participating at any time, you will not be penalized or lose any benefits to which you otherwise qualify.

Is any medical treatment available if I am injured?

The University of Maryland does not provide any medical, hospitalization or other insurance for participants in this research study, nor will the University of Maryland provide any medical treatment or compensation for any injury sustained as a result of participation in this research study, except as required by law.

What if I have questions?

This research is being conducted by Dr. Mark Kantor at the Gemstone Program at the University of Maryland, College Park.

If you have any questions about the research study itself, please contact Dr. Mark Kantor at The University of Maryland, Dept. of Nutrition and Food Science, 0112 Skinner Building, College Park, MD 20742-7640, 301-405-1018, mkanter@umd.edu

If you have questions about your rights as a research subject or wish to report a research-related injury, please contact: Institutional Review Board Office, University of Maryland, College Park, Maryland, 20742; (e-mail) irb@deans.umd.edu; (telephone) 301-405-0678

This research has been reviewed according to the University of Maryland, College Park institutional Review Board (IRB) procedures for research involving human subjects.

* 1. Statement of Age of Subject and Consent:
   By checking below you indicate that:
   - [ ] You are at least 18 years of age
   - [ ] The research has been explained to you
   - [ ] Your questions have been fully answered
   - [ ] You freely and voluntarily choose to participate in this research project

2. Breast Cancer Diagnosis

* 1. Have you ever been diagnosed with breast cancer?
   - [ ] Yes
   - [ ] No

3. Breast Cancer Stage and Treatment

* 1. What was the date of your breast cancer diagnosis?
   - [ ] Month
   - [ ] Year
Breast Cancer and Herbal Dietary Supplement Use

* 2. What is the current stage of your breast cancer?
   - 0 (carcinoma in situ)
   - Stage I
   - Stage II (A)
   - Stage II (B)
   - Stage III (A)
   - Stage III (B)
   - Stage III (C)
   - Stage IV
   - In Remission
   - I don’t know

* 3. Have you been treated for breast cancer with conventional cancer treatments? Conventional cancer treatments include chemotherapy, radiation therapy, and surgery.
   - Yes
   - No

* 4. Have you been treated for breast cancer with conventional cancer treatments in the past 12 months?
   - Yes
   - No

4. Herbal Dietary Supplement Use

The following questions ask about your use of herbal dietary supplements. An herbal dietary supplement is a product that contains a plant or plant part that is used for medicinal or therapeutic properties. They are meant to be taken by mouth as fresh or dried products or in tablet, capsule, powder, softgel, gelcap, or liquid form. Examples include ginseng, green tea, and garlic.

* 1. Have you ever used an herbal dietary supplement?
   - Yes
   - No

5. Black Cohosh
Breast Cancer and Herbal Dietary Supplement Use

1. Have you ever used black cohosh?
   - Yes, for medicinal purposes
   - Yes, for other purposes
   - No

6. Black Cohosh Use

* 1. What was your reason for using this?

2. Did you use this prior to your diagnosis?
   - Yes
   - No
   - I have not been diagnosed with breast cancer

7. Black Cohosh Use 2

1. Did you use this after your diagnosis?
   - Yes
   - No

2. Did you use this to treat side effects of your conventional cancer treatments?
   - Yes
   - No

8. Black Cohosh to Treat Breast Cancer Symptoms

* 1. Which symptoms did you hope to treat?

* 2. How effective was this in treating your symptoms?

<table>
<thead>
<tr>
<th>Effectiveness of Black Cohosh</th>
<th>Not Effective</th>
<th>Somewhat Effective</th>
<th>Very Effective</th>
<th>Don't Know</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

9. Red Clover
Breast Cancer and Herbal Dietary Supplement Use

1. Have you ever used red clover?
   - Yes, for medicinal purposes
   - Yes, for other purposes
   - No

10. Red Clover Use

1. What was your reason for using this?
   [Text field]

2. Did you use this prior to your diagnosis?
   - Yes
   - No
   - I have not been diagnosed with breast cancer

11. Red Clover Use 2

1. Did you use this after your diagnosis?
   - Yes
   - No

2. Did you use this to treat side effects of your conventional cancer treatments?
   - Yes
   - No

12. Red Clover to Treat Breast Cancer Symptoms

1. Which symptoms did you hope to treat?
   [Text field]

2. How effective was this in treating your symptoms?
   [Table]
<table>
<thead>
<tr>
<th>Effectiveness of Red Clover</th>
<th>Not Effective</th>
<th>Somewhat Effective</th>
<th>Very Effective</th>
<th>Don't Know</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
**Breast Cancer and Herbal Dietary Supplement Use**

### 13. Curcumin/Turmeric

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1. Have you ever used curcumin/turmeric?</strong></td>
<td></td>
</tr>
<tr>
<td>○ Yes, for medicinal purposes</td>
<td></td>
</tr>
<tr>
<td>○ Yes, for other purposes</td>
<td></td>
</tr>
<tr>
<td>○ No</td>
<td></td>
</tr>
</tbody>
</table>

### 14. Curcumin/Turmeric Use

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1. What was your reason for using this?</strong></td>
<td></td>
</tr>
<tr>
<td><strong>2. Did you use this prior to your diagnosis?</strong></td>
<td></td>
</tr>
<tr>
<td>○ Yes</td>
<td></td>
</tr>
<tr>
<td>○ No</td>
<td></td>
</tr>
<tr>
<td>○ I have not been diagnosed with breast cancer</td>
<td></td>
</tr>
</tbody>
</table>

### 15. Curcumin/Turmeric Use 2

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1. Did you use this after your diagnosis?</strong></td>
<td></td>
</tr>
<tr>
<td><strong>2. Did you use this to treat side effects of your conventional cancer treatments?</strong></td>
<td></td>
</tr>
<tr>
<td>○ Yes</td>
<td></td>
</tr>
<tr>
<td>○ No</td>
<td></td>
</tr>
</tbody>
</table>

### 16. Curcumin/Turmeric to Treat Breast Cancer Symptoms

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1. Which symptoms did you hope to treat?</strong></td>
<td></td>
</tr>
</tbody>
</table>
Breast Cancer and Herbal Dietary Supplement Use

* 2. How effective was this in treating your symptoms?

<table>
<thead>
<tr>
<th>Effectiveness of Curcumin/Turmeric</th>
<th>Not Effective</th>
<th>Somewhat Effective</th>
<th>Very Effective</th>
<th>Don't Know</th>
</tr>
</thead>
</table>

17. Chamomile

* 1. Have you ever used chamomile?
  - Yes, for medicinal purposes
  - Yes, for other purposes
  - No

18. Chamomile Use

* 1. What was your reason for using this?

* 2. Did you use this prior to your diagnosis?
  - Yes
  - No
  - I have not been diagnosed with breast cancer

19. Chamomile Use 2

* 1. Did you use this after your diagnosis?
  - Yes
  - No

* 2. Did you use this to treat side effects of your conventional cancer treatments?
  - Yes
  - No

20. Chamomile to Treat Breast Cancer Symptoms
21. Flaxseed

* 1. Have you ever used flaxseed?
   - Yes, for medicinal purposes
   - Yes, for other purposes
   - No

22. Flaxseed Use

* 1. What was your reason for using this?

* 2. Did you use this prior to your diagnosis?
   - Yes
   - No
   - I have not been diagnosed with breast cancer

23. Flaxseed Use 2

* 1. Did you use this after your diagnosis?
   - Yes
   - No

* 2. Did you use this to treat side effects of your conventional cancer treatments?
   - Yes
   - No

24. Flaxseed to Treat Breast Cancer Symptoms
Breast Cancer and Herbal Dietary Supplement Use

* 1. Which symptoms did you hope to treat?

* 2. How effective was this in treating your symptoms?

<table>
<thead>
<tr>
<th>Effectiveness of Flaxseed</th>
<th>Not Effective</th>
<th>Somewhat Effective</th>
<th>Very Effective</th>
<th>Don't Know</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

25. Milk Thistle

* 1. Have you ever used milk thistle?

   ○ Yes, for medicinal purposes
   ○ Yes, for other purposes
   ○ No

26. Milk Thistle Use

* 1. What was your reason for using this?

* 2. Did you use this prior to your diagnosis?

   ○ Yes
   ○ No
   ○ I have not been diagnosed with breast cancer

27. Milk Thistle Use 2

* 1. Did you use this after your diagnosis?

   ○ Yes
   ○ No

* 2. Did you use this to treat side effects of your conventional cancer treatments?

   ○ Yes
   ○ No
## Breast Cancer and Herbal Dietary Supplement Use

### 28. Milk Thistle to Treat Breast Cancer Symptoms

1. Which symptoms did you hope to treat?

2. How effective was this in treating your symptoms?

<table>
<thead>
<tr>
<th>Effectiveness of Milk Thistle</th>
<th>Not Effective</th>
<th>Somewhat Effective</th>
<th>Very Effective</th>
<th>Don't Know</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### 29. Ginger

1. Have you ever used ginger?
   - Yes, for medicinal purposes
   - Yes, for other purposes
   - No

### 30. Ginger Use

1. What was your reason for using this?

2. Did you use this prior to your diagnosis?
   - Yes
   - No
   - I have not been diagnosed with breast cancer

### 31. Ginger Use 2

1. Did you use this after your diagnosis?
   - Yes
   - No
Breast Cancer and Herbal Dietary Supplement Use

* 2. Did you use this to treat side effects of your conventional cancer treatments?
   - Yes
   - No

32. Ginger to Treat Breast Cancer Symptoms

* 1. Which symptoms did you hope to treat?
   - [Blank field]

* 2. How effective was this in treating your symptoms?
   - Effectiveness of Ginger
     - Not Effective
     - Somewhat Effective
     - Very Effective
     - Don't Know

33. Evening Primrose

* 1. Have you ever used evening primrose?
   - Yes, for medicinal purposes
   - Yes, for other purposes
   - No

34. Evening Primrose Use

* 1. What was your reason for using this?
   - [Blank field]

* 2. Did you use this prior to your diagnosis?
   - Yes
   - No
   - I have not been diagnosed with breast cancer

35. Evening Primrose Use 2
Breast Cancer and Herbal Dietary Supplement Use

1. Did you use this after your diagnosis?
   - Yes
   - No

2. Did you use this to treat side effects of your conventional cancer treatments?
   - Yes
   - No

36. Evening Primrose to Treat Breast Cancer Symptoms

1. Which symptoms did you hope to treat?

2. How effective was this in treating your symptoms?

37. Ginseng

1. Have you ever used ginseng?
   - Yes, for medicinal purposes
   - Yes, for other purposes
   - No

38. Ginseng Use

1. What was your reason for using this?

2. Did you use this prior to your diagnosis?
   - Yes
   - No
   - I have not been diagnosed with breast cancer

39. Ginseng Use 2
Breast Cancer and Herbal Dietary Supplement Use

* 1. Did you use this after your diagnosis?
   - Yes
   - No

* 2. Did you use this to treat side effects of your conventional cancer treatments?
   - Yes
   - No

40. Ginseng to Treat Breast Cancer Symptoms

* 1. Which symptoms did you hope to treat?

