Biological Activities of Selected Dietary *Apiaceae*, *Lamiaceae*, and *Fabaceae*

By

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Preface

Funding of this project was provided by Dr. Humayoun Akhtar through Agriculture and Agri-Food Canada as part of their ongoing effort to examine the effect of functional foods on human health and wellness. The antimicrobial study was performed at Carleton University in Dr. Tim Xing's laboratory with assistance from Dr. Myron Smith. The Cytochrome P450 inhibition study and the MCF-7 breast cancer proliferation study were performed at the University of Ottawa with the facility and guidance of Dr. John Arnason and Dr. Brian Foster. All experiments were completed in collaboration by Master's candidate Huang Huang of Carleton University and Master's candidate San Nguyen of the University of Ottawa.

Abstract

The effect of functional foods on human cytochrome P450 (CYP) and the gut bacterial microflora may potentially affect drug metabolism and ultimately affect human health and wellness. This study examined a variety of food plants from the *Apiaceae, Fabaceae,* and *Lamiaceae* families for their inhibitory potential on cytochrome 2D6-, 3A4-, 3A5-, and 3A7-mediated metabolism. The antimicrobial effects of these samples were investigated to determine potential affects on the gut microflora. Isoflavone-rich *Fabaceae* extracts were also examined for their potential proliferative effect on MCF-breast cancer cells which are estrogen-sensitive. The highest P450 inhibitory activities were observed from extracts of celery seed, cumin, and fennel seed of the *Apiaceae* family , and basil, oregano, and rosemary of the *Lamiaceae* family. Likewise, the strongest antimicrobial activities were also observed in the *Apiaceae* and *Lamiaceae*. No significant antimicrobial and CYP inhibition was observed with the *Fabaceae* extracts. Results demonstrated the possible risk of food-drug interactions from spice and herb plants, and also the potential effect on the composition of the bacterial gut microflora, which may further affect drug disposition. Soy products from the *Fabaceae* family demonstrated their potential ability to increase the proliferation of estrogen-sensitive breast cancer cells, which may suggest that the consumption of large amount of soys and beans should be avoided in populations exposed to high breast cancer risk factors.

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List of Abbreviations

Absorbance (Abs)

Furanocoumarin (FC)

Cytochrome P450 (CYP)

Degree of Freedom (DF)

3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide (HRT)

Hormone Replacement Therapy (HRT)

Michigan Cancer Foundation (MCF-7)

Phosphate Buffer Saline (PBS)

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Chapter 1: Introduction

1.1 The increase in popularity of natural health products and functional foods

Increased health awareness has led many Canadians to become more vigilant in maintaining good health. Many Canadians have incorporated natural health products (NHPs) and functional foods (foods selected for beneficial health properties) into their daily lives to achieve optimal health and wellness. A report released by Health Canada in 2005 estimated that 71 % of the Canadian population uses NHPs on a daily basis (Murty 2007). At the same time a Canadian survey reported that 47% of those who use prescription drugs and natural health products together had adverse effects ranging from mild rashes to serious effects by those who used blood thinners, etc. (Charrois *et al.*, 2007). Both functional foods and NHPs selected for health benefits contain bioactive secondary metabolites but their roles in promoting human health has not been thoroughly studied (Gurib-Fakim *et al.,* 2006). The high levels of bioactive phytochemicals in some diets have also raised concerns about possible food-drug, NHP-drug interactions, and other adverse health effects.

1.2 The implications of Cytochrome P450 inhibition on human health and drug absorption

Cytochrome P450 (CYP) enzymes have been identified in all kingdoms of life (Roland *et al.*, 2007). CYPs comprise a superfamily of heme-thiolate proteins named for the spectral absorbance peak of their carbon-monoxide-bound species at 450 nm (Danielson *et*

al., 2002). Human CYPs are membrane-associated proteins located in the inner membrane of the mitochondria or the endoplasmic reticulum. They are present in most tissues of the body. Cytochrome p450s support the oxidative, peroxidative and reductive metabolism of such endogenous and xenobiotic substrates as environmental pollutants, agrochemicals, plant allelochemicals, steroids, prostaglandins and fatty acids. All drugs are detoxified and eventually excreted from the body, and many require bioactivation to form the active compound. CYPs are the major enzymes involved in drug metabolism and bioactivation, accounting for ~75% of the total metabolism (Guengerich *et al.,* 2008). In humans, cytochrome p450s are best known for their central role in phase I drug metabolism where they are of critical importance to two of the most significant problems in clinical pharmacology: drug interactions and individual variability in drug metabolism (Danielson *et al.,* 2002). The hepatic CYPs are the most important for metabolizing the majority of endogenous and exogenous molecules, and are the most widely studied.

Drug metabolism generally occurs via two different types of reactions. Phase I reactions, which include oxidation, reduction and hydrolysis of the substrate, and Phase II reactions involving the substrate conjugating with other molecules (e.g. glucoronidation and sulfation) to improve aqueous solubility (Kashuba *et al.,* 2001). A schematic diagram showing the mechanisms of the Phase I reaction occurring at the heme catalytic centre is shown in Figure 1 with Adrenodoxin being the electron carrier.

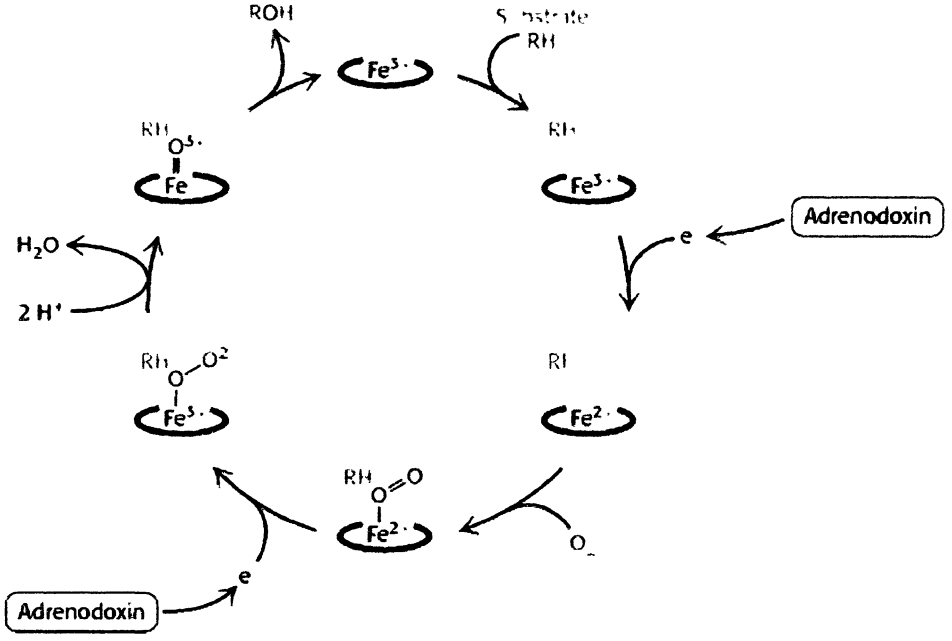


Figure 1. Phase I cytochrome P450 monooxygenase reaction with substrate RH and product ROH. (Guengerich 2008)

Therapeutic agents are metabolised mainly by CYP3A4, 2D6, 2C9 and 2C19 (Wisniewska *et al.,* 2009). CYP3A4 is the most abundant CYP expressed in human liver and small intestine and contributes to the metabolism of approximately half the drugs in use today (Guengerich *et al,,* 1999). Fourteen human families of CYP enzymes have been identified in humans, approximately 95% of all drug oxidation occurs through the action of 6 CYP enzymes: CYP1A2, CYP2C8/9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4/5 (Wrighton *et al.,* 1999).

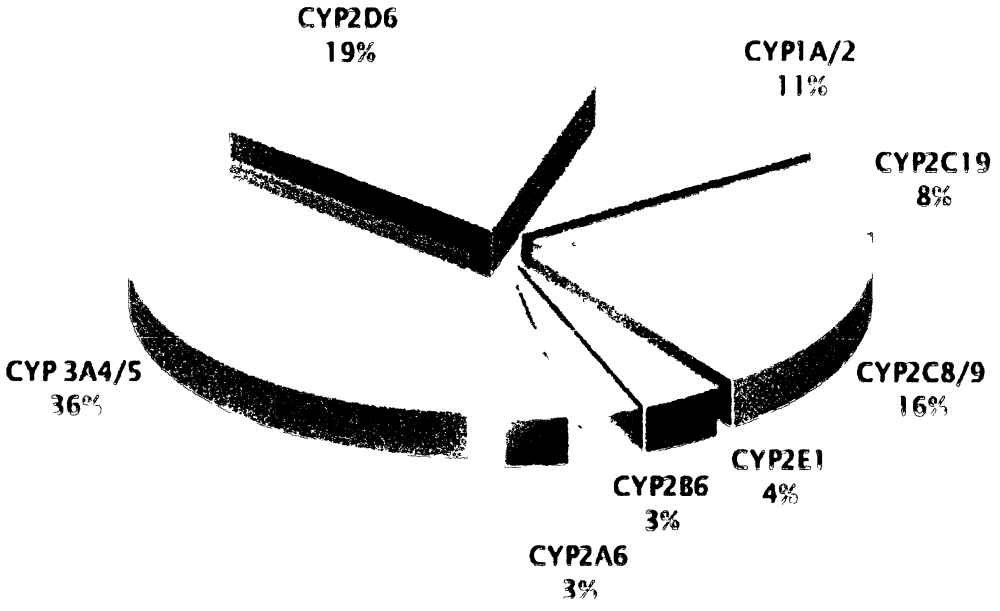


Figure 2. Portion of drugs metabolized by various cytochrome P450 isozymes (Wrighton 1992)

Naturally occurring phytochemicals found in many plants can induce or inhibit the activity of CYPs. These changes may affect the metabolism and clearance of various drugs. If these phytochemicals inhibit the CYP-mediated metabolism of a certain drug, the content of the drug may accumulate within the body to toxic levels or altered plasma causing an overdose.

The "grape juice effect" is a well documented example in which the grapefruit juice furanocoumarin bergamottin and dihydroxybergamottin are responsible for the altered metabolism of drug by mechanism-based inhibition of CYPs (Bailey *et al.*, 1998). Furanocoumarins are produced by *Rutaceae* and *Apiaceae* as a defence mechanism against herbivores such as insects and mammals. *Apiaceae* plants consumed by humans that contain high levels of furanocoumarins should be investigated for their potentially harmful effect on human health and wellness. Other plant families involved in our diet that contain significant

amount of phytochemicals, such as *Fabaceae* and *Lamiaceae*, should also be screened for potential inhibitory effect on Cytochrome P450. Both *Fabaceae* and *Lamiaceae* contain flavonoids, and recent studies have shown that flavonoid-rich plants, such as St. John's Wort, also have inhibitory and inductive effects on Cytochrome P450 (Chaudary and Willet 2006).

1.3 The antimicrobial effects of foods on human gut microflora

The human colon contains over 400 species of bacteria and these bacteria produce a wide spectrum of reductive and hydrolytic enzymes that can metabolize xenobiotics (Jain and Jain 2008). Studies have shown that small amounts of drug metabolites produced from the gut microflora could also alter the P450 enzymes and change the metabolism and toxicity of a drug in the host (Ingleman-Sundberg *et al.,* 2002). One example of the effect of the microflora on drug disposition is with the drug digoxin. A higher percentage of the North American population relative to a population from southern India converts digoxin to reduced metabolites (Nicholson *et al.*, 2005). Due to the symbiotic and mutualistic microflora, and host relationship (Sears *et al.,* 2005), variability in the composition and abundance of the gut microflora may cause variation in P450 response to drugs and toxins. Therefore, bacterial flora in the human gut can play an important role in the absorption, bioactivity and bioavailability of drugs. Foods containing secondary compounds that are antimicrobial may, therefore, alter drug activities. This may further exacerbate the pharmacological action of phytochemicals on the CYP enzymes. Aromatic plants such as *Apiaceae* that are used extensively as culinary spices have high levels of phytochemicals, such as furanocoumarins, which contribute to flavor. Many of these phytochemicals are

produced by plants to defend against herbivores and may have potential antimicrobial effect on the gut microflora when consumed.

1.4 The recent interest in phytoestrogens and breast cancer

The term phytoestrogen refers to substances produced by plants that may have an estrogenic effect on the human body. Phytoestogens can be synthesized in many plants, but their concentrations are generally too low to have any effect on the human body. Only a few plants with phytoestrogens in quantities and have identified compounds that can be used for medicinal purposes. These plants and their main phytoestrogen contents are listed in Table 1.

Table 1. Deliberate intake of phytoestrogen contained in plants or plant extracts (Wuttke et al. 2007)

Deliberately through food	Deliberately as "drugs"
Soy products - isoflavones: genistein, daidzein, formononectin, biochanin A	Soy products - isoflavones
Alfalfa - coumestrol	Red clover - isoflavones, coumestans
Flax seed - lignan: enterodiol, enterolactone	
Hops (beer) - 8-prenylnaringenin	
Grapes (red wine) - resveratrol	

At present, most phytoestrogens identified are not retailed as commercial products in isolated or enriched form. Only isoflavones isolated from soy products and red clover are marketed as food supplements and foods for special medicinal purposes. These products are freely available in pharmacies, health food shops, supermarkets, and via the internet (Eisenbrand *et al.,* 2007).

Isoflavones are heterocyclic phenols with structure similarity to estrogen. All flavonoids share a basic structure consisting of two benzene rings (A and B) linked through a heterocyclic pyrone C ring. The benzenoid B ring position of the isoflavones is in the 3-position, which is different from the 2-position of flavones. (Yuan *et al.,* 2006) Isoflavones do not share a common biosynthetic origin with estrogen, but they do possess two structural features that resemble estrogens: (1) an aromatic A ring with a hydroxyl group and (2) a second hydroxyl group in the same plane of the A ring. (Migliaccio *et al.,* 2003) The structures of the four main isoflavone compounds are shown in Figure 3 along with the structure of estradiol-17β. In biochanin A and formononetin, the hydroxyl group on the A ring is replaced with a methoxy group.

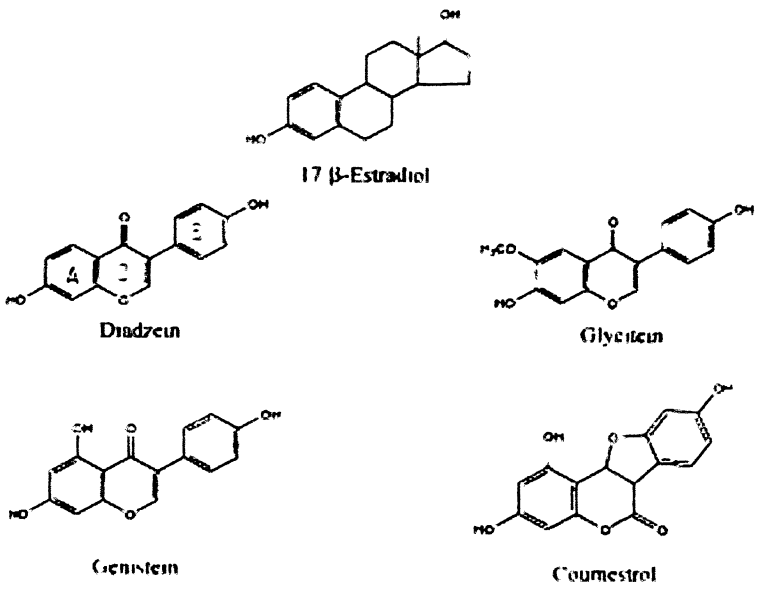
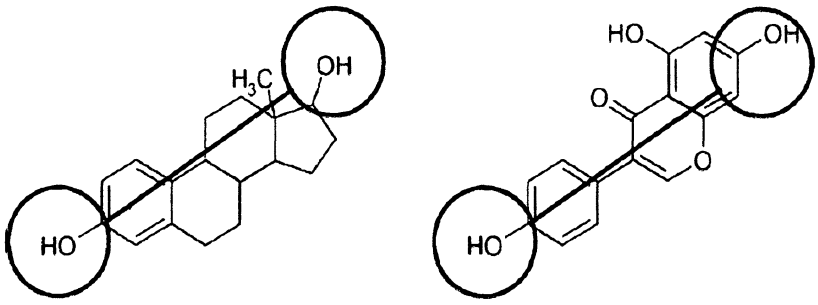


Figure 3. Chemical structure of the isoflavones, genistein, daidzein, coumestrol, and glycitein and estradiol-17β. The A, B, and C rings are indicated in daidzein.

Another important feature is the intramolecular distances between the hydroxyl groups at each end of the molecules in particular being almost identical (Figure 4). These distances determine hydrogen bond interaction with amino acids of the ligand-binding site of the estrogen receptor (Vaya *et al.,* 2004). Both estradiol-17β and genistein have aromatic rings with an OH group in the C3 position and another OH group at an identical distance from the former in Figure 4.



Estradiol 17ß Genistein

Figure 4. The structural similarity between estradiol-17β and genistein.

In Asian countries, such as Japan and China, the incidence of breast cancer is only one-third of North America and Europe and their dietary intake of phytoestrogens, mainly from soy, can produce circulating phytoestrogens and produce estrogenic effects. This led many people to believe that phytoestrogen is a possible replacement for the controversial hormone replacement therapy. Hormone replacement therapy was popular for treating many post-menopausal problems but was associated with an increase risk of breast cancer in women. The Women's Health Initiative (WHI) safety committee decided to interrupt an estrogen replacement therapy study after a 5 year follow-up, because women on the estrogen therapy with synthetic estrogen had an increased risk for breast cancer (Rossouw *et al.*, 2002). In the Million Women Study performed in the UK between 1996 and 2001, over a million women on hormone replacement therapy were recruited and asked about their use of synthetic estrogen. This study found that the risk for developing breast cancer is much higher for women on combined estrogen-progestin therapy than for other types of HRT (Welty *et al.*, 2007). The risk of breast cancer also increased with the duration of the HRT. After publication of these results, many women decided to stop taking synthetic estrogen drugs and started to look at phytoestrogens as a more natural alternative. (Beck *et al.*, 2004)

Although some results are contradictory and remain elusive, the majority of the experimental results and clinical trials show that commercially available isoflavone preparations and soy products have multiple beneficial effects on different disorders relating to estrogen deficiency. Increased isoflavone content in diet was shown to reduce the frequency and severity of hot flushes in menopausal women (Albertazzi 2006). The effectiveness of isoflavone is reversing the effects of osteoporosis was demonstrated by the increase in bone mineral density, alkaline phosphatase, and insulin-like growth factor 1 in menopausal women treated with soy-rich diet (Mazhar *et al.,* 2005; Laya *et al.,* 1995).

With the soaring popularity, interest, and demand for isoflavones, it is very important to understand the potential harmful effects of these products in our diet. This information is especially important for the population exposed to high breast cancer risk, i.e. post-menopausal women, because this will be the same population that are actively seeking phytoestrogen therapy to alleviate the other symptoms associated with estrogen deficiency. Despite the epidemiological studies that favour the consumption of soy products in breast cancer patients and high-risk populations, conflicting experimental data exist in using isoflavones to treat or prevent breast cancer (Jiang *et al.,* 2008; Yellayi *et al.,* 2002). More investigations into the effects of isoflavones are required to qualify soy products as a potential safe replacement for current HRT drugs.

