Antimicrobial and P450 Inhibitory Properties of Common Functional Foods

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ABSTRACT - PURPOSE: To study the effect of functional foods on human cytochrome P450 (CYP) and the gut bacterial microflora that may potentially affect drug metabolism and ultimately affect human health and wellness. METHODS: 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 also investigated with 7 selected bacterial surrogate species to determine potential effects on the gut microflora. RESULTS: The highest CYP inhibitory activities, based upon visual examination, were observed from extracts of celery seed, cumin, fennel seed, basil, oregano, and rosemary belonging to the *Apiaceae* and *Lamiaceae* families, respectively. Likewise, the strongest antimicrobial activities were also observed in the *Apiaceae* and *Lamiaceae*. No significant antimicrobial and CYP inhibition was observed in the *Fabaceae* extracts. CONCLUSION: Results demonstrated the possible risk of food-drug interactions from spice and herb plants may affect drug disposition and safety.

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INTRODUCTION

Increased health awareness has led many consumers to become more vigilant in maintaining good health. Many consumers 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 (1). 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 to severe rashes and serious effects by those who used blood thinners etc (2). 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 (3). The high levels of bioactive phytochemicals in some diets have also raised concerns about possible food-drug and NHP-drug interactions.

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 exceeding the dose required for 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 (4, 5). It was found that furanocoumarins (FC) from grapefruit juice was responsible for mechanism-based inhibition of CYP activity, 6′,7′-dihydroxybergamottin being one of several FCs identified (6). Recent studies have also reported that star fruit and pomegranate juice may also inhibit

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drug metabolism (7, 8).

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 (9). 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 (10). One example of the effect of the microflora on drug disposition is with digoxin. A higher percentage of the North American population relative to a population from southern India converts digoxin to reduced metabolites (11) suggesting that consideration of human gut microbial activities should be part of any treatment regimen. Due to the symbiotic and mutualistic microflora, and host relationship (12), 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.

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, and as such may impact population differently based on diets.

To broaden understanding of 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 1) and affect 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. By testing many common food samples, the assessment of potential food-drug interactions and antimicrobial activities across a broad spectrum of diets and therapeutic use of functional foods was achieved.

MATERIALS AND METHODS

Chemicals and reagents

CYP enzymes 3A4 (Human CYP3A4 + reductase, 1 nM, 500 uL – Cat# 456207), 3A5 (Human CYP3A5 + reductase, 1 nM, 500 uL – Cat# 456235), 3A7 (Human CYP3A7 + reductase + b5, 0.5 nM, 500 uL – 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^{o}C until required. NADPH (β-NADPH reduced tetrasodium salt hydrate – Cat# N7505-1GR), was from Sigma Aldrich (Oakville, ON, Canada) and stored at -20^{o}C under very low light conditions. Ketoconazole was purchased from Calbiochem (Gibbstown, NJ, USA). Methanol was purchased from Fisher Scientific Canada (Ottawa, ON, Canada).

Sample collection

All samples come from local supermarkets or farms in the Ottawa or Guelph areas of Ontario, Canada. 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 1). Each sample was weighed and divided into three portions. One portion was stored in -20^{o}C for archiving at the University of Ottawa Herbarium and the remaining two portions were ground up into 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^{o}C until required and the last portion was stored at -4^{o}C for daily extractions.

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.

Table 1 - Products selected their potential to affect human cytochrome P450-mediated metabolism. NRP, Nutraceutical Research Program number.

NRP #	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*	Lt† Red Kidney bean Var A	*Fabaceae*	Canada
317	*Phaseolus vulgaris*	Lt 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*	seed	*Apiaceae*	N/A
340	*Ocimum basilicum*	Basil leaves	*Lamiaceae*	Imported
347	*Origanum vulgare*	Oregano leaves	*Lamiaceae*	Imported
348	*Rosemarinus officinalis*	Rosemary	*Lamiaceae*	Imported

* this was the designation on the product label. † lt, light.

The sample was then centrifuged in a Fisher Scientific (Ottawa, ON, Canada) Micro12 Centrifuge 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 daily.

Fluorometric microtitre cytochrome P450 inhibition assays

A fluorometric microtitre plate assay was used to assess the inhibitory capacities of the plant extracts against CYP3A4, 3A5, 3A7 and 2D6. The procedure used was adapted and modified from Crespi et al. (13) and Scott et al. (14). 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 triplicate. Each assay was repeated at least once. 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.