* 2. How effective was this in treating your symptoms?

<table>
<thead>
<tr>
<th>Effectiveness of Ginseng</th>
<th>Not Effective</th>
<th>Somewhat Effective</th>
<th>Very Effective</th>
<th>Don't Know</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

41. Green Tea

* 1. Have you ever used green tea?
   - Yes, for medicinal purposes
   - Yes, for other purposes
   - No

42. Green Tea Use

* 1. What was your reason for using this?

* 2. Did you use this prior to your diagnosis?
   - Yes
   - No
   - I have not been diagnosed with breast cancer
Breast Cancer and Herbal Dietary Supplement Use

43. Green Tea Use 2

* 1. Did you use this after your diagnosis?
   - Yes
   - No

* 2. Did you use this to treat side effects of your conventional cancer treatments?
   - Yes
   - No

44. Green Tea to Treat Breast Cancer Symptoms

* 1. Which symptoms did you hope to treat?

* 2. How effective was this in treating your symptoms?

<table>
<thead>
<tr>
<th>Effectiveness of Green Tea</th>
<th>Not Effective</th>
<th>Somewhat Effective</th>
<th>Very Effective</th>
<th>Don't Know</th>
</tr>
</thead>
</table>

45. Kava Kava

* 1. Have you ever used kava kava?
   - Yes, for medicinal purposes
   - Yes, for other purposes
   - No

46. Kava Kava Use

* 1. What was your reason for using this?
Breast Cancer and Herbal Dietary Supplement Use

* 2. Did you use this prior to your diagnosis?
   - Yes
   - No
   - I have not been diagnosed with breast cancer

47. Kava Kava Use 2

* 1. Did you use this after your diagnosis?
   - Yes
   - No

* 2. Did you use this to treat side effects of your conventional cancer treatments?
   - Yes
   - No

48. Kava Kava to Treat Breast Cancer Symptoms

* 1. Which symptoms did you hope to treat?

* 2. How effective was this in treating your symptoms?

<table>
<thead>
<tr>
<th>Effectiveness of Kava Kava</th>
<th>Not Effective</th>
<th>Somewhat Effective</th>
<th>Very Effective</th>
<th>Don't Know</th>
</tr>
</thead>
</table>

49. Saw Palmetto

* 1. Have you ever used saw palmetto?
   - Yes, for medicinal purposes
   - Yes, for other purposes
   - No

50. Saw Palmetto Use
Breast Cancer and Herbal Dietary Supplement Use

* 1. What was your reason for using this?

* 2. Did you use this prior to your diagnosis?
  - Yes
  - No
  - I have not been diagnosed with breast cancer

51. Saw Palmetto Use 2

* 1. Did you use this after your diagnosis?
  - Yes
  - No

* 2. Did you use this to treat side effects of your conventional cancer treatments?
  - Yes
  - No

52. Saw Palmetto to Treat Breast Cancer Symptoms

* 1. Which symptoms did you hope to treat?

* 2. How effective was this in treating your symptoms?

53. Soy

* 1. Have you ever used soy?
  - Yes, for medicinal purposes
  - Yes, for other purposes
  - No

54. Soy Use
Breast Cancer and Herbal Dietary Supplement Use

1. What was your reason for using this?

2. Did you use this prior to your diagnosis?
   - Yes
   - No
   - I have not been diagnosed with breast cancer

55. Soy Use 2

1. Did you use this after your diagnosis?
   - Yes
   - No

2. Did you use this to treat side effects of your conventional cancer treatments?
   - Yes
   - No

56. Soy to Treat Breast Cancer Symptoms

1. Which symptoms did you hope to treat?

2. How effective was this in treating your symptoms?

57. St. John's Wort

1. Have you ever used St. John's Wort?
   - Yes, for medicinal purposes
   - Yes, for other purposes
   - No
### 58. St. John’s Wort Use

1. **What was your reason for using this?**

   ![Reason for using St. John’s Wort](image)

2. **Did you use this prior to your diagnosis?**

   - [ ] Yes
   - [ ] No
   - [ ] I have not been diagnosed with breast cancer

### 59. St. John’s Wort Use 2

1. **Did you use this after your diagnosis?**

   - [ ] Yes
   - [ ] No

2. **Did you use this to treat side effects of your conventional cancer treatments?**

   - [ ] Yes
   - [ ] No

### 60. St. John’s Wort to Treat Breast Cancer Symptoms

1. **Which symptoms did you hope to treat?**

   ![Symptoms list](image)

2. **How effective was this in treating your symptoms?**

<table>
<thead>
<tr>
<th>Effectiveness of St. John’s Wort</th>
<th>Not Effective</th>
<th>Somewhat Effective</th>
<th>Very Effective</th>
<th>Don’t Know</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="Effectiveness options" /></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### 61. Valerian
## Breast Cancer and Herbal Dietary Supplement Use

**1. Have you ever used valerian?**
- [ ] Yes, for medicinal purposes
- [ ] Yes, for other purposes
- [ ] No

### 62. Valerian Use

**1. What was your reason for using this?**

**2. Did you use this prior to your diagnosis?**
- [ ] Yes
- [ ] No
- [ ] I have not been diagnosed with breast cancer

### 63. Valerian Use 2

**1. Did you use this after your diagnosis?**
- [ ] Yes
- [ ] No

**2. Did you use this to treat side effects of your conventional cancer treatments?**
- [ ] Yes
- [ ] No

### 64. Valerian to Treat Breast Cancer Symptoms

**1. Which symptoms did you hope to treat?**

**2. How effective was this in treating your symptoms?**

<table>
<thead>
<tr>
<th>Effectiveness of Valerian</th>
<th>Not Effective</th>
<th>Somewhat Effective</th>
<th>Very Effective</th>
<th>Don’t Know</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
</tr>
</tbody>
</table>

### 65. Other Supplement


**Breast Cancer and Herbal Dietary Supplement Use**

*1. Have you ever used any other herbal dietary supplements besides those previously mentioned? (Those previously mentioned include: Black Cohosh, Red Clover, Curcumin/Turmeric, Chamomile, Flaxseed, Milk Thistle, Ginger, Evening Primrose, Ginseng, Green Tea, Kava Kava, Saw Palmetto, Soy, St. John’s Wort, and Valerian)*

- Yes, for medicinal purposes
- Yes, for other purposes
- No

---

**66. Other Supplement Use**

*1. What was name(s) of this supplement(s)?*


*2. What was your reason(s) for using this(these)?*


*3. Did you use this(these) prior to your diagnosis?*

- Yes
- No
- I have not been diagnosed with breast cancer

---

**67. Other Supplement Use 2**

*1. Did you use this(these) after your diagnosis?*

- Yes
- No

2. Please enter additional comments or explanations about your use of other supplement(s).
Breast Cancer and Herbal Dietary Supplement Use

* 3. Did you use this (these) to treat side effects of your conventional cancer treatments?
   - Yes
   - No

68. Other Supplements to Treat Breast Cancer Symptoms

* 1. Which symptoms did you hope to treat?

* 2. How effective was this in treating your symptoms?

   Effectiveness of Other Supplement
   - Not Effective
   - Somewhat Effective
   - Very Effective
   - Don't Know

3. Please enter additional comments and explanations about your use of other supplements to treat side effects and symptoms.

69. Other Comments

1. Any comments about your use of herbal supplements?

70. Decision to Use Herbal Dietary Supplements

* 1. How influential was each of the following in your decision to either use or not use herbal dietary supplements?

   - Family
   - Friends
   - Physician
   - Religion

   Not Influential
   - Somewhat Influential
   - Very Influential
   - Don't Know/Not Sure
**Breast Cancer and Herbal Dietary Supplement Use**

* 2. Have you ever attempted to seek out information about herbal dietary supplements, such as by doing your own reading or talking to people?

- [ ] Yes
- [ ] No

**71. Sources of Information Regarding Herbal Dietary Supplements**

* 1. How often do/did you consult the following sources when looking for information about herbal dietary supplements?