1.5 Objectives and Hypotheses

To broaden understanding of effects of food on human health and drug-food interactions, this study examined a priority group of pulses, spices and herbs on the Canadian

market selected by Agriculture and Agri-Food Canada (AAFC) to determine their potential risk for inhibiting human CYP enzymes (Table 2) and affecting selected gut microflora. Samples were selected from the *Fabaceae* which contain isoflavones, *Apiaceae* which contain furanocoumarins, and *Lamiaceae* which contain monoterpenes, and tested for potential inhibition against CYP2D6, CYP3A4, CYP3A5 and CYP3A7.
Seven representative gut bacterial genera were selected for the antimicrobial screening. The isoflavone-rich *Fabaceae* products were also tested for proliferative properties on breast cancer cells. By testing many common food samples, the assessment of potential food-drug interactions, antimicrobial activities, and phytoestrogenic effect of phytochemicals across a broad spectrum of diets and therapeutic use of functional foods was achieved. These objectives will test three hypotheses:

1. Food plants containing high levels of phytochemicals and secondary metabolites may have antimicrobial effect on the human gastrointestinal microflora which may potentially affect drug metabolism and human health and wellness.

2. These antimicrobial phytochemicals may have multiple biological effects on the human body and may inhibit the activity of cytochrome P450 enzyme and the inhibitory effect will correlate with the antimicrobial effect.

3. Food plants from the *Fabaceae* family rich in isoflavones may possess phytoestrogenic effect and potentially proliferate estrogen-sensitive breast cancer cells.

Chapter 2: Cytochrome P450 inhibitory effects of *Apiaceae* and *Lamiaceae*

2.1 Cytochrome P450 and drug metabolism

Functional foods and NHPs contain bioactive compounds that are metabolized by cytochrome P450 enzymes (CYP) and may affect drug metabolism thereby resulting in a higher plasma concentration of xenobiotics and drugs and thus causing adverse drug reaction. Some of the major CYP enzymes involved in the metabolism of these products are CYP2D6, CYP3A4, CYP3A5 and CYP3A7. It is well established that grapefruit juice can cause interaction with conventional drugs (Bailey *et al.,* 1998; Bailey *et al.*, 2000). It was found that furanocoumarins (FC) from grapefruit juice were responsible for mechanism-based inhibition of CYP activity, 6′,7′-dihydroxybergamottin being one of several FCs identified (Paine *et al.,* 2006). Recent studies have also reported that star fruit and pomegranate juice may also inhibit drug metabolism (Zhang *et al.,* 2007; Faria *et al.,* 2007). The three plant families selected for this study: *Apiaceae* food plants, rich in furanocoumarins, and *Lamiaceae* and *Fabaceae* food plants rich in flavonoids, are of special interest for investigation into the potential of food-drug interaction due to their high level of phytochemicals. The objective of the cytochrome P450 assay is to assess the potential inhibitory effect of these food plants containing high levels of phytochemicals on the activities of four major CYP isozymes: CYP3A4, CYP3A5, CYP3A7, and CYP2D6.

2.2 Materials and Methods

2.2.1 Sample collection

All samples were obtained from local supermarkets or farms in the Ottawa (ON, Canada) or Guelph (ON, Canada) areas. Each sample was given a Nutraceutical Research Program (NRP) number and all pertinent information such as mass, company name, origin and place of purchase was recorded (see full description and full genus names in main text reference) (Table 2). Each sample was weighed and divided into three portions. One portion was stored permanently at -20 °C for archiving at the University of Ottawa Herbarium and the remaining two portions were ground to a fine powder using a Thomas-Wiley industrial grinder with a 1mm pore industrial grade steel mesh filter for consistency. One of the two portions of ground material was then stored for long term use at -20 °C until required and the last portion was stored at -4 °C for daily extractions.

2.2.2 Sample selection

Samples were selected from three plant families: *Fabaceae, Apiaceae,* and *Lamiaceae*. Plants in these families are used extensively in popular diet both as staple foods in large amounts (e.g. beans), or cooking spices in small amount for flavour (e.g. coriander). There were a total of 46 samples used in this study, including 6 *Apiaceae*, 3 *Lamiaceae*, and 37 *Fabaceae*.

Fabaceae, also called *Leguminosae*, is a large and economically important family of flowering plants. *Fabaceae* species can grow in many different environments and are found throughout the world as very important agricultural plants. Popular food plants such as *Glycine max* (soybean), *Pisum sativum* (pea), and *Phaseolus* (bean) were included in this study. Known to contain high levels of phytoestrogen isoflavones, the focus on this family is to investigate their potential proliferative effect on breast cancer cells.

Apiaceae, also called *Umbelliferae* because of the umbrella shape of the plant inflorescence, is a family of aromatic plants. Many *Apiaceae* plants are used as cooking spices for their flavour. They contain high levels of phytochemicals such as furanocoumarins, which are known inhibitors of CYPs. Some notable species, such as *Anthethum graveolens* (dill), *Cuminum cyminum* (cumin), and *Foeniculum vulgare* (fennel), were included in this study.

Lamiaceae, also known as the mint family, are also aromatic and include many widely used culinary herbs. Many *Lamiaceae* plants are widely cultivated around the world not only due to their flavour but also their ease of cultivation. Popular culinary herbs such as *Rosemarinus officinalis* (rosemary), *Ocimum basilicum* (basil), and *Origanum vulgare* (oregano) were included in this study. All samples selected are listed below in Table 2.

Table 2. Products selected by Agriculture and Agri-Food Canada for their potential to affect human cytochrome P450-mediated metabolism and their assigned NRP No. (Nutraceutical Research Program number).

NRP No.	Botanical Name	Common Name	Family	Country of origin
320	*Glycine max*	Soybean	*Fabaceae*	Canada

321	*Glycine max*	Soybean	*Fabaceae*	Canada
322	*Glycine max*	Soybean	*Fabaceae*	Canada
323	*Glycine max*	Soybean	*Fabaceae*	Canada
324	*Glycine max*	Soybean	*Fabaceae*	Canada
325	*Glycine max*	Soybean	*Fabaceae*	Canada
326	*Glycine max*	Soybean	*Fabaceae*	Canada
327	*Glycine max*	Soybean	*Fabaceae*	Canada
328	*Glycine max*	Soybean	*Fabaceae*	Canada
329	*Glycine max*	Soybean	*Fabaceae*	Canada
330	*Glycine max*	Soybean	*Fabaceae*	Canada
331	*Glycine max*	Soybean	*Fabaceae*	Canada
335	*Phaseolus vulgaris*	Black Bean	*Fabaceae*	Imported
313	*Phaseolus vulgaris*	Black Turtle Bean	*Fabaceae*	Canada
314	*Phaseolus vulgaris*	Cranberry Bean	*Fabaceae*	Canada
356	*Phaseolus vulgaris*	Great Northern Bean	*Fabaceae*	USA
315	*Phaseolus vulgaris*	Dark Red Kidney Bean	*Fabaceae*	Canada
316	*Phaseolus vulgaris*	Light Red Kidney bean Var. A	*Fabaceae*	Canada
317	*Phaseolus vulgaris*	Light Red Kidney bean Var. B	*Fabaceae*	Canada
318	*Phaseolus vulgaris*	White Kidney bean Var. A	*Fabaceae*	Canada
319	*Phaseolus vulgaris*	White Kidney bean Var. B	*Fabaceae*	Canada
339	*Phaseolus vulgaris*	White Kidney bean Var. C	*Fabaceae*	Imported
354	*Phaseolus vulgaris*	White Kidney bean Var. D	*Fabaceae*	Imported
337	*Phaseolus vulgaris*	Navy Bean	*Fabaceae*	Imported
357	*Phaseolus vulgaris*	Pinto Bean	*Fabaceae*	Canada/USA
358	*Phaseolus vulgaris*	Small Red Bean	*Fabaceae*	Canada/USA
355	*Lens culinaris*	Eston Lentil	*Fabaceae*	Imported
350	*Lens culinaris*	Green Lentil	*Fabaceae*	Canada
359	*Lens culinaris*	Red Lentil	*Fabaceae*	Canada
336	*Phaseolus. lunatus*	Lima Bean	*Fabaceae*	Imported
351	*Pisum sativum*	Green Pea	*Fabaceae*	Canada
352	*Pisum sativum*	Yellow Pea	*Fabaceae*	Canada
338	*Pisum sativum*	Yellow Split pea	*Fabaceae*	Imported
332	*Vigna unguiculata*	Black Eyed Pea	*Fabaceae*	Imported
334	*Vigna unguiculata*	Cow Pea	*Fabaceae*	Imported
353	*Cicer arietinum*	Chick pea	*Fabaceae*	Canada
333	*Cicer cayan*	Congo Pigeon pea	*Fabaceae*	Imported
341	*Apium graveolens*	Celery seed A	*Apiaceae*	Imported
342	*Apium graveolens*	Celery seed B	*Apiaceae*	N/A
343	*Coriandrum sativum*	Coriander	*Apiaceae*	N/A
344	*Cuminum cyminum*	Cumin	*Apiaceae*	N/A
345	*Anethum graveoLens*	Dill	*Apiaceae*	N/A
346	*Foeniculum vulgare*	Fennel seed	*Apiaceae*	N/A
340	*Ocimum basilicum*	Basil leaves	*Lamiaceae*	Imported
347	*Origanum vulgare*	Oregano leaves	*Lamiaceae*	Imported
348	*Rosemarinus officinalis*	Rosemary	*Lamiaceae*	Imported

2.2.3 Sample extraction

To prepare stock extracts of each sample, a dry weight of 50 mg/mL aliquot was mixed with 80 % aqueous methanol (v/v) in a 2 mL centrifuge tube and blended on a Fisher Vortex Genie 2 at maximum settings for 2 minutes. The sample was then centrifuged in a Micro12 Centrifuge (Fisher Scientific, Ottawa, ON, Canada) at 13,000 *g* for 20 minutes. The supernatant was stored in an opaque container at -4 °C. Aqueous and ethanol samples were prepared as described above. Extracts were freshly prepared the same day for each assay.

2.2.4 Chemicals and reagents

CYP enzymes 3A4 (Human CYP3A4 + reductase, 1 nM, 500 µL – Cat# 456207), 3A5 (Human CYP3A5 + reductase, 1 nM, 500 µL – Cat# 456235), 3A7 (Human CYP3A7 + reductase + b5, 0.5 nM, 500 µL – Cat# 456237), 2D6 (Human CYP2D6*1 + P450 reductase supersomes – Cat# 455117), dibenzylfluoroscein (DBF), and 3-[2-(N,N-diethyl-N-methylamino)ethyl]-7-methoxy-4-methylcoumarin (AMMC) were obtained from Gentest (Franklin Lakes, NJ, USA). All enzymes were stored at -80 °C until required. NADPH (β-NADPH reduced tetrasodium salt hydrate – Cat# N7505-1GR), was from Sigma Aldrich (Oakville, ON, Canada) and stored at -20 °C under very low light conditions. Ketoconazole was purchased from Calbiochem (Gibbstown, NJ, USA). Methanol was purchased from Fisher Scientific Canada (Ottawa, ON, Canada).

2.3 Fluorometric microtitre cytochrome P450 inhibition assays

A fluorometric microtitre plate assay was used to assess the inhibitory capacities of the plant extracts against cytochrome P450 CYP3A4, 3A5, 3A7 and 2D6. The procedure used was adapted and modified from Crespi *et al.* (1997) and Scott *et al.* (2006). The assays were performed in 96-well plates with white walls and clear, flat bottoms under red-coloured light to minimize the exposure of fluorescent light to photosensitive material (*i.e.* NADPH, quinidine, substrates, extracts). The fluorescence was measured using a Cytofluor 4000 Fluorescence Measurement System (Applied Biosystems, Foster City, CA, USA). The percent inhibition for each extract was calculated relative to the CYP activity in the presence of the vehicle control. A 10 µL aliquot of each extract, at a concentration of 50 mg/mL, was tested in triplicates for all assays. All extracts were freshly made on experimental days and the remainders discarded.

Wells were designated as "control," "control blank," "sample," or "sample blank." The control represented the MeOH vehicle control, whereas the sample represented the extract or positive control. Solution A contained 1.08 mM NADPH and the substrate in 0.25 M potassium phosphate buffer solution, pH 7.4. Solution B contained the CYP in the 0.13 M buffer solution. Solution C was identical to Solution B but instead contained denatured CYP rather than active enzyme ("blank"). A volume of 100 µL of Solution A was added to each well followed by the addition of 10 µL of the extract. Enzyme was thawed prior to its addition to Solution B or C and a 90 µL aliquot of this mixture which was immediately added to each well. The plate was shaken for three seconds, and the initial fluorescence was measured at excitation and emission wavelengths depending on the

substrate and product, respectively, as described below. The plate was then incubated at 37 °C for 20 to 40 minutes depending on the enzyme tested and then final fluorescence was measured.

The concentration of CYP3A4, 3A5, and 3A7 used was 10 μM with DBF as a substrate at concentrations of 1 μM. The positive inhibitor used was ketoconazole at a concentration of 1.9 μM. Samples were read at excitation wavelength of 485 nm and an emission wavelength of 530 nm with gain set at 50. The concentration of CYP2D6 used was 10 μM with AMMC as a substrate at a concentration of 0.12 μM and quinidine as a positive inhibitor at a concentration of 2 μM. The samples tested against CYP2D6 were read with excitation wavelength of 409 nm and emission wavelength of 460 nm with gain set at 50. The incubation time was 20 minutes for CYP3A4 and 3A5 assays and 40 minutes for CYP3A7 and 2D6 assays.

2.4 Results

A total of 46 food samples were examined in this study: 37 *Fabaceae*, 6 *Apiaceae*, and 3 *Lamiaceae*. The CYP inhibitory potential of each sample was categorized as low (<35%), moderate (35-70%) and high (>70%) inhibition. *Apiaceae* and *Lamiaceae* methanolic extracts had the highest CYP3A4 inhibition (Figure 5). Celery seed (var. A), coriander, cumin, and fennel seed of the *Apiaceae* and oregano and rosemary of the *Lamiaceae* inhibited CYP3A5 by over 85%. Among the *Fabaceae*, soybean samples had low to moderate CYP3A4 inhibition. Remaining *Fabaceae* samples displayed low to moderate inhibition with the exception of light red kidney bean (var. B) and yellow pea

having the highest inhibition of 41.2 ± 8.2 % and 44.4 ± 8.8 %, respectively. Aqueous extracts had high inhibition (75-100 %) values in several samples (Figure 6). Fennel seed, cumin, and celery seed (var. A), rosemary, oregano and basil all displayed high levels of inhibition. Soybean samples all moderately inhibited CYP3A4. The remaining *Fabaceae* samples had low to moderate inhibition with the exception of white kidney bean (var. B) having stronger inhibition at 93.6 ± 8.4 %.

Apiaceae and *Lamiaceae* methanolic extracts had high inhibitory levels towards CYP3A5 (Figure 7). Among the *Fabaceae*, soybean samples moderately inhibited CYP3A5 whereas the remaining *Fabaceae* samples displayed low inhibitory levels. Aqueous extracts displayed lower inhibition values in numerous samples (Figure 8). The highest levels of inhibition were observed in cumin, celery seed (var. A) and celery seed (var. B), which were relatively moderate in comparison to the activity observed in the methanolic extracts. Rosemary and oregano were the most active, inhibiting at 99.6 ± 0.5 % and 74.9 ± 3.4 %, respectively. Remaining *Fabaceae* samples had low to moderate inhibition.

In regards to CYP3A7, the *Apiaceae* and *Lamiaceae* methanolic extracts had the highest inhibition as seen previously in CYP3A5 (Figure 9). The highest levels of inhibition were observed with fennel seed, cumin, and celery seed (var. A). Rosemary and oregano and basil also displayed high inhibitory levels. Soybean samples inhibited CYP3A7 at moderate and high levels whereas the remaining *Fabaceae* inhibited at low to moderate levels. The aqueous extracts generally had lower CYP3A7 inhibition values (Figure 10). High levels of inhibition were observed in fennel seed, dill, and celery seed (var. B). Basil oregano and rosemary had moderate inhibition. The activity levels seen in

soybean and the remaining *Fabaceae* samples were low to moderate and were more similar against the CYP3A7 than previously observed in the CYP3A4 and 3A5 assay.

As previously highlighted from the results of CYP3A4, 3A5 and 3A7 data, the *Apiaceae* and *Lamiaceae* methanolic extracts had the highest CYP2D6 inhibition (Figure 11). The highest levels of inhibition were observed in celery seed (var. A), celery seed (var. B), coriander, oregano and rosemary. Interestingly soybean samples had very low inhibition on CYP2D6. The remainder of the *Fabaceae* extracts also had low inhibition with the exception of light red kidney bean (var. B) which inhibited at 94.2 ± 5.6 %. Aqueous extracts were similar to methanolic extracts (Figure 12). The highest levels of inhibition were observed in celery seed (var. A), dill, coriander, rosemary and oregano. The aqueous extracts of basil, on the other hand, had a rather moderately low inhibition activity. Soybean and remaining *Fabaceae* samples also displayed low to moderate levels of inhibition.

Ketoconazole (3A4, 3A5, 3A7) and quinidine (2D6) were used as positive controls and inhibited the respective CYPs at 100%. Results for positive controls were not shown.