Antimicrobial assay

Extracts were examined by antimicrobial assays using the Kirby-Bauer disc diffusion assay (40). 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, Nepean, 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. Ciprofloxacin was used as the positive control against all 7 bacterial species.

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 1A).

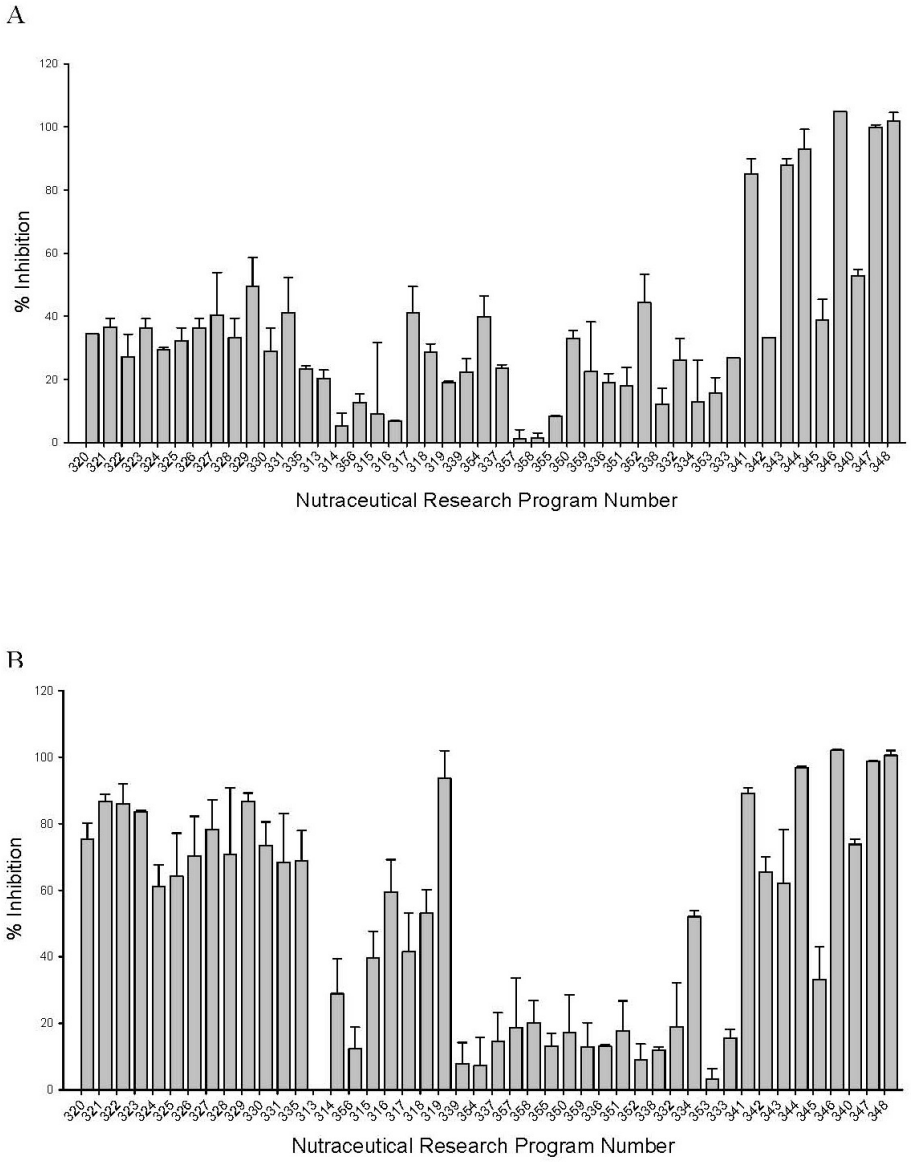

Figure 1 - Percent inhibition of methanolic (A) and aqueous (B) extracts (50 mg/mL) from common food samples on cytochrome P450 3A4 isozyme. Values are presented as means ± standard deviation.

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 1B). 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 2A). 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 2B). 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 3A). 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 3B). 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.