<table>
<thead>
<tr>
<th>Source</th>
<th>Never</th>
<th>Rarely</th>
<th>Sometimes</th>
<th>Often</th>
<th>I don't know what this is</th>
<th>Don't Know/Not Sure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Websites found through internet databases or search engines (Examples: Google, Wikipedia, and Yahoo)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Government websites (Examples: National Institutes of Health (NIH), Food and Drug Administration (FDA), and Centers for Disease Control and Prevention (CDC))</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Information provided by companies or company-sponsored websites (An example is GNC)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breast cancer support groups or other cancer-related non-profit advocacy organizations (An example is Susan G. Komen)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cancer-related health organizations (An example is the American Cancer Society)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Popular magazines or newspapers (Examples: Time, Newsweek, and the Washington Post)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Academic journals (An example is The Journal of the American Medical Association)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Friends</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### Breast Cancer and Herbal Dietary Supplement Use

<table>
<thead>
<tr>
<th>Family Members</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Your physician, oncolgist, nurse practitioner, or other health care provider</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A practitioner specializing in complementary and/or alternative medicine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any Comments?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**2. How reliable do/did you consider information on herbal dietary supplements from the following sources? Reliable information is considered to be accurate, dependable, honest, or trustworthy.**

<table>
<thead>
<tr>
<th>Websites found through internet databases or search engines (Examples: Google, Wikipedia, and Yahoo)</th>
<th>Not Reliable</th>
<th>Somewhat Reliable</th>
<th>Very Reliable</th>
<th>I don't know what this is</th>
<th>Don't Know/Not Sure</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Government websites (Examples: National Institutes of Health (NIH), Food and Drug Administration (FDA), and Centers for Disease Control and Prevention (CDC))</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Information provided by companies or company-sponsored websites (An example is GNC)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breast cancer support groups or other cancer-related non-profit advocacy organizations (An example is Susan G. Komen)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cancer-related health organizations (An example is The American Cancer Society)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Popular magazines or newspapers (Examples: Time, Newsweek, and the Washington Post)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### Breast Cancer and Herbal Dietary Supplement Use

<table>
<thead>
<tr>
<th>Source of Information</th>
<th>Not Concerned</th>
<th>Somewhat Concerned</th>
<th>Very Concerned</th>
<th>Don't Know/Not Sure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Academic journals (An example is The Journal of the American Medical Association)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Friends</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Family Members</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Your physician, oncologist, nurse practitioner, or other health care provider</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A practitioner specializing in complementary and/or alternative medicine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Any Comments?

---

### Safety and Labeling

**1. How concerned are you that herbal dietary supplements are adequately tested for safety?**

<table>
<thead>
<tr>
<th>Concern regarding safety testing</th>
<th>Not Concerned</th>
<th>Somewhat Concerned</th>
<th>Very Concerned</th>
<th>Don't Know/Not Sure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**2. How concerned are you about proper labeling for herbal dietary supplements?**

<table>
<thead>
<tr>
<th>Concern regarding labeling</th>
<th>Not Concerned</th>
<th>Somewhat Concerned</th>
<th>Very Concerned</th>
<th>Don't Know/Not Sure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Demographics

**1. Which of the following do you consider yourself to be? (You can check more than one)**

- [ ] White/Caucasian
- [ ] Black/African-American
- [ ] Hispanic/Latino
- [ ] Asian/Pacific Islander
- [ ] Other

(please specify other)

---

156
# Breast Cancer and Herbal Dietary Supplement Use

**2. How old are you?**
- 18-25
- 26-35
- 36-50
- 51-65
- Over 65

**3. What is your total household income before taxes?**
- Less than $20,000
- $20,000 - $39,999
- $40,000 - $59,999
- $60,000 - $99,999
- More than $100,000
- I don’t know

**4. What is the highest level of education you have completed?**
- Grade school
- High school
- Some college
- Associate’s degree
- Bachelor’s degree
- Advanced/professional degree (beyond bachelor’s degree)
- Other

(please specify other)
Breast Cancer and Herbal Dietary Supplement Use

5. Which of the following religions do you consider yourself to belong to?

- [ ] Christian
- [ ] Jewish
- [ ] Muslim
- [ ] Hindu
- [ ] Buddhist
- [ ] Deist or Agnostic
- [ ] Atheist
- [ ] None
- [ ] Other

(please specify other)

74. Thank You

Thank you for completing our survey! Your results have been received. By pressing 'Done' you will now be redirected to our raffle for a chance to win $50!
Appendix B. Lack of Hepatotoxicity of Tamoxifen and Lack of Interactions Between Actein and SERMs

Figure 46. Tamoxifen does not affect membrane phospholipid accumulation. Tamoxifen does not affect phospholipid accumulation in HepG2/C3A cells. Confluent cells were treated with 0, 0.76, 1.55, 3.1, 6.25, 12.5, 25, 50, and 100 µM tamoxifen for 48 hours, and phospholipid accumulation was measured with the Nile Red assay. Data were normalized as a percentage of the mean value of the 0 µM control, and expressed as mean ± SEM.

Figure 47. Actein and tamoxifen do not have an interaction effect on oxidative stress. Actein and tamoxifen do not have an interaction effect on oxidative stress in HepG2/C3A cells. Confluent cells were treated with the indicated concentrations of actein and tamoxifen for 48 hours, and ROS was measured with the DCFDA assay. Data were normalized as a percentage of the mean value of the 0 µM control, and expressed as mean ± SEM.
**Figure 48.** Actein and tamoxifen do not have an interaction effect on intracellular neutral lipid accumulation.

Actein and tamoxifen do not have an interaction effect on neutral lipid accumulation in HepG2/C3A cells. Confluent cells were treated with the indicated concentrations of actein and tamoxifen for 48 hours, and lipid accumulation was measured with the Nile Red assay. Data were normalized as a percentage of the mean value of the 0 μM control, and expressed as mean ± SEM.

**Figure 49.** Actein and tamoxifen do not have an interaction effect on R123 retention.

Actein and tamoxifen do not have an interaction effect on R123 retention in HepG2/C3A cells. Confluent cells were treated with the indicated concentrations of actein and tamoxifen for 48 hours, and R123 accumulation was measured with the R123 assay. Data were normalized as a percentage of the mean value of the 0 μM control, and expressed as mean ± SEM.
Figure 50. Actein and tamoxifen do not have an interaction effect on albumin production.
Actein and tamoxifen do not have an interaction effect on albumin production by HepG2/C3A cells. Confluent cells were treated with the indicated concentrations of actein and tamoxifen for 48 hours, and albumin production was measured with an albumin ELISA. Data were normalized as a percentage of the mean value of the 0 µM control, and expressed as mean ± SEM.

Figure 51. Actein and raloxifene do not have an interaction effect on oxidative stress.
Actein and raloxifene do not have an interaction effect on oxidative stress in HepG2/C3A cells. Confluent cells were treated with the indicated concentrations of actein and raloxifene for 48 hours, and ROS was measured with the DCFDA assay. Data were normalized as a percentage of the mean value of the 0 µM control, and expressed as mean ± SEM.
Figure 52. Actein and raloxifene do not have an interaction effect CYP1A1 activity.
Actein and raloxifene do not have an interaction effect on CYP1A1 activity in HepG2/C3A cells. Confluent cells were treated with the indicated concentrations of actein and tamoxifen for 48 hours, and CYP1A1 activity was measured with the EROD assay. Data were normalized as a percentage of the mean value of the 0 µM control, and expressed as mean ± SEM.

Figure 53. Actein and raloxifene do not have an interaction effect on intracellular neutral lipid accumulation.
Actein and raloxifene do not have an interaction effect on neutral lipid accumulation in HepG2/C3A cells. Confluent cells were treated with the indicated concentrations of actein and raloxifene for 48 hours, and lipid accumulation was measured with the Nile Red assay. Data were normalized as a percentage of the mean value of the 0 µM control, and expressed as mean ± SEM.
**Figure 54.** Actein and raloxifene do not have an interaction effect on R123 retention. Actein and raloxifene do not have an interaction effect on R123 retention in HepG2/C3A cells. Confluent cells were treated with the indicated concentrations of actein and raloxifene for 48 hours, and R123 accumulation was measured with the R123 assay. Data were normalized as a percentage of the mean value of the 0 µM control, and expressed as mean ± SEM.

**Figure 55.** Actein and raloxifene do not have an interaction effect on albumin production. Actein and raloxifene do not have an interaction effect on albumin production by HepG2/C3A cells. Confluent cells were treated with the indicated concentrations of actein and raloxifene for 48 hours, and albumin production was measured with an albumin ELISA. Data were normalized as a percentage of the mean value of the 0 µM control, and expressed as mean ± SEM.
Appendix C. Howard Hughes Medical Institute Grant Application

Abstract
Conventional cancer treatments are often associated with unpleasant side effects, leading many patients to integrate complementary and alternative medicine (CAM) modalities with their standard therapies. One commonly used and relatively inexpensive CAM is herbal dietary supplements (HDS). However, little information is available regarding the types of HDS used by breast cancer survivors, the perceived effectiveness of HDS, and adverse reactions associated with its use, such as interactions between HDS and prescribed drugs. Using a mixed-methods approach in both a clinical and laboratory setting, we will investigate two aspects of HDS and breast cancer: (1) A survey instrument that we developed and pilot tested will be administered to ~ 200 breast cancer survivors undergoing treatment at the Lombardi Cancer Center (Washington, D.C.) to study their use and perceptions of HDS; (2) The in-vitro effects of a commonly used HDS, black cohosh (*Actaea racemosa*), alone and in combination with the chemohormonal drugs raloxifene and tamoxifen, will be studied using human breast cancer (MCF-7) and liver (HepG2-C3A) cell lines. We expect our investigation to provide a deeper understanding of perceptions, use, and potential effectiveness of HDS in general, and black cohosh in particular, as part of an integrative approach to treating breast cancer.

Rationale
Our research goal is to qualitatively compare local breast cancer survivor use and perceptions of herbal dietary supplements to their proven effects and safety profiles in literature and in our own *in vitro* study of black cohosh. In 2007, an estimated 2.5 million people were diagnosed with cancer in the United States, including 178,480 new cases of breast cancer. Conventional chemotherapy agents treat cancer by targeting rapidly dividing cells, including healthy cells in the lining of the mouth and intestines, bone marrow, and hair follicles, causing side effects such as hair loss, nausea, vomiting, fever, immune system vulnerability, and fatigue. In addition to the side effects associated with general chemotherapy and radiation therapy, breast cancer patients frequently experience amenorrhea, premature menopause, and menopausal symptoms such as hot flashes and changes in the urogenital epithelium due to the endocrine-targeted therapies and the action of chemotherapy on the ovary. Hormone replacement therapy (HRT) with estrogen alone or in combination with progestin has been found to be effective in relieving menopausal symptoms. However, estrogen may also stimulate the growth and development of some types of breast cancer, and there is evidence suggesting that HRT is related to breast cancer recurrence and mortality.