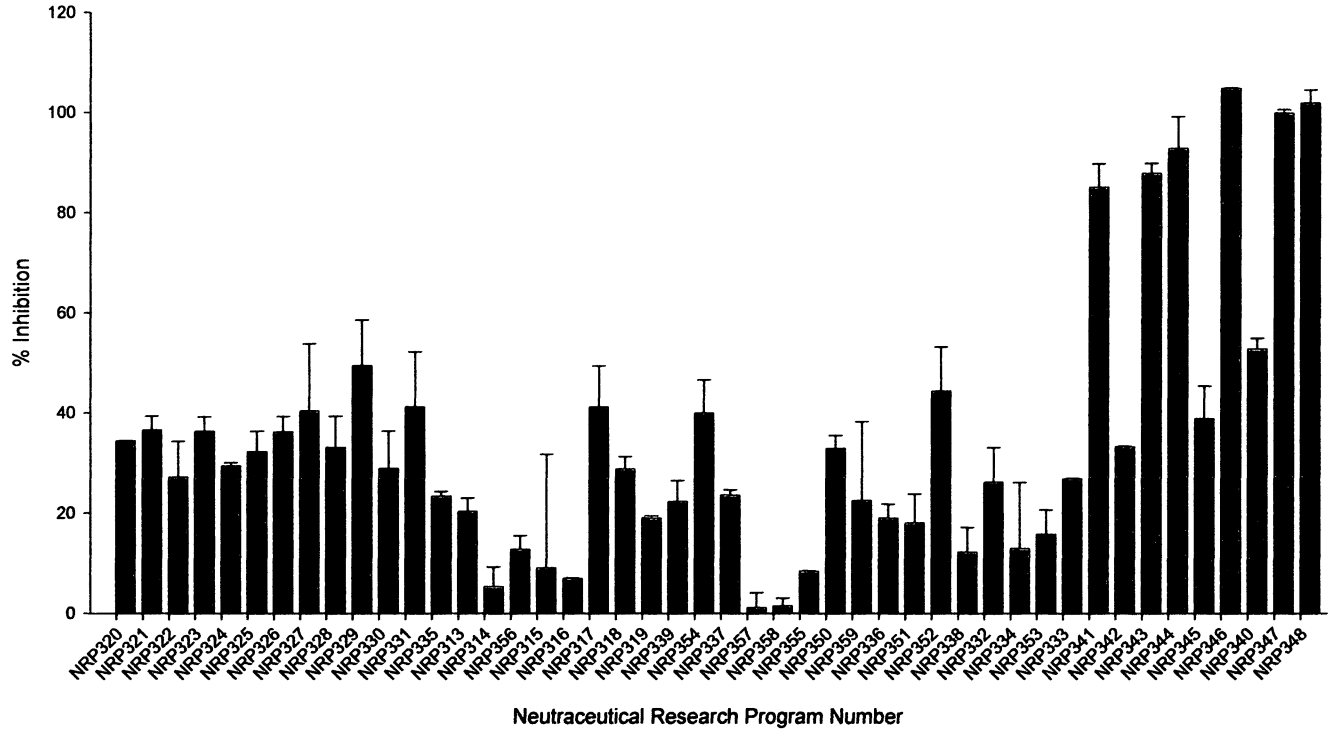


Figure 5. Percent inhibition of methanolic extracts (50 mg/mL) from common food samples on cytochrome P450 3A4 isozyme. Values are presented as means ± standard deviation of extracts tested in triplicates and repeated twice. 46 plants from the *Fabaceae* (red), *Apiaceae* (green), and *Lamiaceae* (blue) families are shown

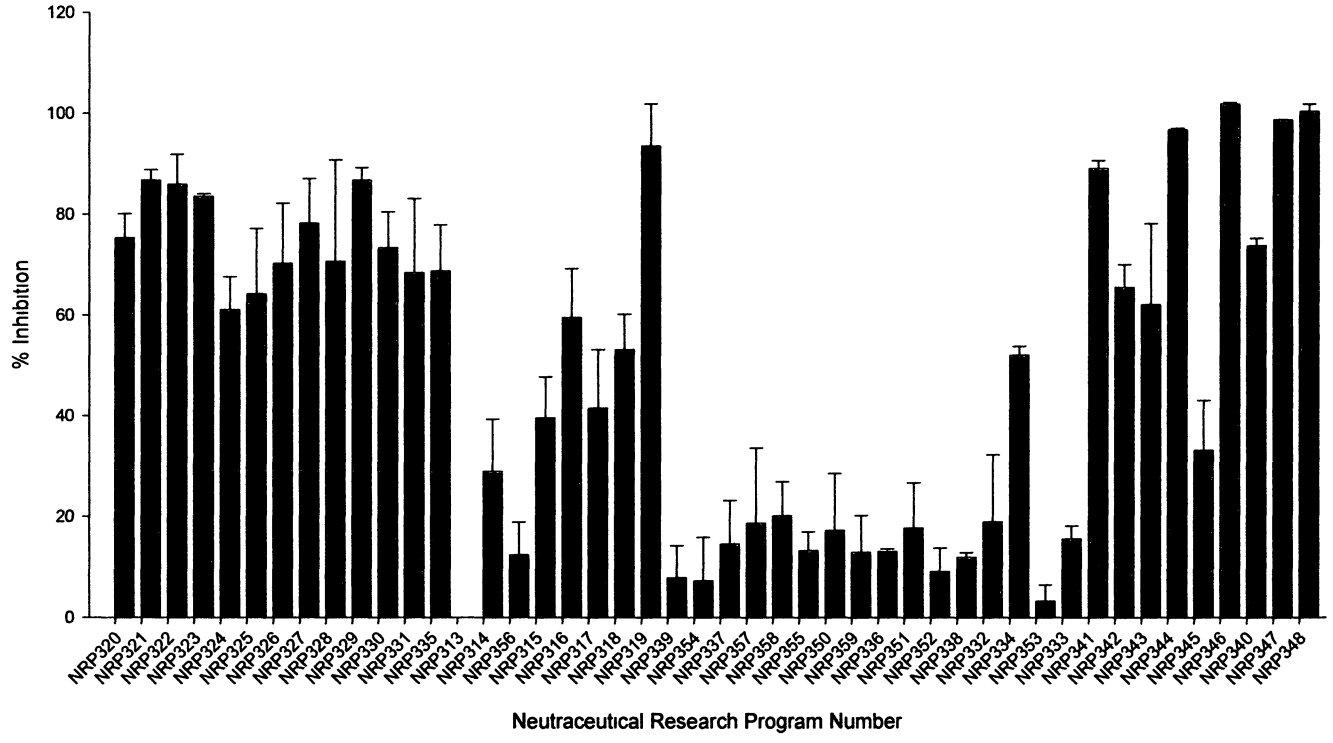


Figure 6. Percent inhibition of aqueous extracts (50 mg/mL) from common food samples on cytochrome P450 3A4 isozyme. Values are presented as means ± standard deviation of extracts tested in triplicates and repeated twice.

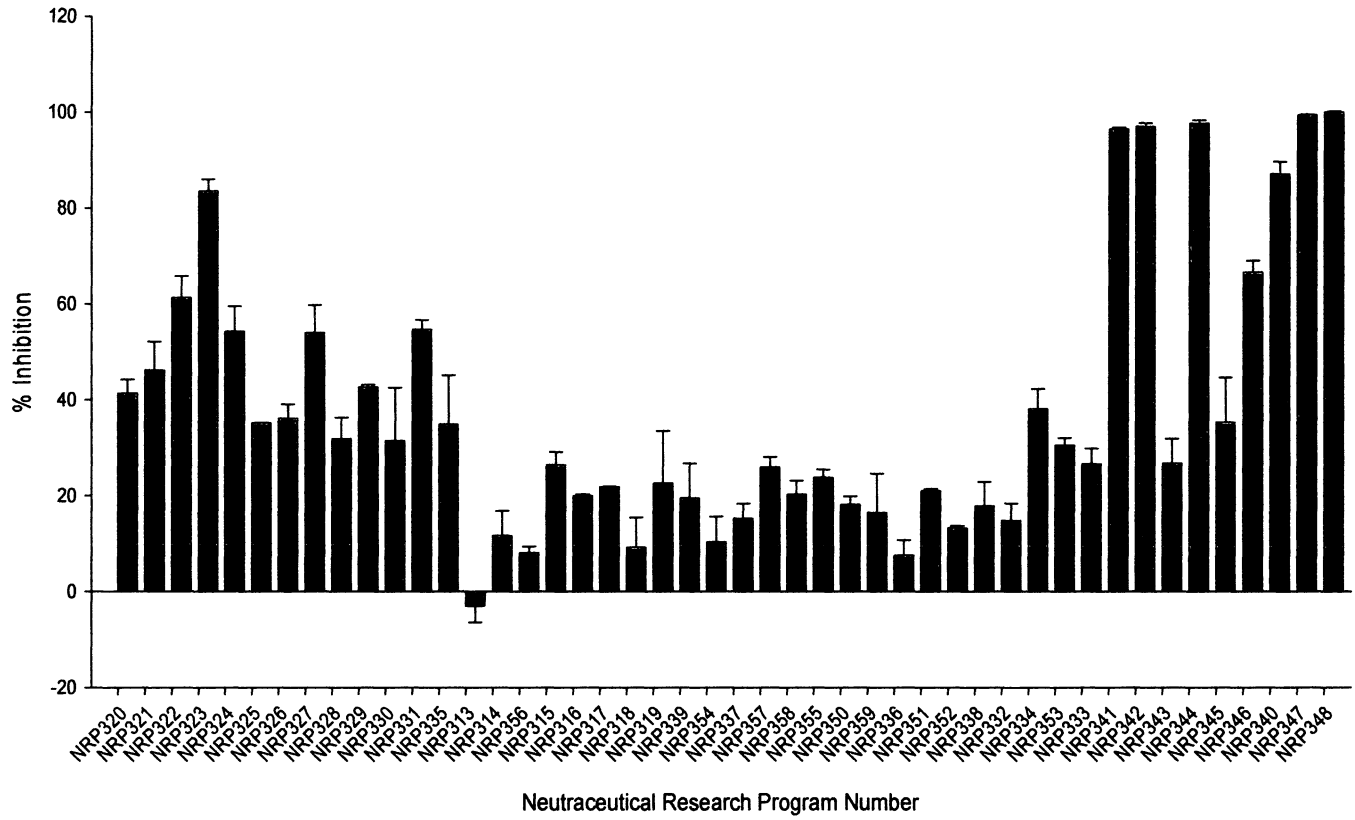


Figure 7. Percent inhibition of methanolic extracts (50 mg/mL) from common food samples on cytochrome P450 3A5 isozyme. Values are presented as means ± standard deviation of extracts tested in triplicates and repeated twice.

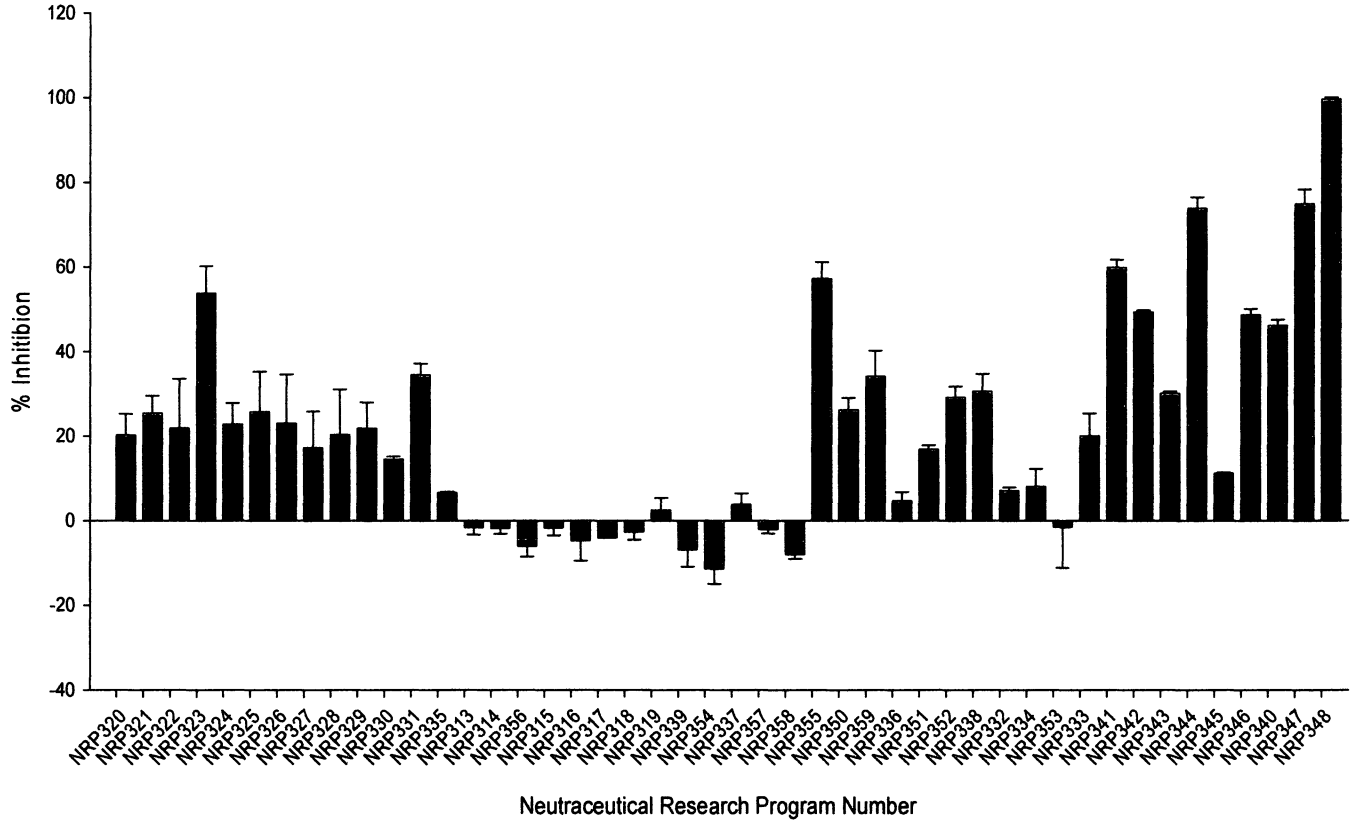


Figure 8. Percent inhibition of aqueous extracts (50 mg/mL) from common food samples on cytochrome P450 3A5 isozyme. Values are presented as means ± standard deviation of extracts tested in triplicates and repeated twice.

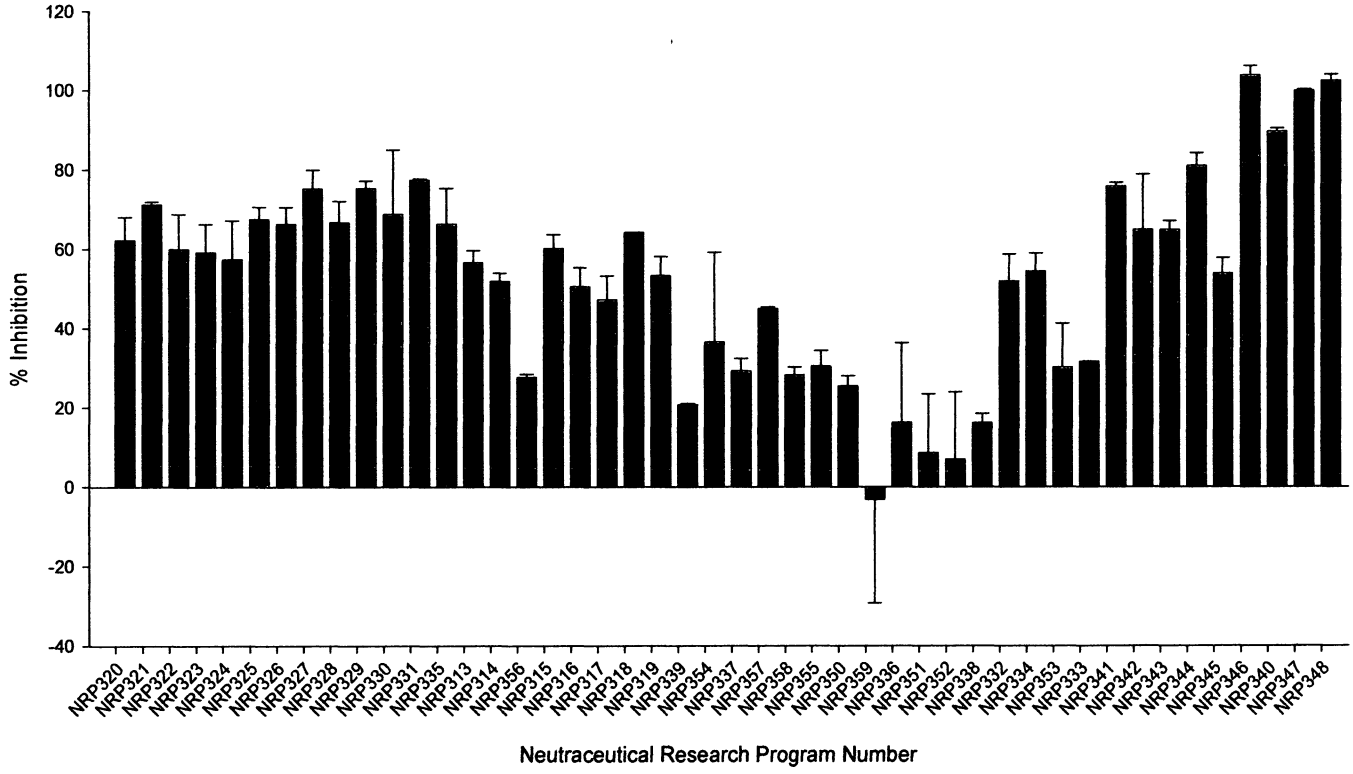


Figure 9. Percent inhibition of methanolic extracts (50 mg/mL) from common food samples on cytochrome P450 3A7 isozyme. Values are presented as means ± standard deviation of extracts tested in triplicates and repeated twice.

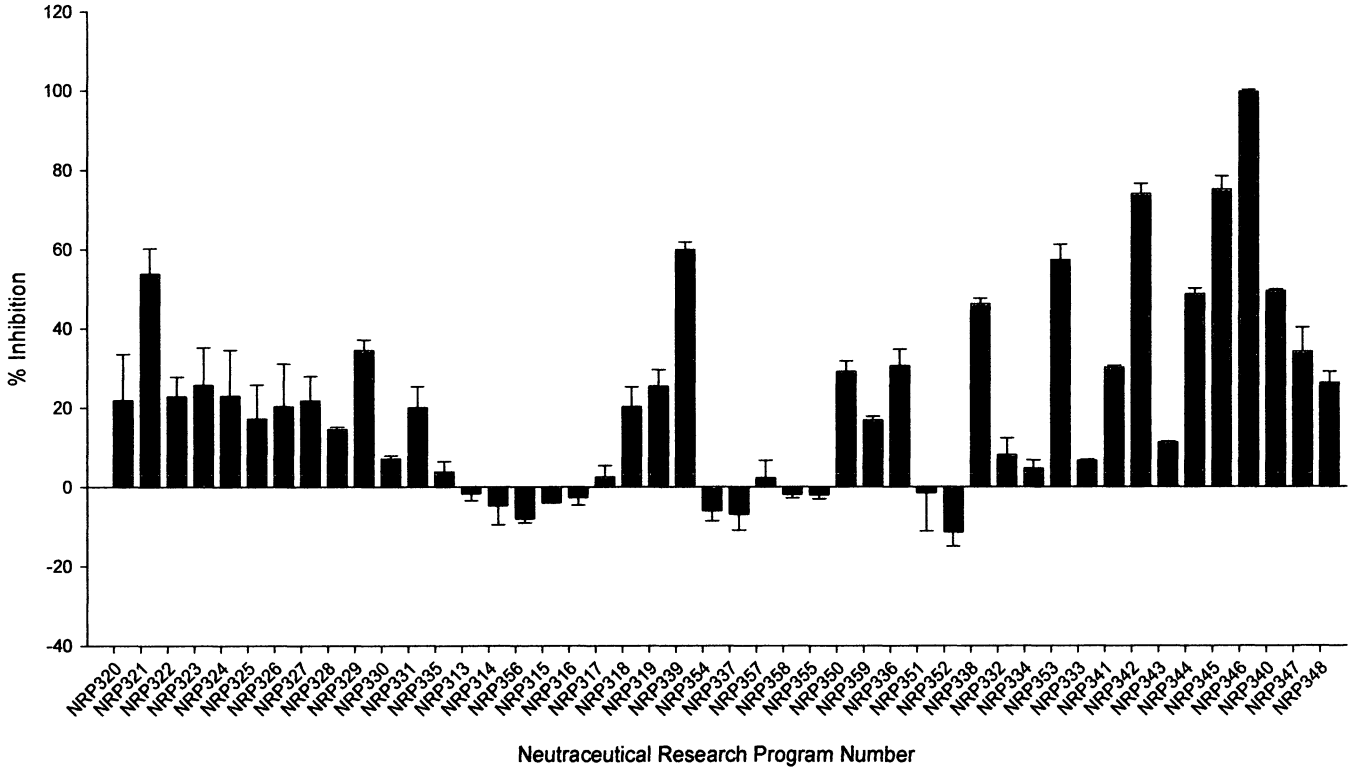


Figure 10. Percent inhibition of aqueous extracts (50 mg/mL) from common food samples on cytochrome P450 3A7 isozyme. Values are presented as means ± standard deviation of extracts tested in triplicates and repeated twice.