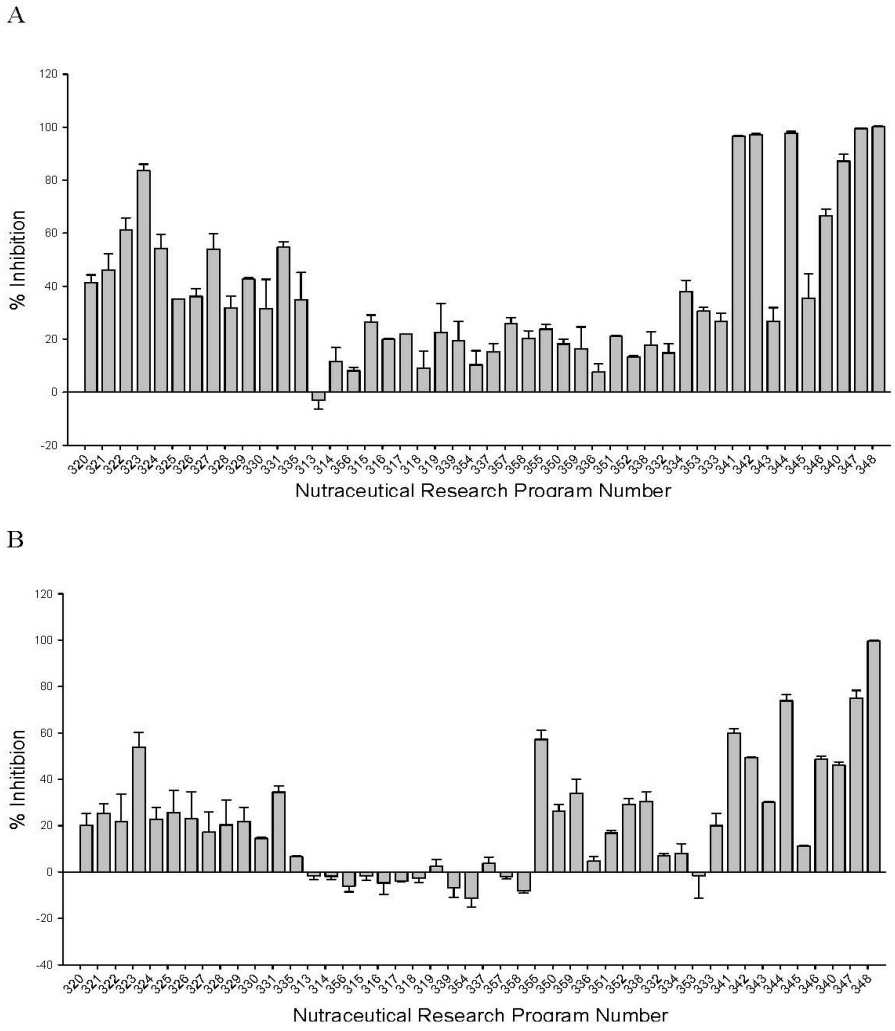

Figure 2. - Percent inhibition of methanolic (A) and aqueous (B) extracts (50 mg/mL from common food samples on cytochrome P450 3A5 isozyme. Values are presented as means ± standard deviation.