The side effects of conventional treatments lead many cancer survivors to seek alternatives to alleviate these side effects, increase immune function, augment overall health, or even replace conventional treatment. A study conducted in 2004 found that over half of the cancer survivors surveyed began using one or more
complementary and alternative medicine (CAM) modalities following cancer diagnosis. A 2006 study of over 2000 women diagnosed with breast cancer during 1998-2003 showed that 62% used some form of CAM, with 19% of the women reporting that they used herbs. In a study comparing 2005 survey data to data from 1998, breast cancer survivors reported using a variety of CAM modalities, most commonly herbal or high-dosage vitamin supplements. A significant increase in the use of these therapies was observed between 1998 and 2005. Because herbal dietary supplements (HDS) are widely available, relatively inexpensive, and a commonly used CAM modality, we will focus our study on HDS, particularly those that have been cited as frequently used by breast cancer survivors: black cohosh or Remifemin, curcumin, echinacea, flaxseed, garlic, ginger, ginkgo, ginseng, kava kava, saw palmetto, soy or soy-derivative (isoflavones, genistein), St. John’s wort, valerian, dong quai, and red clover. To determine the specific HDS that local breast cancer survivors are using and their reasons for using these therapies, we will conduct a survey of survivors at the Lombardi Cancer Center of Georgetown University Medical Center (GUMC).

Concerns regarding HDS use by breast cancer survivors involve a lack of evidence regarding the safety and efficacy of some herbals, lack of communication about HDS use with health care professionals, and potentially harmful herb-drug interactions. In 2005, a survey of over 300 breast and prostate cancer survivors found that 61% reported not discussing CAM use with their physician, while 63% of oncologists said that they bring up the topic of CAM at least some of the time. This can be dangerous as some botanicals are known to affect drug metabolism, increase bleeding risk during surgery, or have estrogenic properties that may stimulate the growth and proliferation of breast cancer cells. Because the Food and Drug Administration does not require pre-market testing or approval of HDS, the safety and efficacy of commercially available botanicals is controversial or largely unknown. Our survey will address such attitudinal and behavioral issues as where breast cancer survivors obtain information about HDS and whether they discuss it with their health care providers.

One dietary supplement frequently used by breast cancer patients is black cohosh (Actaea racemosa or Cimicifuga racemosa), which is controversial with respect to its safety and efficacy. For example, reports in the literature suggest that black cohosh may have estrogenic properties, which would be dangerous to breast cancer patients with estrogen-sensitive cancer. Additionally, there have been several reported cases of hepatotoxicity in individuals using black cohosh. Because existing literature has come to mixed conclusions about the safety and efficacy of black cohosh, we hope to build on the existing knowledge base through further in vitro studies. We will investigate the effects of black cohosh alone and in combination with the selective estrogen receptor modulators tamoxifen and raloxifene – drugs frequently prescribed to breast cancer patients – on breast cancer cell proliferation and on hepatic processes.

Methods

Survey research. Breast cancer survivors’ use and perceptions of HDS will be investigated using a survey instrument that we have developed. This questionnaire
has been validated by expert reviewers and will be further validated by pilot testing with cognitive interviewing in mock interviews among our peers, residents of Spelman House Advanced Care Center, and with other volunteers. The survey and study design and protocol have already been approved by the University of Maryland Institutional Review Board (IRB) and will soon be submitted to the GUMC IRB. Subjects for the study will be recruited and interviewed on site as part of the Lombardi Cancer Center’s ongoing recruitment for the Breast Cancer Biomarker and Clinical Outcomes Research Database. We will use a convenience sample of subjects from a sampling frame of all female breast cancer survivors > 18 years of age who visit the Lombardi Comprehensive Cancer Center at GUMC over the course of a defined 12-month period. The interviewer will read each question from the questionnaire to the subject, using response cards as aids for some questions, and record the responses with a predetermined coding scheme.

**Laboratory research.** The effects of black cohosh will be tested in vitro using the putative active compounds actein, 23-epi-26-deoxyactein, and cimicifugoside. Cell proliferation of estrogen receptor-positive human breast cancer MCF-7 cells treated with the black cohosh compounds alone and in combination with tamoxifen and raloxifene will be tested by plating cells in 24-well plates with 25,000 cells/well, incubating for 24 hours at 37 ºC, and then replacing medium with or without test compounds at physiological concentrations, including appropriate controls. Treatment medium will be replaced twice after 24-hour incubations. 96 hours after the initial treatment, the number of cells in each well will be assessed by fixing, staining with sulforhodamine B, and measuring the absorbance with UV-vis spectrophotometry at a wavelength of 490 nm. The extent that these active compounds have an estrogenic effect will also be examined using the same methodology, but modifying the treatment to include 17β-estradiol.

Potential hepatotoxicity of the black cohosh compounds will be examined in vitro using HepG2/C3A human liver cells grown in a hormonal cocktail to provide a better model of female liver cells. Cells will be plated and treated with the black cohosh compounds as described above. Following treatment, cell death will be measured by determining the amount of double-stranded DNA present in each well. Oxidative stress will be measured by adding dihydrodichlorofluorescein diacetate to the cells, which will be oxidized by reactive oxygen species to dichlorofluorescein, a fluorescent product. The intensity of the fluorescence can be measured and used to determine the concentration of reactive oxygen species. The activity of cytochrome P4501A1, cytochrome P2B, and cytochrome P3A, enzymes involved in xenobiotic metabolism, will be measured by adding substrates that form fluorescent products upon enzyme-catalyzed hydrolysis and then measuring the intensity of the fluorescence. Other endpoint assays may be used to further investigate hepatic effects of the black cohosh compounds.

**Analysis of Results**

Using descriptive statistics, we will describe the attitudes and behaviors of the breast cancer survivors in our sample with respect to HDS. We will use a statistical analysis program (such as SPSS or SAS) to perform multiple regression analyses to relate demographic and health history factors to HDS use overall and for each
individual dietary supplement. Additionally, we will examine relationships between various information sources and HDS use.

To analyze differences in cell proliferation, amount of double stranded DNA, amount of reactive oxygen species, and CYP1A1, CYP2B, and CYP3A activity under the different treatment conditions (black cohosh compounds alone and in combination with either tamoxifen or raloxifene), results will first be expressed as a percent of the appropriate control. Next, an analysis of variance (ANOVA) will be performed to test for differences between treatment condition means. If the existence of significant differences in the means is established, Dunnet’s test for multiple comparisons will be used to determine whether each treatment condition/concentration in a group is significantly different from a shared control. Additionally, the IC$_{50}$ (concentration causing 50% inhibition of cell proliferation) of each of the black cohosh test compounds will be determined.

Finally, the breast cancer survivors’ perceptions of HDS will qualitatively be compared to evidence of safety and efficacy that exists in the published literature. If appropriate, comparisons will also be made to our in-vitro black cohosh studies. The PubMed database will be searched for English articles published in peer-reviewed journals related to breast cancer and each of the specific dietary supplements used by our subjects. Clinical studies, reviews, and primary in vitro and in vivo studies published between 2004-2009 will be included. The reported use and perceptions of HDS in our study will be qualitatively evaluated in the context of existing literature and our black cohosh study, with particular consideration given to the safety and efficacy of each supplement.

References
18. NIH. Workshop on the safety of black cohosh in clinical studies; 11/22/04; Bethesda, MD: NCCAM, NIH Office of Dietary Supplements; 2004.
<table>
<thead>
<tr>
<th>Item</th>
<th>Unit</th>
<th>Unit Price</th>
<th>Quantity</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Filtration units</td>
<td>12/case</td>
<td>$142.00</td>
<td>3</td>
<td>$426.00</td>
</tr>
<tr>
<td>24-well plates</td>
<td>100/case</td>
<td>$242.00</td>
<td>1</td>
<td>$242.00</td>
</tr>
<tr>
<td>T-175 flasks</td>
<td>32/case</td>
<td>$184.68</td>
<td>3</td>
<td>$554.04</td>
</tr>
<tr>
<td>RPMI-1640 media</td>
<td>500 mL</td>
<td>$15.75</td>
<td>20</td>
<td>$315.00</td>
</tr>
<tr>
<td>Fetal bovine serum</td>
<td>500 mL</td>
<td>$250.00</td>
<td>1</td>
<td>$250.00</td>
</tr>
<tr>
<td>Charcoal dextran-treated serum</td>
<td>500 mL</td>
<td>$603.00</td>
<td>1</td>
<td>$603.00</td>
</tr>
<tr>
<td>25 mL pipettes</td>
<td>200/case</td>
<td>$188.99</td>
<td>2</td>
<td>$377.98</td>
</tr>
<tr>
<td>10 mL pipettes</td>
<td>50/case</td>
<td>$29.30</td>
<td>4</td>
<td>$117.20</td>
</tr>
<tr>
<td>Aspirating pipettes</td>
<td>200/case</td>
<td>$159.00</td>
<td>2</td>
<td>$318.00</td>
</tr>
<tr>
<td>Actein</td>
<td>Black cohosh standards kit (ChromaDex), 10 mg of each</td>
<td>$782.50</td>
<td>2</td>
<td>$1,565.00</td>
</tr>
<tr>
<td>23-epi-26-deoxyactein</td>
<td>included in kit</td>
<td></td>
<td></td>
<td>$0.00</td>
</tr>
<tr>
<td>Cimicifugoside</td>
<td>included in kit</td>
<td></td>
<td></td>
<td>$0.00</td>
</tr>
<tr>
<td>Raloxifene</td>
<td>500 mg</td>
<td>$295.00</td>
<td>1</td>
<td>$295.00</td>
</tr>
<tr>
<td>Tamoxifen</td>
<td>1 g</td>
<td>$157.00</td>
<td>1</td>
<td>$157.00</td>
</tr>
<tr>
<td>Transportation to USDA</td>
<td>12 mile/round trip at $0.38/mile</td>
<td>$4.56</td>
<td>250</td>
<td>$1,140.00</td>
</tr>
<tr>
<td>Transportation to Georgetown</td>
<td>round trip metro to Georgetown</td>
<td>$6.85</td>
<td>250</td>
<td>$1,712.50</td>
</tr>
<tr>
<td>Transportation to FDA</td>
<td>16 mile/round trip at $0.38/mile</td>
<td>$6.08</td>
<td>150</td>
<td>$912.00</td>
</tr>
<tr>
<td>Office supplies/photocopying</td>
<td></td>
<td></td>
<td></td>
<td>$100.00</td>
</tr>
<tr>
<td>Postage for correspondence with Georgetown patients</td>
<td></td>
<td></td>
<td></td>
<td>$100.00</td>
</tr>
<tr>
<td>Survey data management and storage</td>
<td></td>
<td></td>
<td></td>
<td>$500.00</td>
</tr>
<tr>
<td>Graduate student for statistical consultation</td>
<td></td>
<td></td>
<td></td>
<td>$200.00</td>
</tr>
<tr>
<td>Conference to present results</td>
<td></td>
<td></td>
<td></td>
<td>$750.00</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
<td></td>
<td><strong>$10,634.72</strong></td>
</tr>
</tbody>
</table>
Budget Rationale