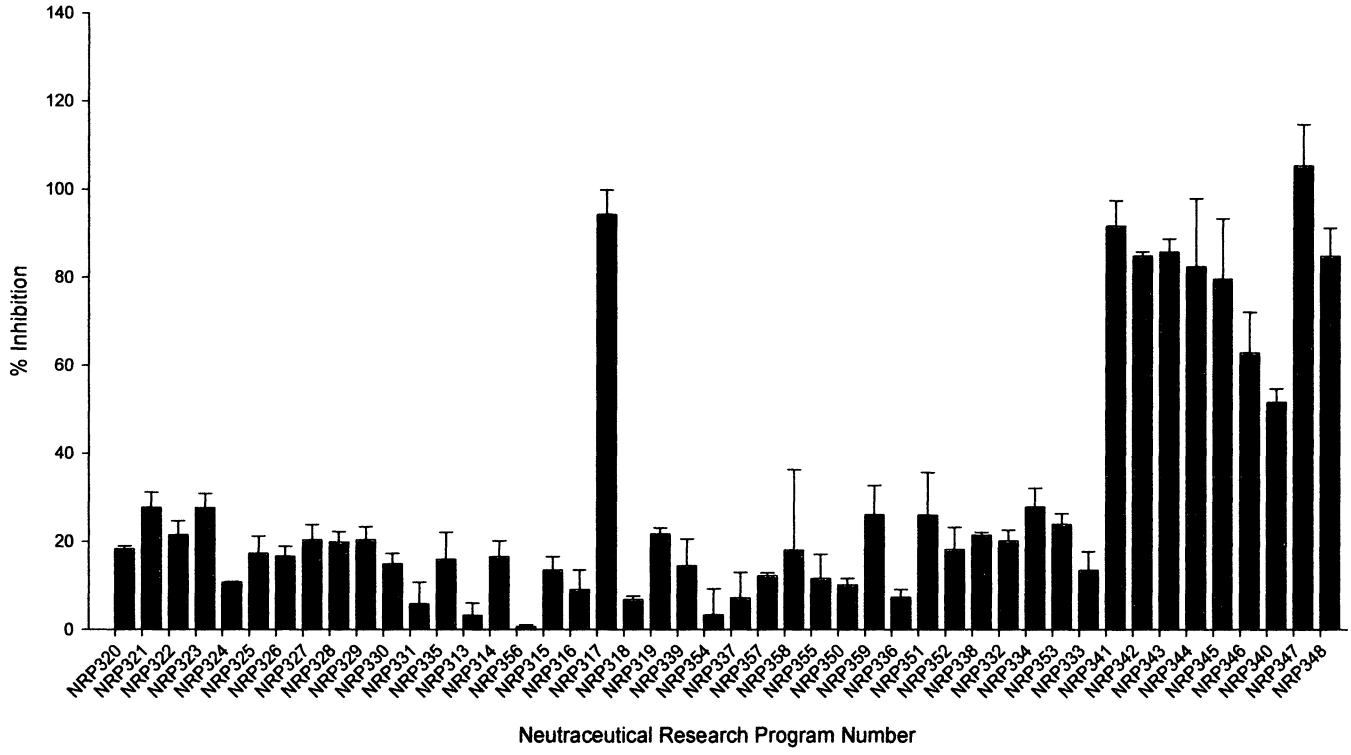


Figure 11. Percent inhibition of methanolic extracts (50 mg/mL) from common food samples on cytochrome P450 2D6 isozyme. Values are presented as means ± standard deviation of extracts tested in triplicates and repeated twice.

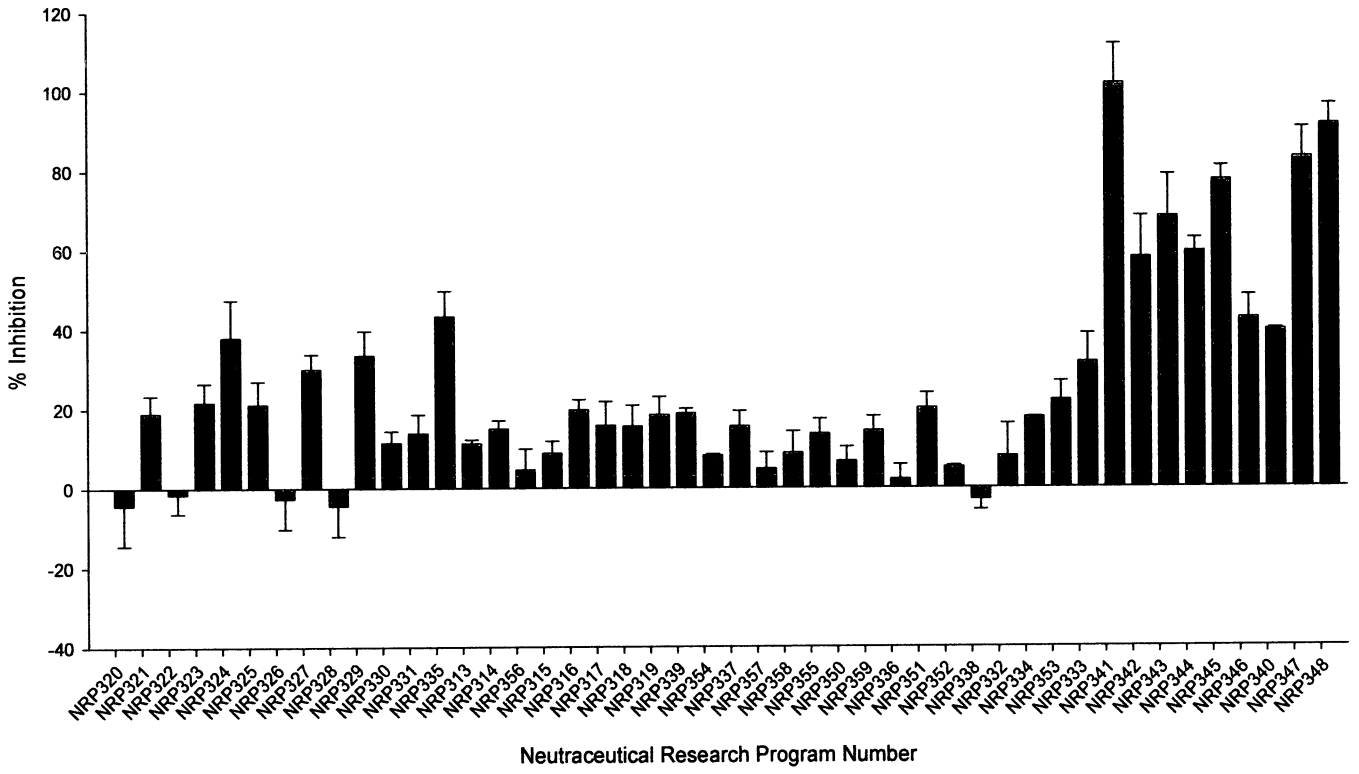


Figure 12. Percent inhibition of aqueous extracts (50 mg/mL) from common food samples on cytochrome P450 2D6 isozyme. Values are presented as means ± standard deviation of extracts tested in triplicates and repeated twice.

2.5 Discussion

Through the evaluation of the 46 food-plant samples using 4 different CYP enzymes to determine the potential risk of food-drug interactions, the findings provide strong evidence that the selected *Apiaceae* and *Lamiaceae* samples have a higher potential at inhibiting CYP activities than the *Fabaceae* products examined. The higher levels of activity in spices and herbs may be due to their selection for flavour, which is associated with a high level of phytochemicals (Lampe *et al.,* 2003). An identical trend was observed in the examination of these plants for their antimicrobial effects with the *Apiaceae* and *Laminaceae* being the most active.

Some products such as fennel seed, celery seed and cumin exhibited consistently high levels of inhibition of all CYP enzymes tested, which may be attributed to high levels of furanocoumarins (FCs) (Subehan *et al.,* 2007). The results obtained from fennel seed are consistent with the report by Subehan that identified 5-methoxypsoralen (5-MOP) as a mechanism-based inhibitor of CYP3A. Coriander and dill of the *Apiaceae* family, although also containing FCs (Ciesla *et al.*, 2008) are not reported to express high levels of activity. The varying levels of inhibitory activity may be due to the concentration or types of FCs in the plant.

The data obtained in this study suggest that both methanolic and aqueous extracts of the *Lamiaceae* plants oregano and rosemary exhibit high levels of inhibition towards CYP enzymes. This high level of inhibitory activity may be attributed to the presence of flavonoids or aromatic monoterpenes and is consistent with studies reported in other

flavonoid rich food plants such as pomegranate and rosemary (Offord *et. al.,* 1995; Faria *et al.,* 2007).

Among the *Fabaceae* in this study, the 12 soybean lines consistently exhibited moderately high inhibition activity against all 4 CYP isozymes. Previous studies have shown that aqueous extracts of soybean have the potential of inhibiting CYP3A4 and CYP3A7 and that hydrolyzed soy extracts at 50 mg/mL can reduce CYP3A4 activity by 22.3 ± 5.9 % compared to that of the control (Anderson *et al.,* 2003; Foster *et al.,* 2003). Lentil and other beans from other genera had lower inhibitory potential.

Chapter 3: Antimicrobial properties of *Apiaceae* and *Lamiaceae*

3.1 Antimicrobial effect and the bacterial gut microflora

The human bacterial gut microflora consists of microorganisms living in the digestive tract. The relationship between the gut microflora and the human body is not commensal, but rather symbiotic where both mutually benefit. The gut microflora has many functions in maintaining the overall health of the host. These includes regulating the growth of the gut, producing vitamins for the human body, preventing growth of pathogenic bacteria, and a variety of metabolic processes that include the break down of xenobiotic drugs. Therefore, any alteration to the content of the gut microflora will potentially have harmful effects to human health and wellness.

Functional foods are complex products and may contain many pharmacologically active phytochemicals, and these active ingredients may possess multiple biological activities rather than having only one effect on human health. The CYP inhibition and antimicrobial activity of foods may be related to the class of phytochemicals present and each activity may react differently to different groups of phytochemicals.

The objective of the antimicrobial assay was to assess the potential effect of food plants containing high levels of phytochemicals on the gut bacterial microflora. Seven bacteria were selected from seven different genera and both Gram (+) and Gram (-) types to include a wide spectrum of bacteria species.

3.2 Materials and methods

3.2.1 Control and maintenance of bacterial cultures

All seven bacterial cultures were provided by Dr. Myron Smith of Carleton University (Table 3). Bacteria were maintained in liquid cultures in 15 mL tubes with 5 mL of Mueller-Hinton medium at 30 °C for day to day usage. A second set of the seven bacterial cultures were kept at 4 °C in Petri dishes as backup in case of contamination or irregular growth. All cultures were sub-cultured every week to maintain optimum growth and homogeneity. Glycerol stocks of the seven cultures were also made and kept at -80 °C.

3.2.2 Kirby-Bauer Disc Diffusion Assay

Extracts were examined by antimicrobial assays using the Kirby-Bauer disc diffusion assay (Omar *et al.,* 2000). Both methanolic and ethanolic extracts were tested. A total of 7 bacterial species were selected in this study from different genera. There were 3 Gram (+) bacterial species: *Bacillus subtilis*, *Enterococcus faecalis*, and *Listeria innocua*, and 4 Gram (-) bacterial species: *Escherichia coli*, *Pseudomonas putida*, *Providencia stuartii*, and *Acetobacter calcoaceticus*. Each bacterial species was inoculated in 10 mL of Mueller-Hinton medium and cultured over night at 37 °C, and then plated using a sterile cotton swab onto Mueller-Hinton agar in Petri dishes. A 20 μL aliquot of the sample extract was transferred onto a 5 mm bacteria susceptibility disc (Oxoid, Ottawa, ON, Canada). Sample discs were then air dried and placed in triplicate onto the inoculated agar surface. The Petri dishes were then incubated at 37°C in dark condition and the zones of

inhibition were measured at 24 hours. The antibiotic Ciprofloxacin™ was used as the positive control.

3.3 Results

The antimicrobial properties of the food samples were examined by the antimicrobial disc-diffusion assay to evaluate potential effect on drug disposition by interacting with the gut bacterial microflora. The largest zones of inhibitions were observed with both methanolic and aqueous extracts of *Apiaceae* and *Lamiaceae* species shown in Table 3, in concordance with high level inhibition of CYP enzyme by extracts of these same plants. Oregano leaves and rosemary demonstrated strong inhibitory activity against 6 of the 7 selected bacterial species, with the exception being *A. calcoaceticus*. *Apiaceae* extracts including cumin, dill, fennel seed, celery seed, and coriander also displayed relatively strong antimicrobial activities. Fennel seed extract showed the most potent antimicrobial effects with the largest zones of inhibitions in six out of the seven bacteria with the exception of *E. coli*. In comparison, celery seed demonstrated weaker antimicrobial effects and was only effective against *P. putida* and *P. stuartii*. None of the *Apiaceae* extracts were active against *E. coli*. No significant antimicrobial effect was observed in the *Fabaceae* extracts although a few *Fabaceae* samples demonstrated weak activity (less than 8 mm) against *A. calcoaceticus*. The antibiotic Ciprofloxacin™ was used as the positive control which tested positive against all 7 bacterial species. Results are shown in Table 3. Sample photos were taken for the two most antimicrobial plant extracts.

The antimicrobial activity of rosemary against *B. subtilis* is shown in Figure 13, and the antimicrobial activity of fennel seed against *P. putida* is shown in Figure 14.

Antimicrobial activities of the extracts were compared to Cytochrome P450 inhibitory activities to examine potential correlation between the two biological activities. The strongest correlations were derived between the antimicrobial activities against E. coli and the inhibitory effects against CYP 3A5 and CYP 3A7. Results are shown in Figure 15 and Figure 16.

Figure 13. Antimicrobial activity of rosemary methonolic extract against *B. subtilis.*

Figure 14. Antimicrobial effect of fennel seed methanolic extracts against *P. putida*.

Table 3. Antimicrobial effects of methanolic and ethanolic extracts of selected *Apiaceae* and *Lamiaceae* (50 mg/mL) against 6 bacterial species. Zones of inhibitions were determined according to the Kirby-Bauer disc diffusion assay. Values represent average inhibition zone diameters (mm+SD) based on triplicate experiments. Ciprofloxacin was used as the positive control. (-) denotes no inhibitory activity or a zone of inhibition of less than 6mm, where disc diameter was 5mm.

	Bacillus subtilis (Gram +) ATCC#23857	*Enterococus faecalis* (Gram +) ATCC#49452	*Listeria innocua* (Gram +) ATCC#51742	*Esherichia coli* (Gram -) ATCC#1157	*Pseudomonas putida* (Gram -) ATCC#12633	*Providencia stuartii* (Gram -) ATCC#33672	*Acetobacter calcoaceticus* (Gram -) ATCC#19011
Cumin	8.3 ± 0.6	-	-	-	11.5 ± 0.0	-	7.5 ± 0.0
	6.5 ± 0.0	-	-	-	10.0 ± 0.0	-	6.3 ± 0.5
Fennel seed	10.7 ± 0.8	10.5 ± 0.0	10.5 ± 0.0	-	12.0 ± 0.0	11.0 ± 0.0	7.0 ± 0.0
	8.0 ± 0.0	9.7 ± 0.6	9.0 ± 0.0	-	9.7 ± 0.6	9.0 ± 0.0	6.0 ± 0.0
Dill	-	7.7 ± 0.5	11.5 ± 0.0	-	7.0 ± 0.0	12.5 ± 0.0	7.3 ± 0.5
	-	6.3 ± 0.4	10.3 ± 0.8	-	6.0 ± 0.0	6.5 ± 0.0	6.0 ± 0.0
Celery seed	8.3 ± 0.6	8.0 ± 0.0	-	-	11.5 ± 0.0	7.7 ± 0.5	11.7 ± 0.9
	6.5 ± 0.0	7.5 ± 0.0	-	-	9.3 ± 0.6	6.0 ± 0.0	8.0 ± 0.0
Coriander	-	-	-	-	6.5 ± 0.0	6.5 ± 0.0	-
	-	-	-	-	6.5 ± 0.0	6.0 ± 0.0	-
Rosemary	10.3 ± 0.8	11.5 ± 0.0	9.7 ± 0.6	8.0 ± 0.0	10.7 ± 0.8	10.5 ± 0.0	-
	8.3 ± 0.6	9.0 ± 0.0	8.5 ± 0.0	6.0 ± 0.0	8.0 ± 0.0	9.3 ± 0.6	-

Oregano	7.0 ± 0.0	12.0 ± 0.0	9.5 ± 0.0	10.5 ± 0.0	10.5 ± 0.0	11.5 ± 0.0	-
	6.3 ± 0.5	10.3 ± 0.8	8.0 ± 0.0	8.0 ± 0.0	8.0 ± 0.0	9.0 ± 0.0	-
Ciprofloxacin	21.5 ± 0.0	23.0 ± 0.0	22.7 ± 1.6	28.5 ± 0.0	31.0 ± 0.0	27.0 ± 0.0	29.5 ± 0.0

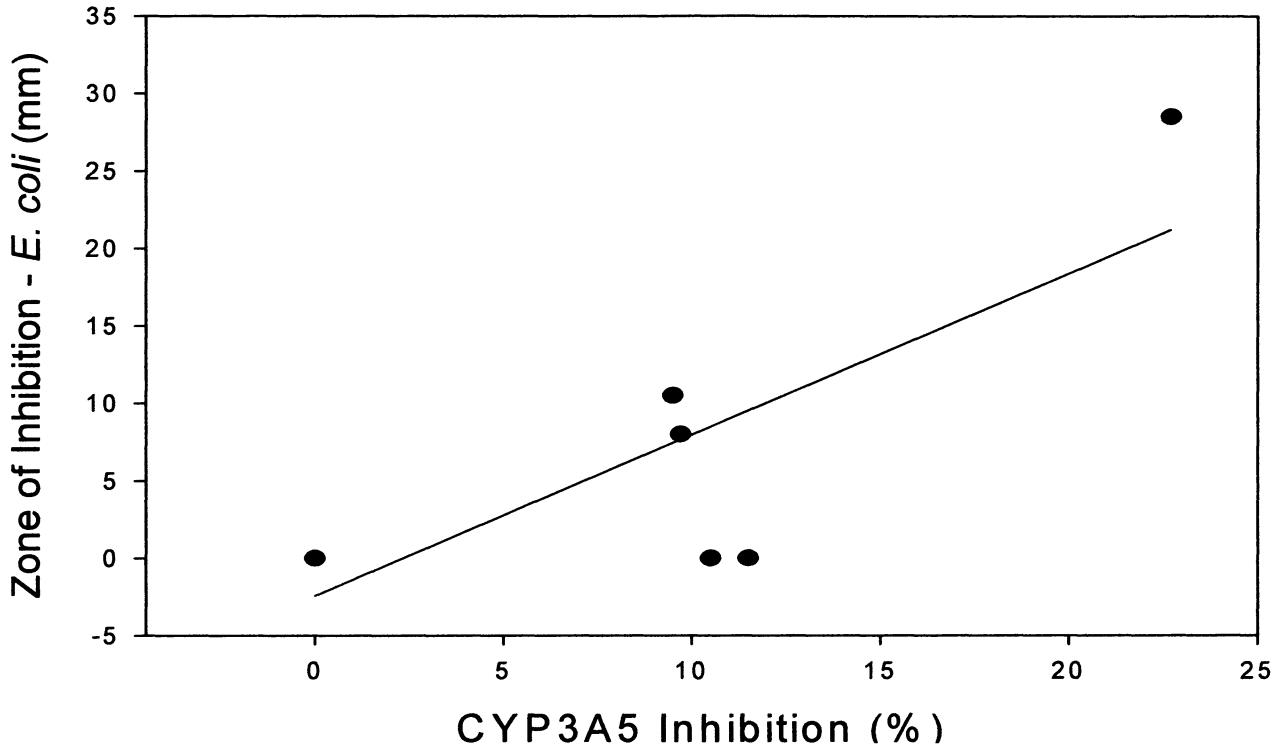


Figure 15. Correlation relationship (r^2=0.67) between cytochrome P450 3A5 isozyme inhibition and anti-microbial activity against *Escherichia coli* of the herbs and spices from the *Lamiaceae* and *Apiaceae* families. y = (1.04)x – 2.43.