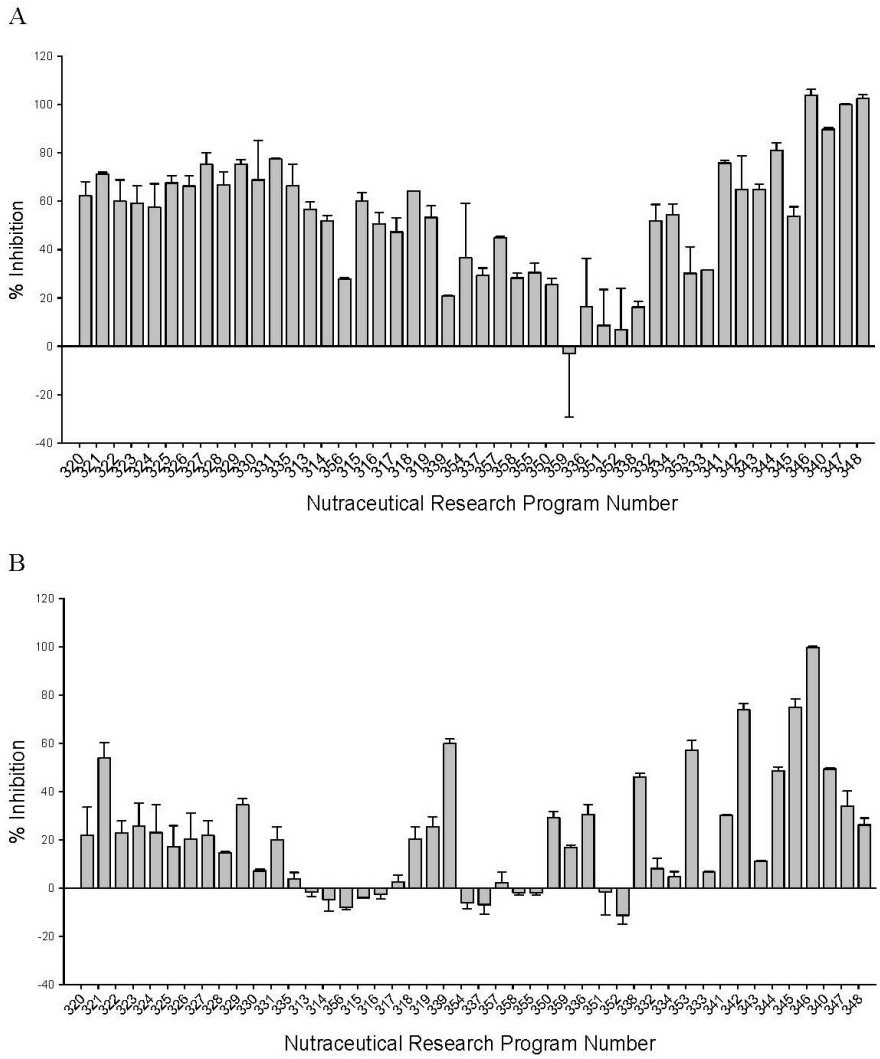

Figure 3. - Percent inhibition of methanolic (A) and aqueous (B) extracts (50 mg/mL) from common food samples on cytochrome P450 3A7 isozyme. Values are presented as means ± standard deviation.

As previously highlighted from the results of CYP3A4, 3A5 and 3A7 data, the *Apiaceae* and *Lamiaceae* methanolic extracts had the highest CYP2D6 inhibition (Figure 4A). 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 4B). 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.

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 was observed with both methanolic and aqueous extracts of *Apiaceae* and *Lamiaceae* species shown in Table 2, 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*.

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, based on a visual examination of the data, have a higher potential than the *Fabaceae* products examined. The higher levels of activity in spices and herbs may be due to their selection for flavor, which is associated with a high level of phytochemicals (15).