Filtration units, 24-well plates, T-175 flasks, RPMI-1640 media, fetal bovine serum, charcoal dextran-treated serum, and pipettes are essential materials for the cell culture portion of our project. Actein, 23-epi-26-deoxyactein, and cimicifugoside are the compounds from black cohosh that we will be testing. Raloxifene and tamoxifen are selective estrogen receptor modulators commonly used in breast cancer treatment that will be used to investigate herb-drug interactions with black cohosh. Transportation costs are a necessary part of our project, as we must be able to transport our team members to Lombardi Cancer Center to interview survey participants, to the toxicology lab at the Food and Drug Administration’s Center for Food Safety and Applied Nutrition, and to the Diet, Genomics, and Immunology lab at the U.S. Department of Agriculture’s Agricultural Research Service facility. Office supplies and postage are for the reproduction of questionnaires and participant consent forms. The fee for survey data management and storage allows us to use Georgetown University Medical Center’s data storage facility, protecting subject confidentiality by ensuring that access to personal information is limited and that personal information is properly separated from questionnaire responses. The statistical consultation will teach our team how to use appropriate statistics software to analyze our data and obtain meaningful results. Financial support for the conference will allow two or three of our team members to travel to a conference such as that held by the American Institute for Cancer Research and present our results in a professional setting.
Appendix D. Institutional Review Board (IRB) Approval

UNIVERSITY OF MARYLAND, COLLEGE PARK
Institutional Review Board
Initial Application for Research Involving Human Subjects
Please complete this cover page AND provide all information requested in the attached instructions.

Name of Principal Investigator (PI) or Project Faculty Advisor: Dr. Mark Kantor
Tel. No: 301-405-1018
(NOT a student or fellow; must be UMD employee)

Name of Co-Investigator (Co-PI): N/A
Tel. No: N/A

Department or Unit Administering the Project: Gemstone Program
E-Mail Address of PI: mkantor@umd.edu
E-Mail Address of Co-PI: N/A

Where should the IRB send the approval letter? Dr. Mark Kantor, Dept. Nutrition & Food Science, 0112 Skinner Building, Univ. Maryland, College Park, MD

Name of Student Investigator: Jessica Stevens
Tel. No: 410-562-5592
E-Mail Address of Student Investigator: jmssteve7@umd.edu

Check here if this is a student master’s thesis or a dissertation research project
Project Duration (mo/yr – mo/yr): 6/08 -- 5/10

Project Title: Herbal Dietary Supplement Use Among Breast Cancer Survivors

Sponsored Project Data Funding Agency: ORAA Proposal ID

(PLEASE NOTE: Failure to include data above may result in delay of processing sponsored research award at ORAA.)

Vulnerable Populations: The proposed research will involve the following (Check all that apply):
pregnant women ☐, human fetuses ☐, neonates ☐, minors/children ☐, prisoners ☐, students ☐, individuals with mental disabilities ☐, individuals with

Exempt or Nonexempt (Optional): You may recommend your research for exemption or nonexemption by completing the appropriate box below. For exempt recommendation, list the

☐ Exempt----List Exemption Category Or ☐ Non-Exempt

If exempt, briefly describe the reason(s) for exemption. Your notation is a suggestion to the IRB Manager and IRB Co-Chairs.

Our study involves survey administration only, which falls under exemption category #2.

Date Signature of Principal Investigator or Faculty Advisor
(PLEASE NOTE: Person signing above accepts responsibility for the research even when data

Date Signature of Co-Principal Investigator

Date Signature of Student Investigator

Date REQUIRED Departmental Signature
Name ____________________________,
Title ____________________________

(Please also print name of person signing above)
(PLEASE NOTE: The Departmental signature block should not be signed by the investigator or the student investigator's advisor.)

*PLEASE ATTACH THIS COVER PAGE TO EACH SET OF COPIES*
Instructions for Completing the Application

The Departmental Signature block should be signed by the IRB Liaison or Alternate IRB Liaison unless there is a conflict of interest. If the Department or Unit does not have an IRB Liaison, the Department Head, Unit Head or Designee should sign the application.

Please provide the following information in a way that will be intelligible to non-specialists in your specific subject area.

1. **Abstract:** Provide an abstract (no more than 200 words) that describes the purpose of this research and summarizes the strategies used to protect human subjects.

   Due to the negative side effects of conventional cancer treatments, many patients are turning to less-understood complementary and alternative medicine (CAM) therapies. One commonly available and frequently inexpensive form of CAM is herbal dietary supplements. We will investigate breast cancer patients’ use and perception of herbal dietary supplements in the Washington, D.C. metropolitan area. We will also determine the metabolic effects of commonly used supplements on cancer cells *in vitro*. Participants will be entered to win one of two fifty dollar visa gift cards. Each subject who volunteers to participate will be asked to complete the survey form. Data for participants will be stored in a locked filing cabinet located at Georgetown. An electronic copy of the survey responses data in which participants are identified only with a study number will be compiled by Georgetown employees and given to team IMAC so that no names or other identifying information will be associated with the results. If quotes of participant responses are used to illustrate key results, no identifying information will be included. Ultimately, we plan to publicize our results either through a peer-reviewed journal or through an informational campaign.
2. **Subject selection:**

   a. Who will be the subjects? How will you recruit them? If you plan to advertise for subjects, please include a copy of the advertisement.

   Subjects will be female breast cancer survivors over the age of 18 that are currently being treated at Georgetown University Medical Center. Following their regular appointment, their physician will refer them to our desk and give them general information about our project. They will be given a brochure explaining the purposes of our research and a general description of what we are studying. (attached) If they are interested, they will approach the desk and will be asked to participate in our survey.

   b. Will the subjects be selected for any specific characteristics (e.g., age, sex, race, ethnic origin, religion, or any social or economic qualifications)?

   The subjects will be female breast cancer survivors over the age of 18. The will not be selected for according to race, ethnic origin, religion, or social or economic qualifications.

   c. State why the selection will be made on the basis or bases given in 2(b).

   Participants will be female because breast cancer most commonly occurs among females. Participants must be over the age of 18 so that they may legally sign the consent form.

   d. How many subjects will you recruit?

   We hope to recruit approximately 200 subjects over the course of about 1 year.

3. **Procedures:**

   What precisely will be done to the subjects? Describe in detail your methods and procedures in terms of what will be done to subjects.

   Following their regular appointment at Georgetown University Medical Center, the subject’s physician will briefly explain our project and refer them to our desk if they are interested. Brochures will also be handed to the patients by the clinic staff when they register for their appointment. Upon approaching our desk, subjects will be given a consent form explaining the purposes of our research as well as the benefits and risks associated. If the subjects agree to participate, they will be asked to fill out a raffle form in order to be entered into our drawing for
one of two fifty dollar visa gift cards as an incentive. Subjects will be interviewed by a member of the Gemstone team for approximately 20 minutes. The interview will be a closed, fixed response interview consisting of 15 questions about their use of herbal dietary supplements during cancer treatment. (See attachment). The questions on the interview come from both the Gemstone team and researchers at Georgetown University. The researchers at Georgetown have already received IRB approval for their interview questions. Each subject will only be interviewed once. They will only be contacted by team IMAC again if they are chosen in the raffle for one of the fifty dollar visa gift cards.

How many subjects are being recruited?

Approximately 200 subjects are being recruited.

What is the total investment of time of the subjects?

The total investment time is approximately 20 minutes.

4. **Risks and Benefits:** Are there any risks to the subjects? If so, what are these risks including physical, psychological, social, legal and financial risks? Please do not describe the risk(s) as minimal. If there are known risks, please list them.

There are no known physical, social, legal, or financial risks involved in completing the questionnaire. There are both psychological and emotional risks that could result from participation in the interview. Since cancer is a very sensitive topic for those who have been personally affected by it, patients may undergo emotional discomfort and psychological distress as they recall their experiences. In some cases completing the questionnaire may cause past feelings of depression, hopelessness, and fear to reoccur. They also may feel uncomfortable discussing their experiences with the interviewer.

What are the benefits? If there are known risks associated with the subject’s participation in the research, what potential benefits will accrue to justify taking these risks?

Although this research is unlikely to benefit subjects with respect to mitigating their disease or improving their long term prognosis, there may be some possible benefits associated with participation in our study. Cancer patients will have a vested interest in furthering research that may improve cancer treatments. Patients may feel a sense of satisfaction and gratification in knowing that their survey contributions could ultimately lead to recommendations for relieving side effects associated with chemotherapy, and therefore help others who are struggling with a similar disease. The process of recalling their experiences may help them discover new ways of coping with their illness. It may also prompt them to search for treatments that are better suited to their needs, and to communicate more openly and productively with their physicians.
5. **Confidentiality:** Adequate provisions must be made to protect the privacy of subjects and to maintain the confidentiality of identifiable information. Explain how your procedures accomplish this objective, including such information as the means of data storage, data location and duration, description of persons with access to the data, and the method of destroying the data when completed.