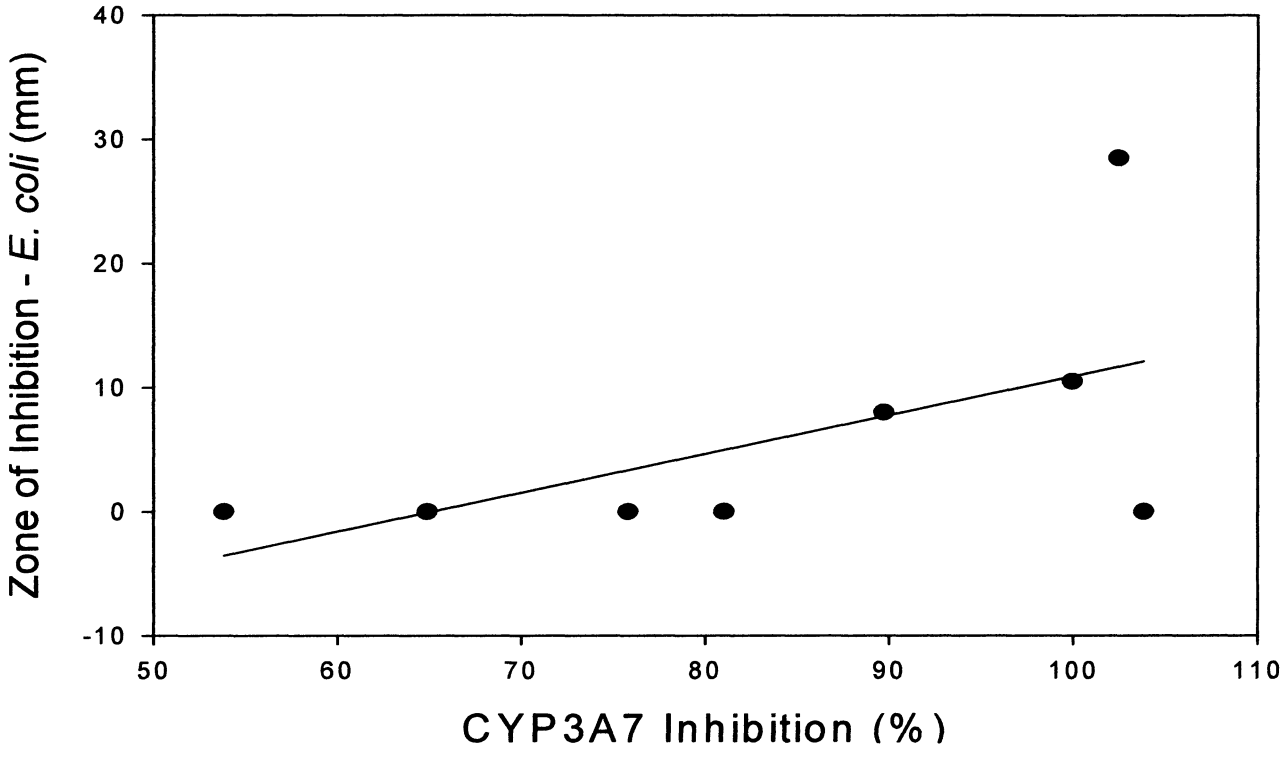


Figure 16. Correlation relationship (r^2=0.33) between cytochrome P450 3A7 isozyme inhibition and anti-microbial activity against *Escherichia coli* of the herbs and spices from the *Lamiaceae* and *Apiaceae* families. y = (0.33)x – 20.38.

3.4 Discussion

The antibacterial activities observed were predominantly from the *Apiaceae* and *Lamiaceae*. Among these, the highest and most broadly antibacterial activity, inhibiting 5 of the 6 bacterial strains, were extracts of rosemary and oregano. Previous studies have shown oregano and rosemary to have high antibacterial activity against *E. coli* (Bozin *et al.*, 2006; Sagdic *et al.*, 2003; Romano *et al.*, 2009). *Apiaceae* extracts also produced high antibacterial activity with fennel possessing the strongest and broadest activity. Extracts from the *Apiaceae* family, namely dill, celery, coriander and fennel, have been shown to contain the antibacterial compounds falcarinol and falcarindiol (Christensen and Brandt 2006). Zones of inhibition observed in this study may be affected by the loss of bioactive volatile phytochemicals and essential oils from the plant material due to processing and drying. A study using fresh plant material will be required to determine their full potential.

The antibacterial activities observed with the *Fabaceae* extracts were relatively low. The majority of the activities were from *Phaseolus vulgaris* varieties such as the light and dark red kidney bean, black bean, and black turtle bean. The data obtained correspond with previous studies and suggest that *Fabaceae* varieties containing coloured seed coats possessed stronger antibacterial activity as a result of secondary metabolites found in the seed coats (Benninger and Hosfield 2003). The coloured seed coats were observed to be a potential indication of bioactive secondary metabolites such as anthocyanins, condensed tannins and flavonoids (Benninger and Hosfield 2003).

By categorizing samples into families and evaluating their activity, two observations may be made about the secondary metabolomic content of a food crop, and its dietary selection as either a staple food or a condiment such as spices and herbs. First, the potential risk of food-drug interactions may be a characteristic of the plant family where there may be constitutive expression of compounds, such as FCs in *Apiaceae* (Ciesla *et al.*, 2008). Likewise, the high activities in the *Lamiaceae* were also observed as a result of their high levels of secondary metabolites such as terpenoids, phenolics, and flavonoids (Wink *et al.,* 2003). Traditionally, spices are used in minute amounts for their flavour and food preserving properties. Food spices and herbs typically contain higher levels of bioactive phytochemicals (Sherman and Hash 2001).

Upon analysis, a strong correlation was derived between the inhibitions of CYP3A5/3A7 (Figure 15 & 16) versus antibacterial activity against *E. coli* from the spice plants. Therefore these data suggest that the selected herbs and spices contain phytochemicals that can influence drug metabolizing enzymes and the gut bacterial flora. Staple foods, consumed in larger volumes than spices, may not cause immediate or extreme biological activities; however this does not imply that they are absolutely safe as concentrations and biological activity may be intensified synergistically with time and volume. When consumed in combination with drugs these food plants may affect drug metabolism, either directly as with human CYP enzymes or indirectly by disrupting gastrointestinal bacteria flora, thereby increasing the pharmacological load on the human system and affecting the patient's wellness.

Chapter 4: Increased proliferation of MCF-7 breast cancer cells treated with *Fabaceae* extracts

4.1 Soys and their potential as a hormone replacement therapy alternative

Isoflavones are not essential nutrients that are required to support life, but they nevertheless exert many health effects. The interests in phytoestrogen research as well as the sales volume of products containing phytoestrogen have increased since the controversy surrounding conventional hormone replacement started. Many women suffering from menopausal disorders, such as hot flushes and osteoporosis, are looking for alternatives to conventional estrogen replacement therapy because they fear the risk of breast cancer. The research on phytoestrogen extracts from soy and red clover as alternatives to conventional estrogen replacement therapy have been debated in the past and point to both beneficial and adverse effects on human health (Jiang *et al.,* 2008; Yellayi *et al.,* 2002). The study of the effect of isoflavones in estrogen-dependent cancers, particularly breast cancer, is important in order to qualify isoflavone as a safe and effective replacement of conventional estrogen replacement therapy. *Apiaceae*, *Lamiaceae*, and *Fabaceae* food plants were subjected to the MTT cell proliferation assay in this study to examine their potential phytoestrogenic effect which may proliferate estrogen-sensitive MCF-7 breast cancer cells. The objective of this estrogenic assay is to test the hypothesis that isoflavone-rich food plants may possess proliferative effect on estrogen-sensitive breast cancer cells and therefore may not be a safe alternative to HRT drugs.

4.2 Materials and methods

4.2.1 Control and maintenance of cell line

MCF-7 breast adenocarcinoma cell line was ordered from ATCC (4410 Paletta Court, Burlington, ON. Canada. L7L 5R2). Cells were thawed and separated into flasks containing Eagle's minimum essential medium. Fifty mL of fetal boving serum, 0.5 mL of bovine insulin, and 5 mL of penstrep was added to every 500 mL of Eagle's minimum essential medium. Flasks were incubated at 37 °C in dark condition. 6 mL of medium was added every two days. Old medium and unattached cells were removed and replaced with fresh medium every week. Attached cells were removed by trypsin after achieving 80% confluent in the flask. The first batch of confluent cells were placed in vials and kept in liquid nitrogen as storage and backup before beginning the experiment. Multiple flasks of cells were maintained simultaneously to avoid possible time delays due to contamination or slow growth.

4.2.2 Harvesting and plating of cells

Attached cells were removed for splitting or plating when they became 80% confluent. Before removal, attached cells were first washed with PBS and incubated with trypsin at 37 °C for 10 minutes. Un-detached cells were washed off the flask with Eagles' Minimum Essential medium to ensure maximum yield. Detached cells were suspended in 50 mL medium and centrifuged at 3,000 rpm for 5 minutes. Centrifuged cells were re-suspended again in 5 mL and mixed gently by tapping. 5 μL of the

suspension was transferred into 45 μL of trypan blue dye for counting using a hemocytometer. After the concentration was determined, cells are pipetted into a 96-well plate and diluted with a calculated amount of medium to achieve the desired concentration.

4.2.3 Chemicals and reagent

MTT reagent solution (25 mL, Cat# 30-1010K), detergent solution (2 x 125 mL, Cat# 30 -1010K), MCF-7 breast adenocarcinoma cells (1 mL, Cat# HTB-22), Eagle's Minimum Essential Medium (10 x 500 mL, Cat# 30-2003), fetal bovine serum (500 mL, Cat# 30-2020), bovine insulin (2 x 10 mL, Cat# I0516) were purchased from Cedarlane Laboratories (Burlinton, ON, Canada). Human β-estradiol (1 g, Cat# E2758) was purchased from Sigma-Aldrich (Oakville, ON, Canada). PBS, trypsin, Pentrep, and trypan blue dye solutions used in cell maintenance were obtained from Dr. John Arnason and Dr. Brian Foster of the University of Ottawa.

4.2.4 MTT cell proliferation assay

MTT (3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide), a yellow tetrazolium salt, can be reduced by metabolically active cells by the dehydrogenase enzymes to generate reducing equivalents such as NADH and NADPH. The product, formazan, can be solubilised and quantified by spectrophotometric means. The process is now widely accepted as a reliable way to examine cell proliferation and viability in response to external factors.

The assay was performed based on the procedure provided by ATCC MTT Cell Proliferation Assay Kit (CAT# 30-1010K). The cells were plated at 100,000 cells per well in a 96-well clear bottom plate. Extracts were added at various concentrations in labelled wells. Human β-estradiol (Sigman-Aldrich, CAT# E2578), which is known to proliferate estrogen-sensitive breast cancer cells, was used as the positive control and stimulated a strong response at 50 μg/mL. Wells without treatment were used to measure unstimulated spontaneous proliferation. Cells were incubated with the treatments for 76 hrs at 37 °C. 10 μL of MTT reagent was added to each well after incubation. The plate was then returned to incubation for another 3 to 4 hours until the purple formazan precipitate became visible. 100 μL of detergent reagent was added to each well in the end, and the plate was incubated overnight at room temperature in the dark. The absorbance of each well was recorded using the Cytofluor 4000 Fluorescence Measurement System at 570 nm (Applied Biosystems, Foster City, CA, USA).

Percent proliferation was calculated using the following equation:

$$\% \text{ Proliferation} = \frac{\text{Final Abs. of treated cells at 72 hrs} - \text{Initial Abs.}}{\text{Initial Abs.}} \times 100\%$$

Adjusted percent proliferation (Spontaneous growth removed) was calculated using the following equation:

$$\% \text{ Proliferation} = \frac{\text{Final Abs. of treated cells at 72 hrs} - \text{Initial Abs.}}{\text{Final Abs. of spontaneous growth} - \text{Initial Abs.}} \times 100\%$$

4.3 Results

All 46 samples were subjected to the MTT cell proliferation assay. Each ethanolic extract (50 μg/mL) was tested initially to screen for possible increased proliferation and selection for dose response study. Results are shown in Figure 13 where food samples were grouped based on plant families.

A clear pattern emerges upon analysis of the three plant families. None of the *Fabaceae* extracts hindered the rate of proliferation of the breast cancer cells. In fact, *Fabaceae* extracts showed proliferation rate ranges from 16% to 23% in 72hrs. Comparing to the spontaneous growth rate of 16.4%, a few *Fabaceae* extracts showed weak proliferative effects and a slight increase in the spontaneous growth rate of the breast cancer cells. Upon close examination of the different species, *Glycine max* extracts had the strongest effect on breast cancer proliferation ranging from 17.4% to 21.8% proliferation. Beans (*Phaseolus*) had the second highest activity ranging from 16.5% to 18.8% proliferation. Lentils (*Lens*) extracts demonstrated relatively weak proliferative effect compared to soys and beans. Species from the pea group, including the genera *Pisum*, *Vigna,* and *Cicer*, did not demonstrate a significant proliferative effect. Results are shown in Figure 17 and Appendix Table 6.

Soybean extracts from the *Fabaceae* family demonstrated statistically significant proliferative effect on the MCF-7 breast cancer cells compared to the proliferation rate of the untreated cells ($t = -2.579$, $DF = 11$, $P = 0.026$). In the initial screen, soy bean extract (NRP #329), demonstrated the highest proliferative effect on MCF-7 breast cancer cells at 21.8%. The dose-response analysis of this particular extract revealed a positive relationship between the concentration of *Fabaceae* extracts and the rate of

proliferation of breast cancer cells (Figure 18). For *Glycine max*, when the treatment concentration increased from 50 μg/mL to 200 μg/mL, the rate of proliferation increased from 21.8% to 28.5%. When compared to the 72.0% proliferation by 50 μg/mL β-estradiol, 200 μg/mL of soy extract achieved more than one third of the effect of human β-estradiol. The adjusted percent proliferation was calculated by subtracting the spontaneous percent proliferation from the treatment proliferation. The adjusted percent proliferation was 12.1% for soybean, significantly higher than the other genera of *Fabaceae* ($t = 8.948$, $DF = 45$, $P = <0.0001$). Results are shown in Figure 18 and 22.

Bean extracts (genus *Phaseolus*) had the second highest activity compared to other genera. Black bean (NRP# 335) demonstrated the highest proliferative effect on breast cancer cells within the *Phaseolus* genus at 18.8% during the initial screening. A clear dose-response can be seen when the concentration of black bean extract increased first from 50 μg/mL to 100 μg/mL, then doubled to 200 μg/mL. The rate of proliferation increased from 18.8% to 21.8%. The rate of proliferation appeared to decrease between 100 μg/mL to 200 μg/mL indicating an end point of the dose-response curve where the reaction reached a plateau possibly due to saturation. The adjusted percent proliferation for black bean was 5.4% at 200 μg/mL. When comparing between genera, the proliferative effect of *Phaseolus* extracts is statistically less than the effect of *Glycine* extracts on breast cancer cells ($t = -6.103$, $DF = 24$, $P = <0.001$). Results are shown in Figure 20 and 24.

Lentils extracts (genus *Lens*) had weak proliferative effect compared to beans and soybeans and the percent proliferation was statically insignificant compared to the negative control. The three lentils' extracts increased breast cancer cell proliferation by an average of 17.5% at 50 μg/mL. Percent proliferation of red lentil (NRP #359) on

breast cancer cells increased to 20.9% and 21.1% at 100 μg/mL and 200 μg/mL respectively. Similar to black bean, the rate of proliferation also decreased between 100 μg/mL and 200 μg/mL indicating another possible end point of the does-response curve. The adjusted percent proliferation for red lentil was 4.3% at 200 μg/mL. Results are shown in Figure 19 and 23.

The extracts of other genera including *Pisum*, *Vigna*, and *Cicer* of the pea group did not demonstrate any proliferative effect on the breast cancer cells at 50 μg/mL concentration. For these genera, the average percent proliferation of breast cancer cells at 72hrs of after the treatment was approximately 16.3%, identical to the spontaneous percent proliferation of 16.4%. Chickpea (NRP# 353), a popular food, was selected for tests at higher concentrations, but no proliferative effect was detected at 100 μg/mL and 200 μg/mL. Results are shown in Figure 21.

The *Apiaceae* and *Lamiaceae* extracts exhibited opposite effect on MCF-7 breast cancer cells when compared to the *Fabaceae* extracts. The results demonstrated negative percent of proliferation indicating the *Apiaceae* and *Lamiaceae* extracts were toxic to the breast cancer cells and were killing the cells slowly during the 72hrs of incubation. The *Lamiaceae* extracts appeared to be more toxic to the MCF-7 breast cancer cells than the *Apiaceae* extracts. The *Apiaceae* extracts demonstrated a negative proliferation of -43.5% on average while the *Lamiaceae* extracts demonstrated a negative proliferation of -57.1% on average.

In the *Apiaceae* extracts, fennel seed (NRP #346) extract demonstrated the highest toxicity towards MCF-7 breast cancer cells at -60.3%, followed by dill at -56.8% (NRP #345), and celery seed at -46.5% (NRP #341). In the *Lamiaceae* extracts, rosemary (NRP #348) had the strongest toxicity towards breast cancer cells at -68.9%,

followed by basil leaves (NRP #347) at -52.9%, and oregano leaves (NRP #340) at -49.6 %.

Overall, the results obtained from the MTT- cell proliferation study demonstrated similar trend when compared to the results of the Cytochrome P450 inhibition study and the antimicrobial effect study. In the CYP inhibition study, the average inhibitory effect of *Lamiaceae* extracts was stronger than the average inhibitory effect of *Apiaceae* extracts. The *Fabaceae* extracts had relatively weak CYP inhibition effect. In the MTT cell proliferation study, *Lamiaceae* extracts were also more toxic to the MCF-7 breast cancer cells compared to the *Apiaceae* extracts, while the *Fabaceae* extracts had a positive effect on breast cancer cell proliferation. A similar trend was observed in the antimicrobial study as well where *Fabaceae* extracts did not have any antimicrobial effect, while *Apiaceae* and *Lamiaceae* extracts did demonstrate significant antimicrobial effect with *Lamiaceae* being the stronger of the two.