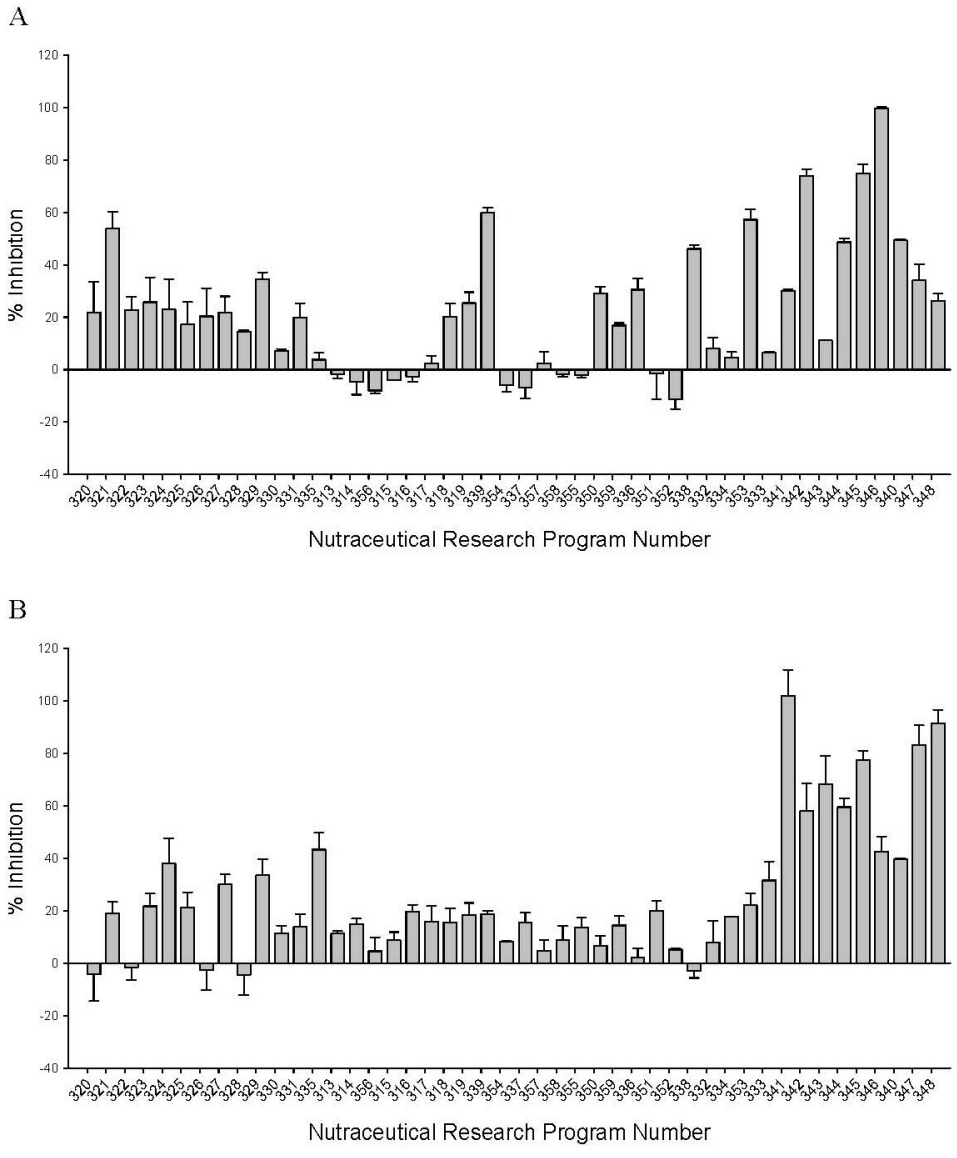

Figure 4. - Percent inhibition of methanolic (A) and aqueous (B) extracts (50 mg/mL) from common food samples on cytochrome P450 2D6 isozyme. Values are presented as means ± standard deviation.

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 FCs (16). The results obtained from fennel seed consistent with the report by Subehan et al. (16) that identified 5-methoxypsoralen (5-MOP) as a mechanism-based inhibitor of CYP3A. Coriander and dill of the *Apiaceae* family, although also containing FCs (17) are not reported to express high levels of activity. The varying levels of inhibitory activity may be due to strain or chemotype differences, environmental factors related to growth and harvest conditions, instability during storage or transport, or processing (manufacturing) affecting the concentration or types of FCs present.

The data obtained in this study suggests 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 (8, 18). 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 (19) strongly cautions the possibility of flavonoid-drug interactions in functional foods and herbal supplements, and counsels the need for advisory labeling of unregulated products.

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 (20, 21). Lentil and other beans from other genera had lower inhibitory potential.

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, belonged to rosemary and oregano. Previous studies have shown oregano and rosemary to have high antibacterial activity against *E. coli* (22-24). *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 (25). 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 (26). The coloured seed coats were observed to be a potential indication of bioactive secondary metabolites such as anthocyanins, condensed tannins and flavonoids (26).

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 spice and herb. 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* (17). 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 (27). 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 (28). Therefore this data suggests that the selected herbs and spices contain phytochemicals that can influence drug metabolizing enzymes and some bacterial species suggesting that there may also be an effect on 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. The results

presented in this study are consistent with and extend earlier findings by our group that foodstuffs such as garlic (29), soy (30) and traditional West African plants (31) also have the potential to after CYP-mediated metabolism.

CONCLUSIONS

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 deemed safe when consumed in reasonable amounts, but when consumed in larger amounts or together with other therapeutic products, the data obtain from this study indicates that there is a potential for drug interactions between functional foods and other therapeutic products. This may be an underlying cause or contributing factor for some drug overdoses, drug resistance or therapeutic failure. Although the majority of healthy individuals will see very little, if any, effect when consuming common food products, patients undergoing serious medical care should become more aware of potential risks identified with certain foods. More work is needed to examine the inhibitory properties of these samples under different sample selection criteria and extraction conditions. Studies are required to determine if there is an effect on the complex human gut microbiome.

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Table 2. - Antimicrobial effects of methanolic and ethanolic extracts of selected *Apiaceae* and *Lamiaceae* (50 mg/mL) *against Bacillus subtilis, Enterococcus faecalis, Listeria innocua, Escherichia coli, Pseudomonas putida, Providencia sturatii,* and *Acetobacter calcoaceticus*. 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 6 mm, where disc diameter was 5 mm.

	B. subtilis (Gram +)	*En. faecalis* (Gram +)	*L. innocua* (Gram +)	*E.coli* (Gram -)	*Ps. putida* (Gram -)	*P. stuartii* (Gram -)	*A. calcoaceticus* (Gram -)
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