Subjects’ names and telephone number will be recorded on raffle form. The completed survey instruments and other written documents containing information about the subjects and associated with this research project will be stored in a locked file cabinet at Georgetown University Medical Center. Employees at Georgetown will transfer the data to an electronic system at which point they will give each participant a number. This electronic data will be given to team IMAC. The names of the participant will no longer be linked with their survey, thus maintaining confidentiality. However, team IMAC will have a list of the participants’ names and phone numbers for purposes of the raffling off the incentive. The subject (s) that is chosen in the raffle drawing will be contacted by team IMAC. At UMCP, only the 12 members of the Gemstone team, the Gemstone mentor Dr. Mark Kantor, and a statistician will have access to the electronic data. Team IMAC will store the data in an electronic database using SQL that will be password protected. Following graduation of the Gemstone team and successful defense of the team’s thesis, the database will be first sent back to the Georgetown University investigators (e.g. so they can acquire any enhanced features such as summary scores and other created variables, to merge with their data) and then destroyed at UMCP by deleting the electronic files no later than December 2010.

If the research involves audio taping, videotaping or digital recordings, state who will have access to the tapes or recordings, where the tapes or recordings will be kept, and state the final disposition of the tapes or recordings (i.e. Will the tapes or recordings be destroyed? If so, when will the tapes or recordings be destroyed?).

The research does not involve audio or videotaping.

6. **Information and Consent Forms:** State specifically what information will be provided to the subjects about the investigation.

The subjects will be told that the purpose of the research is to study the attitudes, beliefs, and perceptions of herbal dietary supplements among women who are breast cancer survivors. Other specific information, including a description of the research and its purpose is provided on the consent form that the subjects will receive.

Is any of this information deceptive?
All information provided to the subjects will be straightforward and not at all deceptive.

State how the subjects’ informed consent will be obtained. Will you obtain informed
<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
</table>
| **consent in a language other than English?** | **Informed consent will be obtained with a written consent form and will only be obtained in English. All participants will be fluent English speakers.**  
**As per federal guidelines, consent forms are to be kept for at least 3 years after the completion of the study.** |
| **7. Conflict of Interest:** Describe the potential conflict of interest, including how such a conflict would affect the level of risk to the study participants. Please consult the University of Maryland policy on conflict of interest as defined by the University of Maryland Policies and Procedures III-1.11and II-3.10. These may be viewed at: http://www.usmh.usmd.edu/Leadership/BoardOfRegents/Bylaws/SectionIII/III111.html | **There is no known conflict of interest.** |
| **8. HIPAA Compliance:** State whether you are using HIPAA protected health information or “PHI”. Currently, researchers employed by the University of Maryland Center or who are working within or under the auspices of the University Health Center are subject to specific HIPAA requirements regarding the creation, use, disclosure, or access of PHI. Please consult the University of Maryland’s Summary of HIPAA’s Impact on University Research. | **HIPAA protected health information or PHI is not being used. The staff at Georgetown University are required to obtain signed HIPAA releases from every participant, under the GU-IRB requirements for their study. No PHI will be provided to the UM personnel.** |
9. **Research Outside of the United States:** Provide responses to the following questions. Separate responses are required for each country where the research will be conducted. If you are not conducting research outside the U.S., please state “Not Applicable”

Not applicable.

10. **Research Involving Prisoners:** Provide responses to the following additional IRB criteria for research involving prisoners. If you are not conducting research involving prisoners, please state “Not Applicable”

Not applicable.
## SUPPORTING DOCUMENTS

Each copy of the application should include the IRB application cover form, the information required in items 1-10 above, and all relevant supporting documents including: consent forms, letters sent to recruit participants, questionnaires completed by participants, the ORAA Internal Routing Form for Proposals (if any), and any other material germane to human subjects review. Please also include a mailing label for the IRB to send the approval notification.

## NUMBER OF COPIES

Please send 1 original application with signatures and 1 copy of the signed, original application unless your research requires full Board Review. Please submit 1 signed original application and seventeen (17) copies of any application which will require the review of the full IRB. Full Board reviews are required for initial applications involving greater than minimal risk to the subjects (i.e. more risk than subjects would generally encounter in their routine daily activities).

IRB Campus Mailing Address: 2100 Lee Building, Zip -5125.

## IRB MEETING DATES AND APPLICATION SUBMISSION DEADLINES

To view the dates for upcoming meetings and the final date for submission of applications to be considered for each meeting, please check the following URL: http://www.umresearch.umd.edu/IRB/IRBdates.html.

You may send an e-mail to irb@deans.umd.edu to inquire about the status of an application.
**CONSENT FORM**

<table>
<thead>
<tr>
<th>Project Title</th>
<th>Herbal Dietary Supplement Use Among Breast Cancer Survivors</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Why is this research being done?</strong></td>
<td>This is a research project being conducted by Gemstone Team Integrative Medicine and Cancer (IMAC) at the University of Maryland, College Park. We are inviting you to participate in this research project because you are a female breast cancer survivor over the age of 18. The purpose of this research project is to investigate the uses and perceptions of herbal dietary supplements among a group of female breast cancer survivors and test the effects and safety of the most commonly used herbal dietary supplements in a laboratory setting.</td>
</tr>
</tbody>
</table>
| **What will I be asked to do?** | The procedures involve completing a short interview with one of our team members. The interview will be completed while at Georgetown University Medical Center, will take approximately 20 minutes to complete, and will only be completed once. The questions will relate to the use of herbal dietary supplements during and after cancer treatment. They will include reasons for use, sources of information about the reliability of herbal dietary supplements, duration of use, perceived efficacy, and perceived safety. We also will ask you some basic demographic questions, such as your race and education level, so that we can describe the group of participants when we report the results of this study. Your participation in this interview will place you in a drawing to win one of two fifty dollar visa gift cards, unless you decide not to participate in the raffle. Please check one of the choices below:  
[ ] I wish to be entered into the drawing  
[ ] I do not want to be entered into the drawing. |
| **What about confidentiality?** | We will do our best to keep your personal information confidential. To help protect your confidentiality, your surveys will be kept in a locked filing cabinet at Georgetown University Medical Center. Employees at Georgetown will assign your survey form an identification number, and they will never give out other information that may personally identify you. The answers on your survey form will be given to team IMAC where it will be stored in an electronic database. The database will be password protected and will only be accessible to members of the Gemstone team, the team mentor Dr. Mark Kantor, and a professional statistician. If you decided to participate in the drawing, we will keep your name and telephone number in a separate list so that we may contact you if you are selected to receive a prize in the raffle drawing. After the drawing is over that list will be destroyed. If we write a report or article about this research project, your identity will not be revealed. The information you share with us will be |
summarized along with all of the study participants’ responses. Your information may be shared with representatives of the University of Maryland, College Park or governmental authorities if you or someone else is in danger or if we are required to do so by law.
<table>
<thead>
<tr>
<th><strong>Project Title</strong></th>
<th>Herbal Dietary Supplement Use Among Breast Cancer Survivors</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>What are the risks of this research?</strong></td>
<td>There may be some risks from participating in this research study. There are both psychological and emotional risks that could result from participation in the questionnaire. Since cancer is a very sensitive topic for those who have been personally affected by it, patients may undergo emotional discomfort and psychological distress as they recall their experiences. In some cases completing the questionnaire may cause past feelings of depression, hopelessness, and fear to reoccur.</td>
</tr>
<tr>
<td><strong>What are the benefits of this research?</strong></td>
<td>This research is not designed to help you personally, but the results may help the investigators learn more about dietary supplements that help relieve some of the side effects of chemotherapy. The benefits to you may include feeling a sense of satisfaction and gratification in knowing that your contribution may help others in the same situation. We hope that, in the future, other people might benefit from this study through improved understanding of the benefits and safety of herbal dietary supplement use during cancer treatment.</td>
</tr>
<tr>
<td><strong>Do I have to be in this research? May I stop participating at any time?</strong></td>
<td>Your participation in this research is completely voluntary. You may choose not to take part at all. If you decide to participate in this research, you may stop participating at any time. If you decide not to participate in this study or if you stop participating at any time, you will not be penalized or lose any benefits to which you otherwise qualify.</td>
</tr>
<tr>
<td><strong>Is any medical treatment available if I am injured?</strong></td>
<td>The University of Maryland does not provide any medical, hospitalization or other insurance for participants in this research study, nor will the University of Maryland provide any medical treatment or compensation for any injury sustained as a result of participation in this research study, except as required by law.</td>
</tr>
<tr>
<td><strong>What if I have questions?</strong></td>
<td>This research is being conducted by Dr. Mark Kantor at the Gemstone Program at the University of Maryland, College Park. If you have any questions about the research study itself, please contact Dr. Mark Kantor at: The University of Maryland, Dept. of Nutrition and Food Science, 0112 Skinner Building, College Park, MD 20742-7640, 301-405-1018, <a href="mailto:mkantor@umd.edu">mkantor@umd.edu</a> If you have questions about your rights as a research subject or wish to report a research-related injury, please contact: Institutional Review Board Office, University of Maryland, College Park, Maryland, 20742; (e-mail) <a href="mailto:irb@deans.umd.edu">irb@deans.umd.edu</a>; (telephone) 301-405-0678 This research has been reviewed according to the University of Maryland, College Park IRB procedures for research involving human subjects.</td>
</tr>
<tr>
<td><strong>Statement of Age of Subject and Consent</strong></td>
<td>Your signature indicates that: you are at least 18 years of age; the research has been explained to you; your questions have been fully answered; and</td>
</tr>
</tbody>
</table>
you freely and voluntarily choose to participate in this research project.

<table>
<thead>
<tr>
<th>Signature and Date</th>
<th>NAME OF SUBJECT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SIGNATURE OF SUBJECT</td>
</tr>
<tr>
<td></td>
<td>DATE</td>
</tr>
</tbody>
</table>

183
Appendix E. IRB Addendum

To whom it may concern:

We plan to modify the protocol for the project titled “Herbal Dietary Supplement Use Among Breast Cancer Survivors”. We will no longer use the survey supplement titled: “Appendix B Survey Supplement Page” and these questions have been incorporated into the survey instrument originally titled “Appendix A Survey Instrument” as noted below.