Out of the 47 plants under investigation, rosemary of the *Lamiaceae* and fennel seed of the *Apiaceae* consistently exhibited the highest activities in all three assays. The two plant extracts demonstrated the strongest antimicrobial activity against all 7 bacteria, the highest inhibitory effect on cytochrome P450, as well as the most toxicity towards MCF-7 breast cancer cells. The *Fabaceae* extracts demonstrated some proliferative effects on the growth of the MCF-7 breast cancer cells but the growth were insignificant statistically. Only the *Glycine* genera consisted of soybeans demonstrated statistically significant proliferative effect on MCF-7 breast cancer cells.

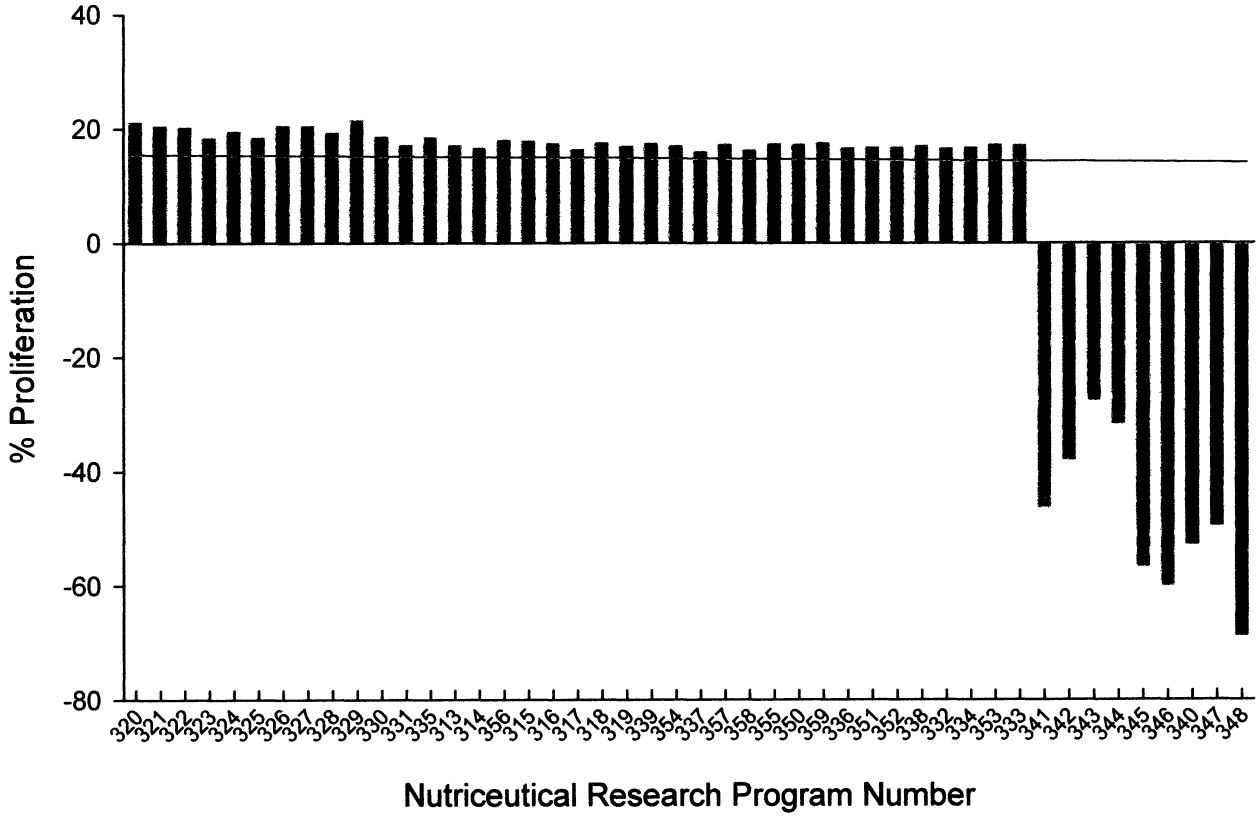


Figure 17. Cell proliferation of MCF-7 breast cancer cells measured with the MTT proliferation assay with standard deviation shown. 46 samples of plant extracts from *Fabaceae* (red), *Apiaceae* (green), and *Lamiaceae* (blue) family were initially screened for possible proliferative activity. Cells were treated with 50 µg/mL ethanolic extract and incubated at 37°C for 72 hours. The solid line indicates spontaneous growth (untreated cells) at 16.4%.

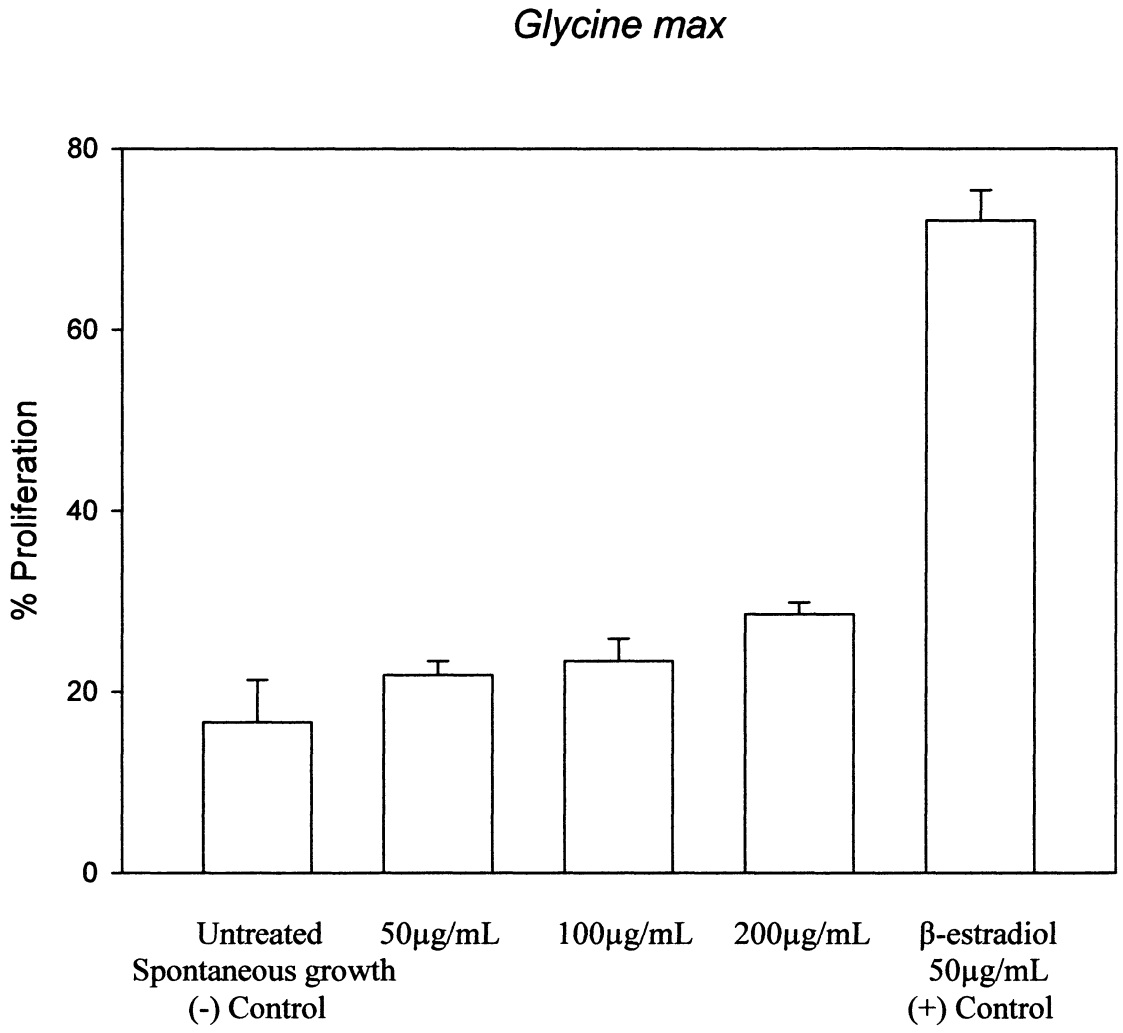


Figure 18. Cell proliferation of MCF-7 breast cancer cells measured with the MTT proliferation assay. Cells were treated with various concentrations of ethanolic extract of soybean (NRP#329) and incubated at 37 °C for 72 hours. Beta-estradiol was used as the positive control.

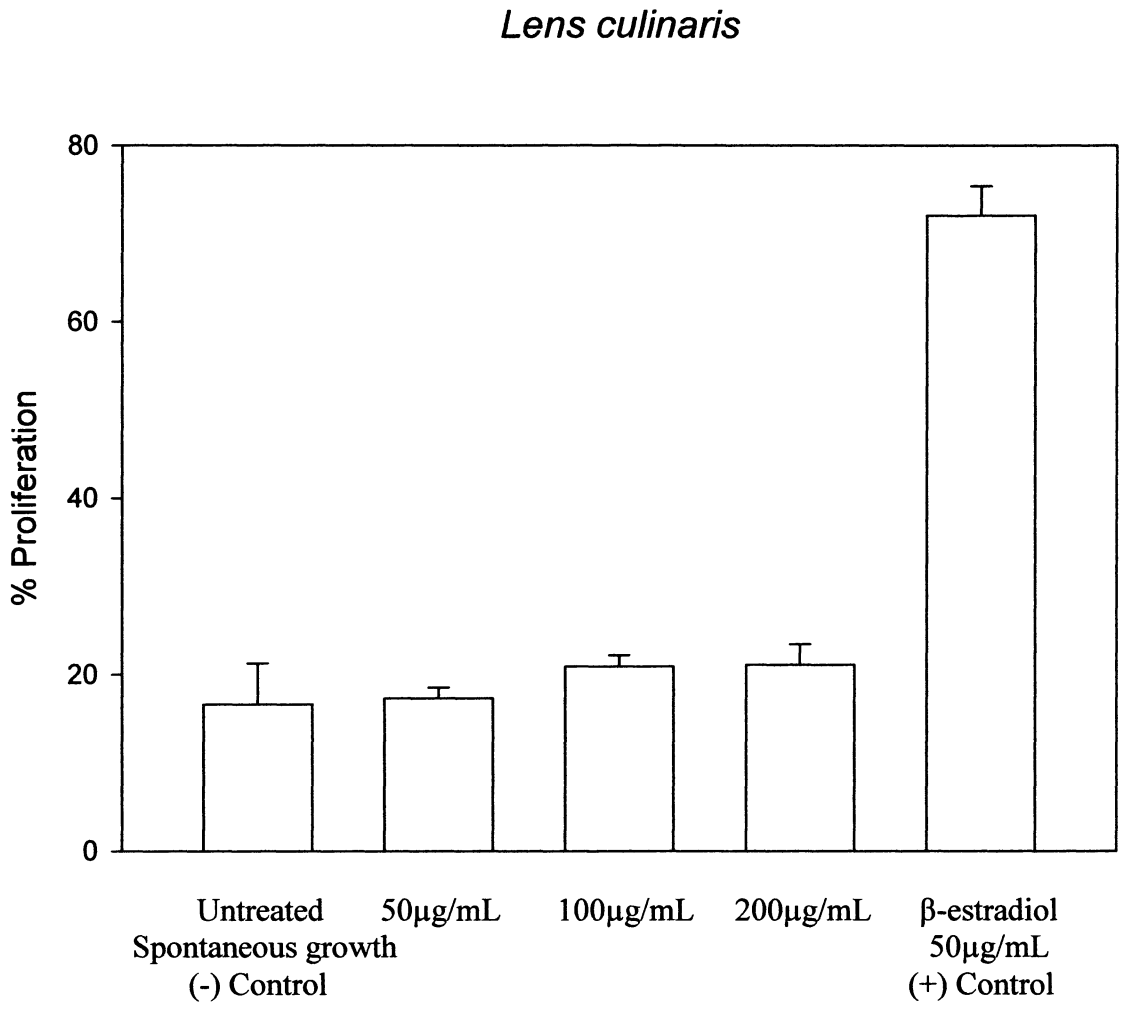


Figure 19. Cell proliferation of MCF-7 breast cancer cells measured with the MTT proliferation assay. Cells were treated with various concentrations of ethanolic extract of *Lens culinaris* (NRP#359) and incubated at 37 °C for 72 hours. Beta-estradiol was used as the positive control.

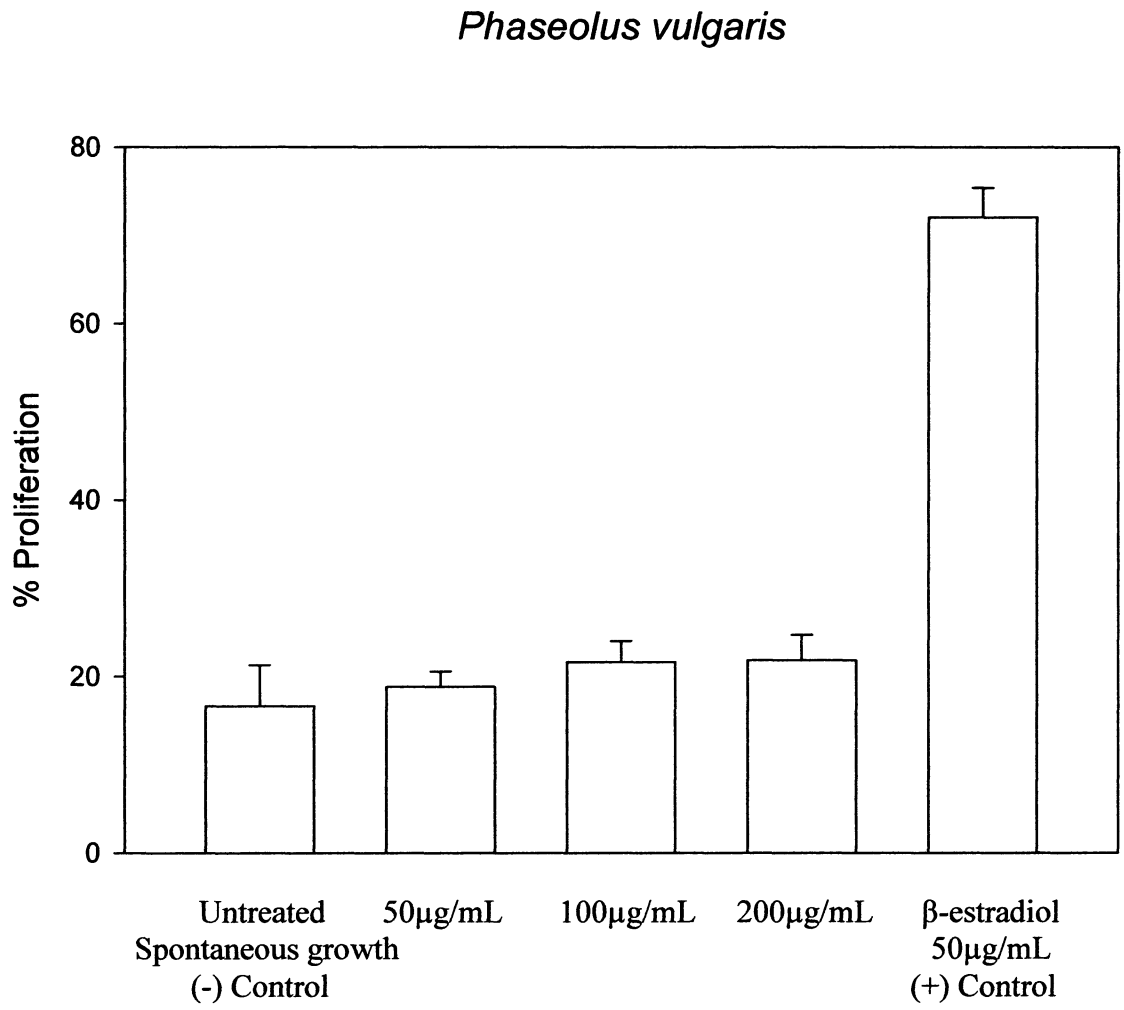


Figure 20. Cell proliferation of MCF-7 breast cancer cells measured with the MTT proliferation assay. Cells were treated with various concentrations of ethanolic extract of black bean (NRP#335) and incubated at 37 °C for 72 hours. Beta-estradiol was used as the positive control.

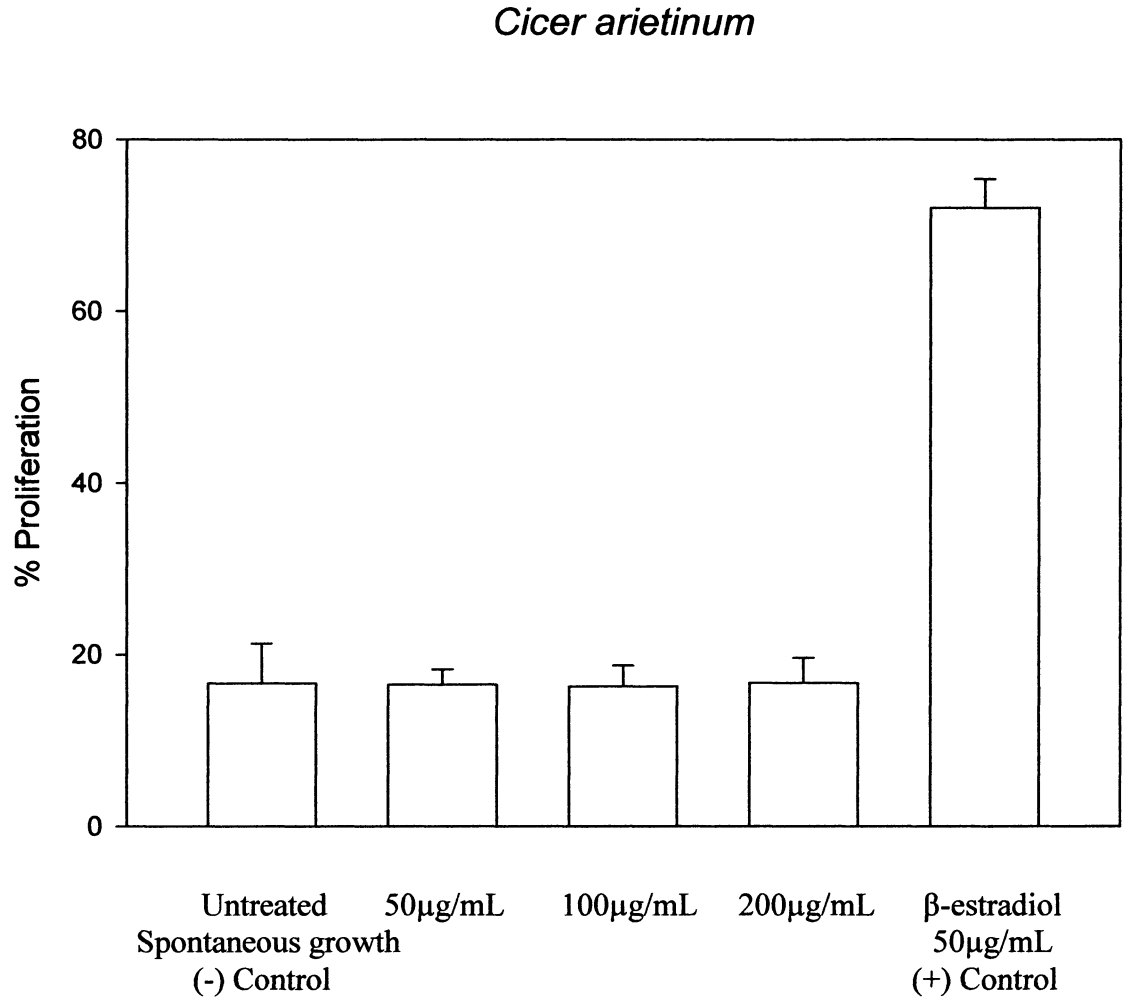


Figure 21. Cell proliferation of MCF-7 breast cancer cells measured with the MTT proliferation assay. Cells were treated with various concentrations of ethanolic extract of chickpea (NRP#353) and incubated at 37 °C for 72 hours. Beta-estradiol was used as the positive control.