Question 4 is now formatted as a chart, rather than a series of questions. The questions numbered 5-10 originally included in Appendix B are now included in the chart for Question 4. Using a chart format will simplify survey administration by facilitating the interviewer’s recording of responses, so the interview will run more smoothly.

For question 4, we now ask whether the supplements are used specifically for medicinal purposes, used for other reasons, or not used rather than asking whether or not they are used. Since we are interested in supplement use for medicinal purposes and many of the supplements are used for other reasons, it is necessary to clarify whether the subjects’ reason for use is in fact medicinal purposes.

The supplements that we ask about in question 4 are also changed. Red clover, chamomile, milk thistle, evening primrose are included in place of blue cohosh, echinacea, garlic, and gingko. After further reviewing the most recent literature, we have decided that these supplements are more pertinent to our research since they are used more often or more specifically by breast cancer patients.

For questions 11a-11d, 15, and 16 the answer choice “don’t know/no response” is changed to two separate answer choices “don’t know” and “no response”. For questions 13 and 14a-14j, the answer choice “don’t know/no response” is changed to three separate answer choices “don’t know”, “I don’t know what this is”, and “no response”. This allows us to clarify the reason that the subject does not provide an answer for statistical and coding purposes.

The procedure for survey administration has not changed. The survey will still be administered in interview format, so that the subjects do not see the survey itself. Since the majority of the changes are formatting changes and the subjects will not see the survey, these changes do not affect the risk to the subjects. The changes in the supplements of interest do not change the risk to the subjects, as these are all common supplements similar to the original supplements of interest.

Sincerely,

Dr. Mark Kantor
Mentor, Gemstone Team IMAC
Dept. Nutrition & Food Science
0112 Skinner Building
Univ. Maryland, College Park, MD 20742-7640
301-405-1018
E-mail: TeamIMAC@gmail.com
Appendix F. IRB Renewal

UNIVERSITY OF MARYLAND COLLEGE PARK
Institutional Review Board
Renewal Application Cover Sheet

<table>
<thead>
<tr>
<th>Protocol Expiration Date</th>
<th>June 9, 2009</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protocol Number</td>
<td>08-0330</td>
</tr>
<tr>
<td>Protocol Title</td>
<td>Herbal Dietary Supplement Use Among Breast Cancer Survivors</td>
</tr>
<tr>
<td>Risk Classification</td>
<td></td>
</tr>
</tbody>
</table>

REQUIRED SIGNATURES
The Principal Investigator, Co-Investigator, and Student Investigator, in signing this renewal application, certify that they have conducted research in accordance with the IRB-approved protocol and that any consent forms used in connection with the project have been retained by the Principal Investigator unless otherwise indicated in this renewal application.
1) Participant Enrollment to Date

- This is required to ensure equitable subject selection according to the populations identified in the approved IRB protocol. If race was not collected as a demographic, please include recruitment numbers in the All Races Included Row. If gender was not collected, please include recruitment numbers under the Total Column. If race and gender were not collected, please include recruitment numbers in the All Races Included/Total cell.

No participants have been interviewed to date.

<table>
<thead>
<tr>
<th>Population (If known)</th>
<th>Adults</th>
<th>Children/Adolescents</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td>Male</td>
</tr>
<tr>
<td>All Races Included</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>White/Caucasian</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>African American</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Hispanic</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Native American</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Asian/ Pacific Islander</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Other</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

2) Data and Safety Monitoring

- Required for protocols presenting greater than minimal risk. State if project was monitored during the previous approval period and provide a summary of the reviews findings, if any.

N/A. Project presented minimal risk and was not monitored.
3) Open to Enrollment

- State if protocol will remain open to participant enrollment. If not, please state why. Also state the number of participant withdrawals, if any, and the reason for withdrawal.

The protocol will remain open to participant enrollment because no participants have been interviewed to date. This is due to complications with gaining access to our subjects at Georgetown, and not due to participant withdrawals.

4) Project Summary

- Provide a summary of the study progress to date. This should include any interim findings (positive/negative), problems encountered, goals for upcoming approval period and a projected completion date (i.e. March 2011).

There has been no progress to date with data collection. Due to complications with gaining access to subjects at Georgetown, we have decided to use alternative subjects (see section 8). During the upcoming approval period, we hope to collect at least 50 surveys and plan to complete the project by December 2009.

5) Problem History

- Provide a summary of any adverse events and/or unanticipated problems involving risks to participants or others. This should also include any participant complaints. Please discuss how these problems were handled.

There have been no adverse events or unanticipated problems involving risks to participants or others.

6) Additional Information

- Provide a summary of any relevant literature, additional risks to participation that have been identified and any other relevant information.

No other risks have been identified.

7) Deviations

- Indicate whether there were deviations to the currently IRB Approved protocol and why these occurred. A deviation is any difference in study conduct from the criteria or activities prescribed in the IRB Approved protocol, which may or may not affect the participants’ rights, safety, welfare and/or the integrity of the study.

No deviations have occurred because there have been no subject interviews yet.
8) **Request for Approval of New Changes**

- The IRB Office does not recommend submitting Addendum requests during the Continuing Review process as this will increase the turnaround time for these applications. However, if the requested modifications are minor (for example: editorial or research staff changes) please make the changes to the appropriate section of the protocol and clearly identify the changes in the box below.

<table>
<thead>
<tr>
<th>New Online Survey Protocol:</th>
</tr>
</thead>
</table>
| Due to the difficulty in obtaining an adequate number of subjects from the Georgetown clinic to complete our survey, we need to find a different source of subjects. Therefore, we have decided to administer our survey (Appendix A) to a different population. Subjects will be female breast cancer survivors over the age of 18 that currently subscribe to the website BreastCancer.net. An advertisement for our research and survey will appear on the website's home page (See Appendix E). Website users can click on the advertisement and will be brought to our website, where they will complete the survey and consent form. Patients will not be able to complete the survey until they have completed the consent form. The survey (Appendix A) will be modified slightly because questions 13 and 14 will be changed to the past tense. We feel that this makes more sense when reading the survey online, since many subjects are no longer receiving treatments for their cancer. We have also removed any interviewer instructions previously included, since the format will not be an interview. Minor changes have been made to the consent form (See Appendix C) to better explain the current project. Subjects will select a check box after reading the consent form prior to beginning the survey.
|  |
| There will be no additional risks to participants, since the survey questions have not changed. The risks may even be less, since the participants will be able to answer the questionnaire from their own home and will not experience any discomfort or emotional stress that may occur during a physical interview. The consent form will only contain a check box not a signature box. No personal identifying information will be collected on either the consent form or the survey. Each survey and consent form will contain an assigned number for identification purposes. Patients will still be able to enter the raffle previously described by completing a short information form after the survey (See Appendix F). This form contains personally identifying information, but the information will in no way be linked to their survey or consent form. The raffle will be for a $50 cash prize, not a $50 gift card as indicated in our previous IRB submission. Completed surveys will be stored in a secure file on a password protected computer in Dr. Mark Kantor’s office. |

9) **Conflict of Interest**

- Indicate if any conflict of interest (COI) issues exist that were not previously reported to the IRB Office. If there is a new COI issue, describe the potential COI, including a plan to mitigate the conflict and how the conflict may affect the level of risk to study participants. Please see the UMCP policy on COI at: http://www.usmh.usmd.edu/leadership/BoardOfRegents/Bylaws/SectionIII/III111.html

| There are no changes in the COI. |
10) **Funding Sources/Research Support**

- Provide the names of any organization, including Federal agencies, providing funding/support for the research protocol.

| The Howard Hughes Medical Institute Undergraduate Research Program has provided a grant for $5220 to support our research. The University of Maryland Gemstone Program will also be providing additional funds. |

11) **Protocol/Consent Forms**

- If changes have been made during this Renewal Application a copy of the update documents (protocol, surveys, advertisements, etc.) must be included with the application. A copy of the consent form must be submitted as well. The consent should be the version the IRB will stamp when the project is ready for approval. If more than one consent form (Parent Consent, Assent, Group A, Group B, etc.) will be used for this protocol please list them below:

| Please note that missing information and documents will result in a delay of IRB review. Please DO NOT include a copy of your IRB Approval Letter or stamped consent form with your Renewal Application. |

**New Online Survey:**

- The original survey (Appendix A) will be used with minimal modifications explained above. Questions from Appendix B were added to Appendix A. Appendix B is no longer used. We received approval for this change in January 27th, 2009.
- The original consent form (Appendix C) will be used with minimal modifications explained above.
- A new advertisement (Appendix E) will be used.
- A raffle form (Appendix F) will be used for the drawing.
- Appendix D will no longer be used.
Glossary

actein: a triterpene glycoside that is a bioactive component of the HDS black cohosh; the ingredient chosen for the focus of our lab studies

albumin: protein that carries many compounds, such as some hormones and certain drugs, through the blood; made in the liver - low albumin levels suggest liver problems

ANalysis Of VAriance (ANOVA): a basic statistical test that tests for significant differences between means

angiogenesis: the growth of new blood vessels from pre-existing ones; increased angiogenesis provides the blood supply to tumors

anti-estrogenic: having properties that block, inhibit, or decrease the effect of estrogen

black cohosh: common name for Actaea racemosa; a plant native to eastern North America, traditionally used as a Native American medicine; an HDS, often in tablet form, commonly used to alleviate hot flashes and menopausal symptoms; the chosen HDS for our mixed methods study

Bonferroni post-test: post hoc test used to determine the significant differences between group means in an analysis of variance setting

CDKN1A: a gene that encodes a potent cyclin-dependent kinase inhibitor, which regulates cell cycle progression at stage G1; plays an important role in the cell’s response to DNA damage

cell proliferation: an increase in the number cells as a result of cell growth and cell division

chamomile: the common name for several daisy-like plants which are often made into a tea that helps with sleep; an HDS traditionally used to treat many ailments such as skin inflammation, common cold, and gastrointestinal conditions; used by survey participants for its calming effects to alleviate insomnia and anxiety

chemotherapy: a common treatment for cancer using cytotoxic chemicals/drugs that target and kill rapidly dividing cells

complementary and alternative medicine (CAM): the group of diverse medical and healthcare systems, practices, and products that are not generally considered part of conventional medicine, including herbal dietary supplements for medicinal purposes
confluence: in cell culture, refers to the extent of coverage of a surface (in a dish or flask) by cells; 100% confluence indicates that the surface is completely covered by cells and there is no room for cells to grow and divide

curcumin: the principal curcuminoid and bioactive component of the Indian spice turmeric of the ginger family; an HDS that has been suggested to have therapeutic or preventive properties for many ailments; used by many survey participants to directly treat their cancer

CYPIA1: a gene that encodes a member of the cytochrome P450 superfamily of enzymes, which catalyze many reactions involved in drug metabolism; mutations to this gene have been associated with cancer risk

CYP1A1: an enzyme of the cytochrome P450 superfamily of enzymes, encoded by the gene CYPIA1 (see CYPIA1)

DCFDA assay: a test that measures levels of reactive oxygen species with fluorescence.

double-stranded DNA assay: a test that measures the levels of viable DNA in cells

Dunnett’s test: statistical test that compares all group means to the mean of the control.

endothelial cell growth: growth of the cells that line blood vessels

EROD assay: test that measures cytochromeP4501A1 activity in cells

estradiol: natural estrogen receptor agonist (activator); this molecule binds to the estrogen receptor on cells to regulate the menstrual cycle, help develop secondary sex characteristics in females, and stimulate a variety of pathways (see estrogen)

estrogen receptor negative (ER-): cells lacking estrogen receptors, which thus do not rely on estrogen for growth (see estrogen)

estrogen receptor positive (ER+): cells with estrogen receptors, which thus do rely on estrogen for growth (see estrogen)

estrogen: a group of steroid compounds that function as the primary female sex hormone; estrogen-dependent breast cancer is dependent on estrogen for growth (see estradiol)

estrogenic: having estrogen-like properties or mimicking the effect of estrogen (see estrogen)
evening primrose: common name for *Oenothera*; a genus of herbaceous flowering plants native to North and South America; used to heal asthmatic coughs, whooping cough, gastrointestinal disorders, and as a sedative pain-killer; used by survey participants to alleviate hot flashes and other symptoms of menopause

ex vivo: in scientific research, refers to experimentation done in or on tissue in an artificial environment outside the organism, as close to natural conditions as possible

Fisher LSD Test: statistical test that finds significant differences between all group means.

flaxseed: a common name for *Linum usitatissimum*, also known as common flax

ginger: the rhizome of the plant *Zingiber officinale*; a tuber originally cultivated in Asia that is consumed as a medicine or spice; an HDS used by many survey participants to treat digestive problems and nausea

ginseng: also known as Ginnsuu; any one of 11 species of slow growing plants with fleshy roots; a member of the *Panax* genus and the family *Araliaceae*; native to north eastern Asia; thought to possibly have antioxidant and anticarcinogenic properties; used by survey participants to improve cognition and treat fatigue

glyceraldehyde 3-phosphate dehydrogenase (GAPDH): a gene that encodes an enzyme in glycolysis; used as a normalizing gene in observing gene expression because it is universally expressed in cells

green tea: a type of tea, originated from China, made with the leaves of *Camellia sinensis* that have undergone minimal oxidation during processing; an HDS with limited evidence of lowering risks of heart disease, weight loss management, and prevention against cancer; used by many survey participants to directly treat their cancer and for its antioxidant properties

hepatotoxicity: a general term for liver damage that is caused by chemicals or drugs

HepG2/C3A: a clonal derivative of the HepG2 human hepatoma cell line that is commonly used in liver studies

herbal dietary supplement (HDS): An herb or herbal blend taken to maintain or improve overall wellness.

in vitro: in scientific research, refers to experimentation done in a controlled environment outside of the organism, such as a test tube or Petri dish

in vivo: in scientific research, refers to experimentation done in a whole, living organism as opposed to a partial or dead organism or an in vitro controlled environment
**integrative medicine (IM):** any healing practice that is not considered conventional medicine.

**kava kava:** common name for *Piper methysticum*; an ancient plant from the western Pacific; an HDS commonly used to relax and as a nausea remedy

**linseed:** a plant native to the region from the eastern Mediterranean to India; an HDS with a high fiber content that may possess anti-cancer properties; used by many survey participants to directly treat cancer, to help lower cholesterol, and to alleviate digestive problems

**lipid:** an organic molecule that is not soluble in water; there are many types of lipids in the body, including steroid hormones such as estrogen and estradiol

**MCF-7:** a breast cancer cell line isolated in 1970 from a 69-year-old Caucasian woman that has been the source of much current knowledge about breast cancer; an acronym of Michigan Cancer Foundation-7, the institute where the cell line was established in 1973

**menopausal symptoms:** symptoms that occur during the permanent cessation of reproductive fertility in women around the age of 50, most commonly hot flashes, night sweats, irregular periods, loss of libido, and vaginal dryness

**milk thistle:** a flowering plant in the daisy family that has thistles; native to Europe, northern Africa, and the Middle East; the seeds are often used to treat liver disease and to protect against liver toxins; an HDS used by survey participants to improve liver function

**mitochondrial membrane depolarization:** when the charge inside the cell membrane of the mitochondria becomes positive compared to the area outside of the cell

**mixed methods:** multimethodology; an approach to professional research that combines the collection and analysis of quantitative and qualitative data

**Nile Red assay:** a test that measures accumulation of phospholipids and neutral lipids in cells

**oncogenic:** cancer-causing

**oxidative stress:** a term used to describe the effect of oxidation, in which an abnormal level of reactive oxygen species lead to cell damage
**p-Glycoprotein:** a 170-KDa transmembrane glycoprotein that serves as an ATP-dependent efflux pump for a variety of chemicals; overexpression of these glycoproteins is associated with multidrug resistance

**phospholipid:** type of lipid with a polar head and a nonpolar tail that is a major part of the cell’s membrane

**polymerase chain reaction (PCR):** technique to amplify exponentially a single or few copies of a sequence of DNA, using thermal cycling and enzymatic replication of DNA

**premenstrual syndrome (PMS):** wide range of physical or emotional symptoms that occur about 5 to 11 days before a woman starts her monthly menstrual cycle, such as mood swings, cramps, swelling, bloating, food cravings, constipation, anxiety, depression, and more

**R123 assay:** test that measures mitochondrial membrane depolarization in cells

**raloxifene:** sold under the trade name Evista®; a second generation SERM that acts as an antagonist in breast cancer, taken orally, and has been found to be just as effective as tamoxifen in reducing breast cancer incidences

**reactive oxygen species (ROS):** highly reactive molecules containing an oxygen atom, which may function in cell signaling processes at lower levels and at higher levels, are capable of damage to cell structures

**real-time polymerase chain reaction (RT-PCR):** a technique based on PCR, where amplification of DNA and quantification of a targeted DNA molecule occurs simultaneously (see polymerase chain reaction)

**red clover:** the common name for *Trifolium pratense*; a species of clover native to Europe, western Asia, and northwest Africa; an HDS used to treat symptoms of menopause and to treat many other ailments; used by some survey participants to alleviate hot flashes

**rhodamine:** a group of synthetic dyes ranging in color from red to pink, obtained by condensation of phthalic anhydride with an amino derivative of phenol

**saw palmetto:** common name for *Serenoa repens*; also known as Sabal serrulatum; a small fruit bearing palm native to the southeastern United States; an HDS used to treat urinary tract infections; used by very few survey participants

**selective estrogen receptor modulator (SERM):** class of compounds that act on the estrogen receptor and have different effects in different tissues, enabling selective inhibition or stimulation in tissues; SERMs chosen for our study are tamoxifen and raloxifene
soy: common name for *Glycine max*; a member of the legume family native to East Asia; a common crop used in many foods; an HDS commonly used as a source of protein and omega-3 fatty acids; used by many survey participants to treat symptoms of menopause, especially hot flashes

**St. John's Wort:** common name for *Hypericum perforatum*, also known as Tipton's Weed or Klamath weed; an HDS commonly used to treat depression; native to temperate and subtropical regions of North America, Europe, Asia Minor, Russia, India, and China; used by many survey participants to improve mood and to alleviate anxiety

steatosis: the collection of excessive amounts of fats such as triglycerides inside liver cells

**sulforhodamine B (SRB) assay:** a technique using the red fluorescent dye sulforhodamine B to quantify cellular proteins of cultured cells

synergistic effect: the cooperative effect of two or more drugs, which is more than the additive effect

**tamoxifen:** sold under the trade name Nolvadex®; a SERM that has been used to treat breast cancer for over 30 years, by acting as an antagonist of estrogen in breast tissue

**trefoil factor 1 (TFF1):** a gene that encodes secretory proteins expressed in the gastrointestinal mucosa and in human tumors

**Tukey post-test:** statistical post-test that compares all pairs of columns following an ANOVA analysis

two-way ANOVA: statistical test that measures the effects of two factors in an experiment, simultaneously

valerian: common name for *Valeriana officinalis*; a perennial flowering plant with pink or white flowers; native to Europe and parts of Asia; an HDS prepared from the roots of the plant used to treat insomnia and sleep disorders; used by many survey participants to treat insomnia and anxiety

**vascular endothelial growth factor A (VEGFA):** protein that specifically acts on endothelial cells to increase vascular permeability, induce angiogenesis and vasculogenesis, promote cell migration and endothelial cell growth, and inhibit apoptosis

**vasculogenesis:** process of blood cell formation without the use of pre-existing cells
xenobiotic: foreign chemical in an organism, such as a drug or environmental pollutant
Works Cited


Cancer Center and the Implications for Oncology. *J Clin Oncol*, 18(13), 2505-2514.