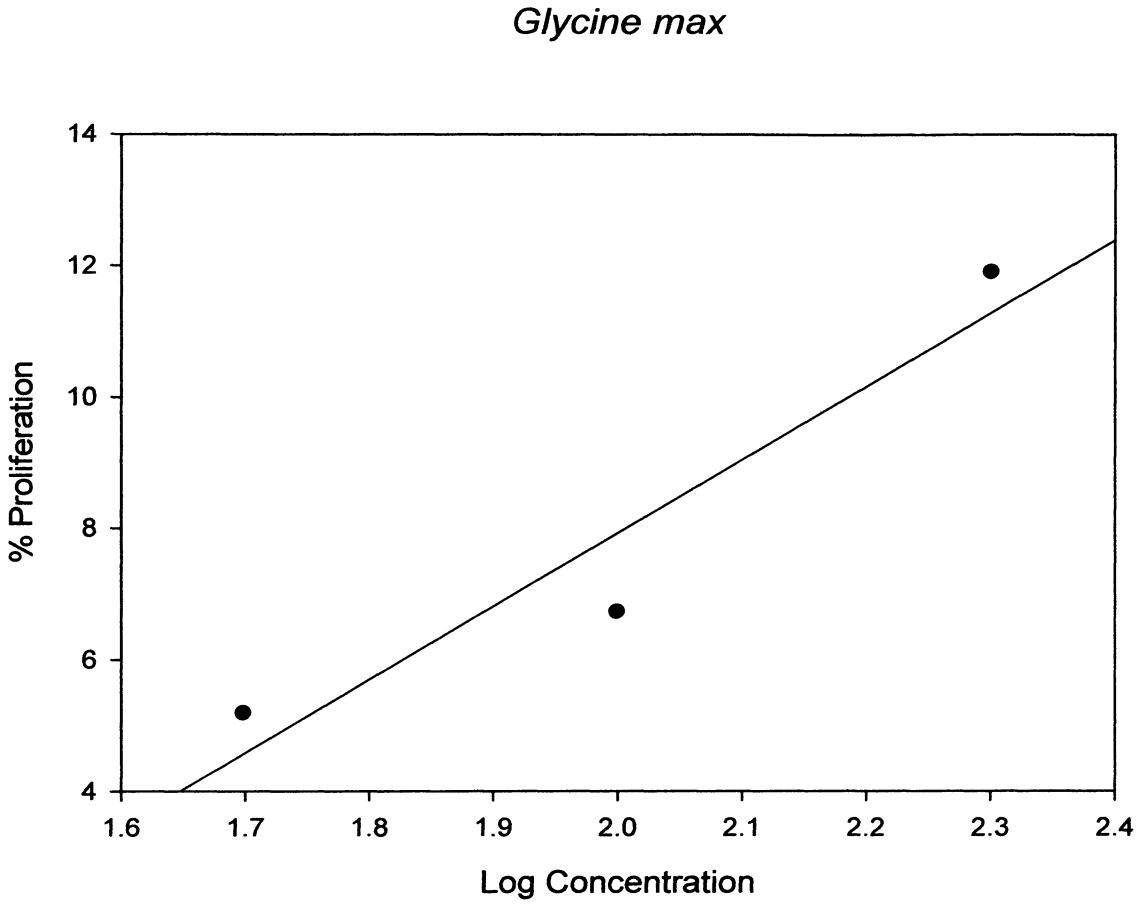


Figure 22. Dose-response study of the effect of *Glycine max* on MCF-7 breast cancer cells. 50 µg/mL, 100 µg/mL, and 200 µg/mL concentrations were tested. Percent proliferation was graphed against the log of concentration with regression line shown. y = 11.167x - 14.167

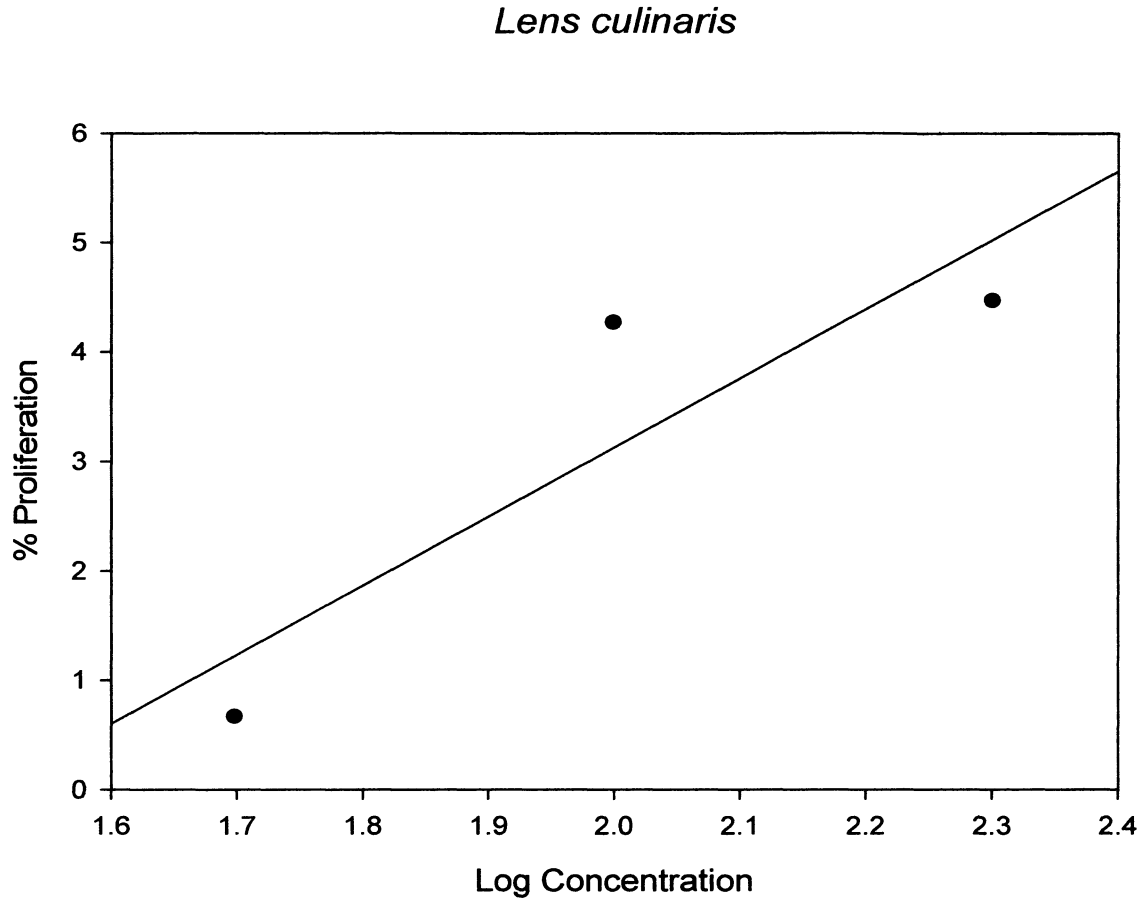


Figure 23. Dose-response study of the effect of *Lens culinaris* on MCF-7 breast cancer cells. 50 μg/mL, 100 μg/mL, and 200 μg/mL concentrations were tested. Percent proliferation was graphed against the log of concentration with regression line shown. $y = 6x - 8.5667$

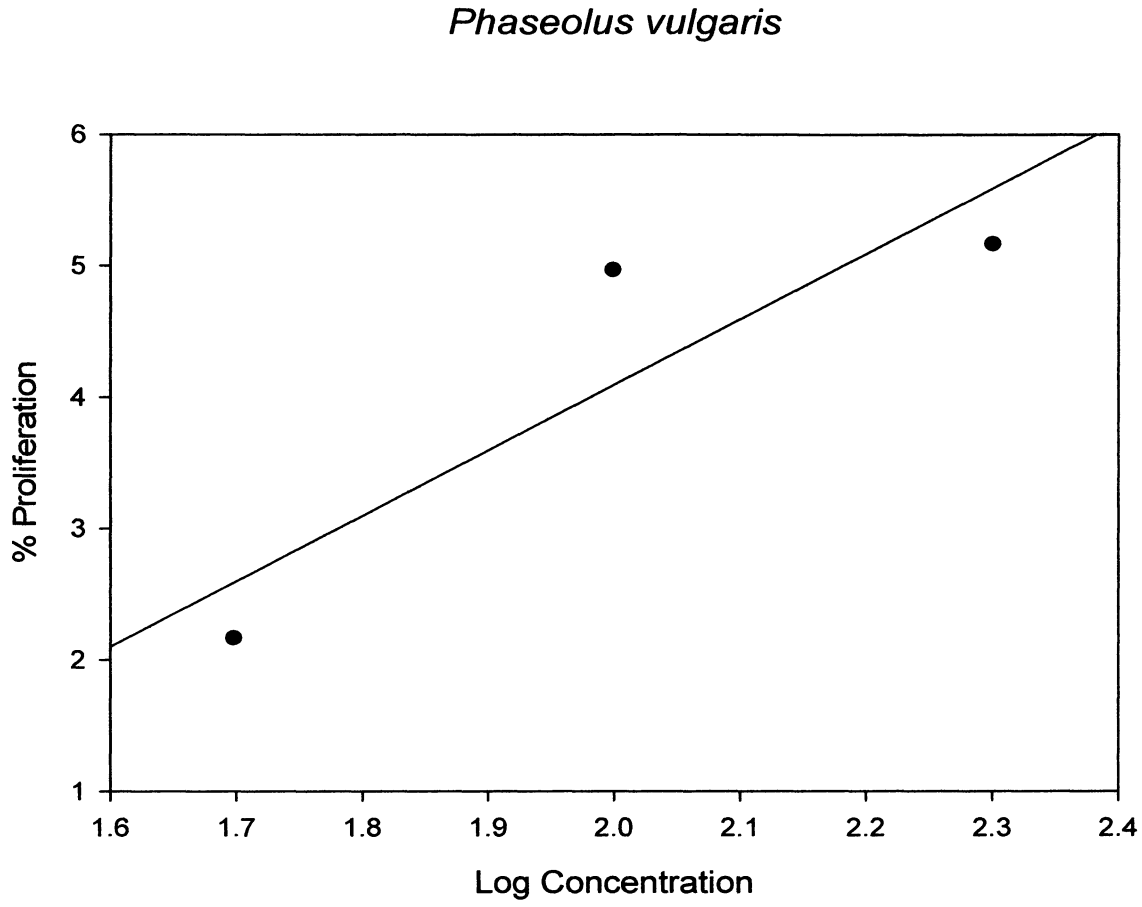


Figure 24. Dose-response study of the effect of *Phaseolus vulgaris* on MCF-7 breast cancer cells. 50 μg/mL, 100 μg/mL, and 200 μg/mL concentrations were tested. Percent proliferation was graphed against the log of concentration with regression line shown. y = 5x - 5.9

4.4 Discussion

The breast cancer cell proliferation effect was observed exclusively from the *Fabaceae* plants. Within the *Fabaceae* family, the genus *Glycine* consisting of soybeans was the most active at accelerating breast cancer cell proliferation, followed by the genus *Phaseolus* consisting of beans, and the genus *Lens* consisting of lentils. This result corresponds with previous studies that documented most members of the *Fabaceae* family contain significant amounts of isoflavones with soybeans being the most significant source of isoflavone genistein in our diet (Kaufman *et al.*, 1997). A study done by Boker *et al.* indicated that on average the European/North Amerian diet contain less than 2 mg of isoflavones per day (Boker *et al.*, 2002), but the concentrations tested in this study is significant taken into account of isoflavone's bioavailability at plasma concentration of approximately 1500 nmol/L (Chanteranne *et al.*, 2008) and that non-purified samples were tested.

The toxicity of *Apiaceae* and *Lamiaceae* plants extracts towards the breast cancer cells was also expected because of the high level of phytochemicals in these two plant families. Phytochemicals such as furanocoumarins in *Apiaceae*, and flavonoids in the *Lamiaceae* were produced by the host plants to defend against predation, therefore these chemicals should exhibit toxicity towards cells *in vitro*. It should be noted that the toxicity level of *Lamiaceae* and *Apiaceae* towards the breast cancer cells were consistent with their inhibitory effect on CYPs and antimicrobial effect, indicating these phytochemicals have multiple biological effects in the human body.

The ability of isoflavones to mimic the effect of human estrogen is well documented. Genistein and daidzein at low concentrations were found to stimulate

breast tumor growth in *in vitro* and *in vivo* animal studies, and antagonize the antitumor effect of tamoxifen in vitro (de Lamos *et al.*, 2001). When it binds to estrogen-receptors in estrogen-dependent breast cancer cells, genistein increases the proliferation by increasing the production of key proteins such as IGF-IR and IRS-1 in MCF-7 cells (Chen *et al.*, 2004). These effects may be enhanced in an estrogen-limited environment, such as post-menopausal women, because more estrogen receptors are available for genisteins to bind. Another study using an animal model, demonstrated that genistein acts in an additive manner to the effect of β-estradiol in proliferating breast tumor growth *in vivo* (Ju *et al.*, 2006). There are studies showing other harmful effects of phytoestrogens besides proliferating breast cancer cells. Studies have shown genistein can stimulate the development and maintenance of female characteristics. *In vitro* studies have proven genistein to induce apoptosis of testicular cells at certain levels, thus raising concerns about effects it could have on male fertility (Kumi *et al.*, 1998). Yet, the epidemiological evidence still suggests a link between soy consumption and the reduction of breast cancer incidence. Some recent researches have demonstrated that the contradictory effects of isoflavones are attributed to the timing of dietary isoflavone exposure. Isoflavone exposures during puberty may play an important role in determining later risk by inducing epigenetic changes that modify vulnerability to breast cancer, while exposure to isoflavone at a later age may have the opposite effect (De Assis *et al.*, 2006).

Phytoestrogens, specifically isoflavones, have attracted much attention recently as being a safer alternative to HRT. Many epidemiological studies point to isoflavones to have preventive effect on breast cancer development because dietary soybeans have been correlated to reduction of risk in Asian populations. The results of this study

demonstrated the potential effect of *Fabaceae* products in proliferating breast cancer and advised against the consumption of soybean products in breast cancer patients and population exposed to high breast cancer risk, such as post-menopausal women. While many studies have shown the benefits of soybeans in treatment of diseases related to estrogen deficiency, the cancer risk identified in this *in vitro* study warrants much closer study of soy products in animal trials and clinical epidemiology.

Chapter 5: General Discussion and Conclusion

This study was the first to examine and attempted to correlate three important biological effects of a variety of food samples from the *Fabaceae*, *Lamiaceae*, and *Apiaceae* family. The results demonstrated that food plants with high levels of phytochemicals have the potential of affecting human health and wellness. All three plant families exhibited their ability in inhibiting the activity of Cytochrome P450 with *Apiaceae* and *Lamiaceae* plants being the most potent inhibitors. The antimicrobial results presented a similar pattern in which the *Apiaceae* and *Lamiaceae* demonstrated strong antimicrobial effects toward selected bacteria due to the plants' high phytochemical content. When consumed in combination with drugs these food plants may affect drug metabolism, either directly as with human CYP enzymes or indirectly by disrupting gastrointestinal bacteria flora, thereby increasing the pharmacological load on the human system and affecting the patient's wellness. While the *Fabaceae* plants did not affect Cytochrome P450 and bacterial growth, the data obtained suggested that isoflavone-rich *Fabaceae* plants can stimulate breast cancer proliferation. *Fabaceae* products may not be a safe alternative to hormone replacement therapy as patients are exposed to the same risks as using HRT.

The results of this study will contribute to the growing field of neutraceutical research that include many recent studies that examined phytochemicals in functional foods' biological effects on human health and wellness. Similar to the effect of grapefruit juice, a study by Zhang *et al.* have demonstrated that starfruit juice can also inhibit the activity of Cytochrome P450 (Zhang *et al.*, 2007). Sulforaphane, isolated from broccoli, is another antimicrobial agent that has exhibited some health benefits

such as anti-cancer and anti-diabetic effects (Yanaka *et al.*, 2009; Dashwood *et al.*, 2007). Beside the three biological effects demonstrated by phytochemicals in this study, the antioxidant properties of many phytochemicals have garnered much attention because of their reported anti-cancer effects. Antioxidant Resveratrol, a phytoalexin produced by several plants (i.e. grapes), has demonstrated many beneficial health properties including anti-cancer, anti-inflammatory, and anti-aging effects (Kode *et al.*, 2008; Lagouge *et al.*, 2006). Another antioxidant Catechin, found in green tea, has also shown promising anti-cancer results (Qiao *et al.*, 2009; Sartippour *et al.*, 2006). Given the heightened popularity of antioxidants among the general public, the flavonoid-drug interactions may also be a problem that will increase. A review by Cermak strongly cautions the possibility of flavonoid-drug interactions in functional foods and herbal supplements, and counsels the need for advisory labelling of unregulated products (Cermak 2008).

Future studies should focus on the identification and quantification of the active phytochemicals in the plant extracts. This can be achieved by HPLC analysis. The active phytochemicals can be isolated and tested in animal models to examine the effect of the pure compounds *in vivo* to validate the effects of these food plants on human health and wellness. A thorough correlation study should be done with purified compounds to examine the link between the biological effects. Only a few correlations were examine with the most active plant extracts in the antimicrobial assay and the cytochrome p450 inhibition assay in this study. Future work should include all bacterial species and CYP isozymes. The mechanisms of action of these phytochemicals should also be investigated to further elucidate their biological effects. For example, by determining whether the inhibitory effects on Cytochrome P450s were caused by

mechanism-based inhibition or competitive inhibition, we can better understand how these phytochemicals affect human biologically and how we may potentially manipulate them to improve human health and wellness.

With the soaring popularity of NHPs and functional foods, many individuals are consuming larger quantities of these products such as soy, fresh herbs and spices. These products are safe when consumed in reasonable amounts, but when consumed in larger amounts or together with other therapeutic products, they may cause a drug interaction and undesired health effects. Food-drug interactions may be the underlying cause for some drug overdoses, drug rejection, and therapeutic failure as a result of direct systemic CYP inhibition or disruption of the bacterial flora. Although the majority of healthy individuals will see very little, if any, effect when consuming common food products, patients undergoing serious medical care or exposed to risk factors should become more aware of potential risks identified with certain foods.

Appendix

Table 4. Activity of methanolic extracts (50 mg/mL) of plant samples against Cytochrome P450 3A4, 3A5, 3A7 and 2D6 isozymes expressed as mean percent inhibition (%) ± standard deviation.

NRP No.	3A4	3A5	3A7	2D6
320	34.44 ± 0.06	41.35 ± 2.90	62.22 ± 5.90	18.31 ± 0.67
321	36.61 ± 2.77	46.15 ± 6.01	71.25 ± 0.74	27.71 ± 3.46
322	27.17 ± 7.17	61.30 ± 4.53	60.00 ± 8.80	21.50 ± 3.20
323	36.35 ± 2.89	83.55 ± 2.48	59.15 ± 7.16	27.62 ± 3.28
324	29.42 ± 0.67	54.30 ± 5.23	57.43 ± 9.81	10.76 ± 0.13
325	32.31 ± 4.09	35.15 ± 0.07	67.53 ± 3.15	17.24 ± 3.94
326	36.20 ± 3.12	36.10 ± 2.97	66.29 ± 4.30	16.63 ± 2.29
327	40.43 ± 13.40	54.00 ± 5.80	75.26 ± 4.71	20.36 ± 3.43
328	33.12 ± 6.26	31.85 ± 4.46	66.70 ± 5.40	19.86 ± 2.33
329	49.46 ± 9.14	42.65 ± 0.50	75.34 ± 1.86	20.32 ± 2.98
330	28.89 ± 7.49	31.45 ± 11.10	68.78 ± 16.27	14.91 ± 2.35
331	41.22 ± 10.99	54.70 ± 1.98	77.45 ± 0.30	5.80 ± 4.93
335	23.40 ± 0.96	34.87 ± 10.29	66.33 ± 9.07	15.92 ± 6.12
313	20.30 ± 2.71	-2.95 ± 3.47	56.60 ± 3.07	3.20 ± 2.80
314	5.30 ± 3.97	11.65 ± 5.16	51.84 ± 2.17	16.49 ± 3.64
356	12.74 ± 2.75	8.08 ± 1.32	27.66 ± 0.80	0.66 ± 0.42
315	9.03 ± 22.66	26.40 ± 2.69	60.16 ± 3.49	13.48 ± 3.05
316	6.90 ± 0.11	19.95 ± 0.35	50.49 ± 4.83	9.05 ± 4.46
317	41.17 ± 8.21	21.85 ± 0.07	47.23 ± 6.00	94.23 ± 5.58
318	28.76 ± 2.52	9.15 ± 6.29	64.19 ± 0.02	6.78 ± 0.80
319	19.00 ± 0.45	22.60 ± 10.89	53.29 ± 4.82	21.70 ± 1.37
339	22.32 ± 4.20	19.46 ± 7.26	20.75 ± 0.27	14.46 ± 6.09
354	39.95 ± 6.62	10.33 ± 5.33	36.51 ± 22.63	3.39 ± 5.84
337	23.55 ± 1.07	15.22 ± 3.10	29.24 ± 3.14	7.20 ± 5.78
357	1.12 ± 3.02	25.94 ± 2.11	44.98 ± 0.41	12.22 ± 0.64
358	1.47 ± 1.58	20.27 ± 2.86	28.23 ± 1.99	18.01 ± 18.26
355	8.40 ± 0.08	23.77 ± 1.73	30.46 ± 3.90	11.58 ± 5.50
350	32.91 ± 2.63	18.15 ± 1.73	25.44 ± 2.61	10.14 ± 1.48
359	22.47 ± 15.80	16.45 ± 8.17	-3.10 ± 26.17	26.00 ± 6.65
336	18.99 ± 2.81	7.55 ± 3.18	16.24 ± 20.07	7.33 ± 1.75
351	18.02 ± 5.81	21.01 ± 0.38	8.58 ± 14.90	25.97 ± 9.69
352	44.38 ± 8.81	13.24 ± 0.47	6.89 ± 17.06	18.16 ± 5.07
338	12.20 ± 4.97	17.82 ± 5.06	16.17 ± 2.33	21.44 ± 0.65
332	26.16 ± 6.92	14.80 ± 3.54	51.92 ± 6.74	20.09 ± 2.44

334	12.89 ± 13.19	38.05 ± 4.17	54.40 ± 4.57	27.83 ± 4.29
353	15.77 ± 4.84	30.52 ± 1.55	30.10 ± 11.10	23.88 ± 2.45
333	26.80 ± 0.11	26.60 ± 3.25	31.53 ± 0.14	13.43 ± 4.26
341	85.09 ± 4.72	96.48 ± 0.37	75.79 ± 1.01	91.63 ± 5.78
342	33.22 ± 0.11	97.02 ± 0.74	64.87 ± 13.97	84.84 ± 1.00
343	30.22 ± 39.34	26.74 ± 5.14	64.89 ± 2.23	85.78 ± 3.01
344	92.84 ± 6.36	97.74 ± 0.64	81.01 ± 3.24	82.43 ± 15.48
345	10.85 ± 31.07	35.30 ± 9.35	53.81 ± 3.92	79.63 ± 13.71
346	104.83 ± 0.08	66.62 ± 2.46	103.83 ± 2.40	62.78 ± 9.26
340	52.75 ± 2.16	87.20 ± 2.55	89.71 ± 0.81	51.57 ± 3.13
347	99.91 ± 0.68	99.50 ± 0.11	99.94 ± 0.38	105.29 ± 9.47
348	101.95 ± 2.59	100.11 ± 0.22	102.46 ± 1.59	84.80 ± 6.34

Table 5. Activity of aqueous extracts (50 mg/mL) of plant samples against Cytochrome P450 3A4, 3A5, 3A7 and 2D6 isozymes expressed as mean percent inhibition (%) ± standard deviation.

NRP No.	3A4	3A5	3A7	2D6
320	75.29 ± 4.80	20.22 ± 5.01	21.85 ± 11.65	-4.31 ± 10.15
321	86.76 ± 2.08	25.38 ± 4.17	53.77 ± 6.42	18.92 ± 4.43
322	85.94 ± 5.95	21.85 ± 11.65	22.76 ± 5.04	-1.54 ± 4.77
323	83.57 ± 0.48	53.77 ± 6.42	25.69 ± 9.52	21.67 ± 4.82
324	61.06 ± 6.53	22.76 ± 5.04	22.98 ± 11.58	37.95 ± 9.56
325	64.19 ± 12.95	25.69 ± 9.52	17.18 ± 8.63	21.14 ± 5.82
326	70.28 ± 11.91	22.98 ± 11.58	20.32 ± 10.73	-2.65 ± 7.58
327	78.23 ± 8.86	17.18 ± 8.63	21.79 ± 6.12	30.00 ± 3.78
328	70.67 ± 20.11	20.32 ± 10.73	14.53 ± 0.58	-4.40 ± 7.74
329	86.74 ± 2.48	21.79 ± 6.12	34.44 ± 2.65	33.48 ± 6.17
330	73.35 ± 7.14	14.53 ± 0.58	7.06 ± 0.80	11.42 ± 2.94
331	68.36 ± 14.74	34.44 ± 2.65	19.99 ± 5.35	13.81 ± 4.79
335	68.75 ± 9.16	6.58 ± 0.27	3.78 ± 2.66	43.27 ± 6.44
313	58.37 ± 58.44	-1.59 ± 1.71	-1.66 ± 1.82	11.26 ± 0.98
314	28.94 ± 10.37	-1.79 ± 1.35	-4.704.82	14.91 ± 2.09
356	12.36 ± 6.49	-6.04 ± 2.53	-8.00 ± 1.03	4.53 ± 5.39
315	39.57 ± 8.14	-1.66 ± 1.82	-3.99 ± 0.06	8.71 ± 3.11
316	59.47 ± 9.70	-4.70 ± 4.82	-2.62 ± 1.92	19.72 ± 2.54
317	41.49 ± 11.64	-3.99 ± 0.06	2.46 ± 2.91	15.77 ± 5.98
318	53.08 ± 7.08	-2.62 ± 1.92	20.22 ± 5.01	15.49 ± 5.33
319	93.55 ± 8.37	2.46 ± 2.91	25.38 ± 4.17	18.42 ± 4.56
339	7.85 ± 6.36	-6.91 ± 4.00	59.82 ± 1.90	18.76 ± 1.25
354	7.25 ± 8.63	-11.37 ± 3.62	-6.04 ± 2.53	8.15 ± 0.28
337	14.54 ± 8.64	3.78 ± 2.66	-6.91 ± 4.00	15.50 ± 3.88
357	18.70 ± 14.89	-2.03 ± 0.99	2.26 ± 4.43	4.77 ± 4.17
358	20.13 ± 6.77	-8.00 ± 1.03	-1.89 ± 0.90	8.73 ± 5.45
355	13.23 ± 3.74	57.20 ± 3.90	-2.03 ± 0.99	13.56 ± 3.82
350	17.22 ± 11.32	26.13 ± 2.86	29.06 ± 2.64	6.64 ± 3.67
359	12.93 ± 7.30	34.03 ± 6.11	16.80 ± 1.00	14.39 ± 3.61
336	13.05 ± 0.52	4.65 ± 2.11	30.49 ± 4.17	2.12 ± 3.65
351	17.76 ± 8.98	16.80 ± 1.00	-1.53 ± 9.65	20.04 ± 3.78
352	9.09 ± 4.69	29.06 ± 2.64	-11.37 ± 3.62	5.17 ± 0.37
338	11.92 ± 0.94	30.49 ± 4.17	46.08 ± 1.46	-2.99 ± 2.60
332	18.92 ± 13.33	7.06 ± 0.80	8.03 ± 4.22	7.87 ± 8.25
334	52.07 ± 1.78	8.03 ± 4.22	4.65 ± 2.11	17.64 ± 0.11
353	3.19 ± 3.25	-1.52 ± 9.65	57.20 ± 3.90	22.03 ± 4.67
333	15.57 ± 2.63	19.99 ± 5.35	6.58 ± 0.27	31.54 ± 7.22
341	89.17 ± 1.58	59.82 ± 1.90	30.03 ± 0.48	101.79 ± 9.93

342	65.52 ± 4.57	49.31 ± 0.40	73.83 ± 2.64	57.93 ± 10.50
343	62.08 ± 16.14	30.03 ± 0.48	11.14 ± 0.24	68.26 ± 10.64
344	96.84 ± 0.34	73.83 ± 2.64	48.62 ± 1.44	59.45 ± 3.30
345	33.14 ± 9.93	11.14 ± 0.24	74.89 ± 3.41	77.44 ± 3.51
346	101.99 ± 0.30	48.62 ± 1.44	99.56 ± 0.53	42.54 ± 5.77
340	73.78 ± 1.51	46.08 ± 1.46	49.31 ± 0.40	39.58 ± 0.37
347	98.81 ± 0.05	74.89 ± 3.41	34.03 ± 6.11	83.06 ± 7.57
348	100.55 ± 1.44	99.56 ± 0.53	26.13 ± 2.86	91.42 ± 5.09

Table 6. Percent proliferation (±standard deviation) of 50 µg/mL ethonolic extracts of plant samples measure by MTT cell proliferation assay.

NRP No.	Botanical Name	Common Name	% Proliferation
320	*Glycine max*	Soybean	21.4 ± 2.1
321	*Glycine max*	Soybean	20.7 ± 1.9
322	*Glycine max*	Soybean	20.5 ± 2.5
323	*Glycine max*	Soybean	18.6 ± 1.8
324	*Glycine max*	Soybean	19.8 ± 2.0
325	*Glycine max*	Soybean	18.7 ± 2.7
326	*Glycine max*	Soybean	20.8 ± 3.2
327	*Glycine max*	Soybean	20.7 ± 1.6
328	*Glycine max*	Soybean	19.6 ± 2.7
329	*Glycine max*	Soybean	21.8 ± 2.2
330	*Glycine max*	Soybean	18.9 ± 2.4
331	*Glycine max*	Soybean	17.4 ± 2.6
335	*Phaseolus vulgaris*	Black Bean	18.8 ± 3.1
313	*Phaseolus vulgaris*	Black Turtle Bean	17.4 ± 2.6
314	*Phaseolus vulgaris*	Cranberry Bean	16.9 ± 2.5
356	*Phaseolus vulgaris*	Great Northern Bean	18.3 ± 1.9
315	*Phaseolus vulgaris*	Dark Red Kidney Bean	18.1 ± 3.7
316	*Phaseolus vulgaris*	Light Red Kidney bean Var. A	17.7 ± 3.1
317	*Phaseolus vulgaris*	Light Red Kidney bean Var. B	16.6 ± 3.8
318	*Phaseolus vulgaris*	White Kidney bean Var. A	17.8 ± 2.5
319	*Phaseolus vulgaris*	White Kidney bean Var. B	17.2 ± 1.9
339	*Phaseolus vulgaris*	White Kidney bean Var. C	17.7 ± 1.7
354	*Phaseolus vulgaris*	White Kidney bean Var. D	17.3 ± 2.6
337	*Phaseolus vulgaris*	Navy Bean	16.2 ± 2.9
357	*Phaseolus vulgaris*	Pinto Bean	17.5 ± 2.1
358	*Phaseolus vulgaris*	Small Red Bean	16.5 ± 1.6
355	*Lens culinaris*	Eston Lentil	17.6 ± 2.5
350	*Lens culinaris*	Green Lentil	17.5 ± 2.7
359	*Lens culinaris*	Red Lentil	17.8 ± 1.4
336	*Phaseolus. lunatus*	Lima Bean	16.9 ± 1.9
351	*Pisum sativum*	Green Pea	16.3 ± 1.3
352	*Pisum sativum*	Yellow Pea	16.3 ± 2.3
338	*Pisum sativum*	Yellow Split pea	16.1 ± 1.8
332	*Vigna unguiculata*	Black Eyed Pea	16.8 ± 2.5
334	*Vigna unguiculata*	Cow Pea	16.2 ± 1.2
353	*Cicer arietinum*	Chick pea	16.5 ± 2.3
333	*Cicer cayan*	Congo Pigeon pea	15.8 ± 2.1
341	*Apium graveoLens*	Celery seed A	-46.5 ± 7.6
342	*Apium graveoLens*	Celery seed B	-38.1 ± 5.3
343	*Coriandrum sativum*	Coriander	-27.8 ± 5.9

344	*Cuminum cyminum*	Cumin	-31.8 ± 4.3
345	*Anethum graveoLens*	Dill	-56.8 ± 6.6
346	*Foeniculum vulgare*	Seed	-60.3 ± 8.2
340	*Ocimum basilicum*	Basil leaves	-52.9 ± 7.6
347	*Origanum vulgare*	Oregano leaves	-49.6 ± 7.1
348	*Rosemarinus officinalis*	Rosemary	-68.9 ± 5.2

Table 7. Antimicrobial effects of selected *Fabaceae* methanolic and ethanolic extracts (50 mg/mL) against 7 bacterial species. Zones of inhibitions are measured according to the Kirby-Bauer disc diffusion assay. Values represent average diameters between triplicates of and measured in millimetres. Ciprofloxacin is used as the positive control. (-) denotes no inhibitory activity or a zone of inhibition of less than 6 mm.

Common Name	NRP#	*Bacillus subtilis* (Gram +) ATCC#23857	*Enterococcus. faecalis* (Gram +) ATCC#49452	*Listeria innocua* (Gram +) ATCC#51742	*Escherichia coli* (Gram -) ATCC#1157	*Pseudomonas putida* (Gram -) ATCC#12633	*Provedencia stuartii* (Gram -) ATCC#33672	*Acetobacter calcoaceticus* (Gram -) ATCC#19011
Soybean	320	-	-	-	-	-	-	-
		-	-	-	-	-	-	-
Soybean	321	-	-	-	-	-	-	-
		-	-	-	-	-	-	-
Soybean	322	-	-	-	-	-	-	-
		-	-	-	-	-	-	-
Soybean	323	-	-	-	-	-	-	-
		-	-	-	-	-	-	-
Soybean	324	-	-	-	-	-	-	-
		-	-	-	-	-	-	-
Soybean	325	-	-	-	-	-	-	-
		-	-	-	-	-	-	-
Soybean	326	-	-	-	-	-	-	-
		-	-	-	-	-	-	-
Soybean	327	-	-	-	-	-	-	-
		-	-	-	-	-	-	-
Soybean	328	-	-	-	-	-	-	-
		-	-	-	-	-	-	-
Soybean	329	-	-	-	-	-	-	-

		-	-	-	-	-	-	-
Soybean	330	-	-	-	-	-	-	-
		-	-	-	-	-	-	-
Soybean	331	-	-	-	-	-	-	-
		-	-	-	-	-	-	-
Black bean	335	-	-	-	-	6.0 ± 0.0	-	7.5 ± 0.0
		-	-	-	-	6.0 ± 0.0	-	7.0 ± 0.0
Black Turtle bean	313	-	-	-	6.5 ± 0.0	6.5 ± 0.0	6.7 ± 0.5	6.0 ± 0.0
		-	-	-	6.5 ± 0.0	6.5 ± 0.0	6.5 ± 0.0	6.0 ± 0.0
Cranberry bean	314	-	-	-	-	-	-	-
		-	-	-	-	-	-	-
Great Northern bean	356	-	-	-	-	6.5 ± 0.0	-	-
		-	-	-	-	6.5 ± 0.0	-	-
Dark Red Kidney bean	315	-	-	-	-	6.7 ± 0.5	6.5 ± 0.0	-
		-	-	-	-	6.0 ± 0.0	6.0 ± 0.0	-
Light Red Kidney bean Var. A	316	-	6.5 ± 0.0	-	-	6.5 ± 0.0	-	8.7 ± 0.7
		-	6.5 ± 0.0	-	-	6.5 ± 0.0	-	8.0 ± 0.0
Light Red Kidney bean Var. B	317	-	-	-	6.3 ± 0.4	-	-	-
		-	-	-	6.0 ± 0.0	-	-	-
White Kidney bean Var. A	318	-	6.0 ± 0.0	-	-	-	-	8.5 ± 0.0
		-	6.0 ± 0.0	-	-	-	-	8.5 ± 0.0
White Kidney bean Var. B	319	-	-	-	-	-	-	-
		-	-	-	-	-	-	-
White Kidney bean Var. C	339	-	-	-	-	-	-	-
		-	-	-	-	-	-	-
White Kidney bean Var. D	354	-	-	-	-	-	-	-
		-	-	-	-	-	-	-
Navy bean	337	-	-	-	-	-	-	-
		-	-	-	-	-	-	-
Pinto bean	357	-	-	-	-	-	-	-
		-	-	-	-	-	-	-
Small Red bean	358	-	-	-	-	-	-	-
		-	-	-	-	-	-	-

Eston lentil	355	-	-	-	-	-	-	-
		-	-	-	-	-	-	-
Green lentil	350	-	-	-	-	-	6.5 ± 0.0	-
		-	-	-	-	-	6.5 ± 0.0	-
Red lentil	359	-	-	-	-	-	6.7 ± 0.5	-
		-	-	-	-	-	6.0 ± 0.0	-
Lima bean	336	-	6.5 ± 0.0	-	6.7 ± 0.5	-	-	7.7 ± 0.6
		-	6.0 ± 0.0	-	6.0 ± 0.0	-	-	7.0 ± 0.0
Green pea	351	-	-	-	-	-	-	-
		-	-	-	-	-	-	-
Yellow pea	352	-	-	-	-	-	-	-
		-	-	-	-	-	-	-
Yellow split pea	338	-	-	-	-	-	-	6.5 ±0.0
		-	-	-	-	-	-	6.3 ± 0.4
Black-eyed pea	332	-	6.3 ± 0.4	-	-	-	-	-
		-	6.0 ± 0.0	-	-	-	-	-
Cow pea	334	-	-	-	-	-	-	-
		-	-	-	-	-	-	-
Chick pea	353	-	-	-	-	-	6.0 ± 0.0	-
		-	-	-	-	-	6.0 ± 0.0	-
Congo Pigeon pea	333	-	-	-	-	-	-	6.5 ± 0.0
		-	-	-	-	-	-	6.0 ± 0.0

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