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Matthew George Menkart March 15, 2023

Inhibition of Cytochrome P450 Isoforms by Ethanolic Extracts of *Cinnamomum* spp. (Lauraceae)

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An abstract of

a thesis submitted to the Faculty of Emory College of Arts and Sciences

of Emory University in partial fulfillment

of the requirements of the degree of

Bachelor of Science with Honors

Biology

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Abstract

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Herbal medicine is a key component of both traditional and Western medicines, but many herbs lack sufficient research on their ability to inhibit or induce cytochrome P450 enzymes (CYPs). Usage in conjunction with FDA-approved medication may lead to drug-herb interactions and potentially cause the accumulation of toxic metabolites, or other dangerous side effects. One of the most used traditional herbal medicines, Cinnamon (*Cinnamomum* spp.), was found to possess CYP inhibitory activity through prior high-throughput screening efforts. These studies aimed to determine species variation in CYP inhibition by *C.* species and identify which CYP isoforms are most affected. Five isoforms, CYP2B6, CYP2C9, CYP2C19, CYP2D6, and CYP3A5, were tested against crude ethanolic extracts of four *Cinnamomum* species: *C. burmannii, C. verum, C. cassia,* and *C. camphora*. Extracts were prepared at concentrations ranging from 2 µg/mL to 64 µg/mL and inhibition was quantified using a half-maximal inhibitory concentration value. The greatest inhibition was observed in CYP2C9, with *C. burmannii* and *C. verum* being more inhibitory than *C. cassia* and *C. camphora*. This trend was seen across CYP2B6 and CYP3A5, CYP2C19 was most inhibited by *C. camphora*, while CYP2D6 showed negligible inhibition by the *Cinnamomum* extracts. Subsequent liquid-liquid partitioning and fractionation via reverse-phase high-performance liquid chromatography of *C. burmannii* suggest that inhibition of CYP2C9 may be attributed to only a few, non-polar phytochemicals. These results suggest that cinnamon has the ability to inhibit multiple CYP isoforms, but the extent of their inhibition varies across species, with *C. burmannii* and *C. verum* being the most inhibitory.

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Acknowledgements

To Dr. Quave – I am so incredibly thankful that you selected me to join your team. Throughout my time at the Quave Research Group, you have helped me grow from a student who learned to pipette virtually during a pandemic to an honors researcher with a thesis under their belt. You helped me look at medicine and the sciences in a completely different light by introducing me to the field of ethnobotany and all of its many applications. The Quave Research Group has been one of the highlights of my undergraduate experience, so I would like to thank you for entrusting me with this project and guiding me to where I am today.

To William Crandall and Emily Edwards – Thank you for being such great mentors. Without your patience and guidance, I would have never been able to develop into the researcher that I am now, nor learned any of the skills that I will continue to use throughout my career. Whether it was teaching me how to pipette into a black plate in the dark or teaching me how to use (and not be afraid of) the HPLC, you two continued to guide me. I am indebted to you two and hope I can as good of a mentor to someone else one day.

To the Quave Research Group – Thank you for making my past two years in the lab an incredible and welcoming experience. To Cate, Lewis, Marco, Dr. Gras, Dr. Samarakoon, Dr. Woo, and the other undergrads, thank you for offering me your wisdom and guidance to help me see my project from so many perspectives, help me troubleshoot my experiments when they went wrong, and simply being a support team when I needed it.

To my committee members – Thank you for serving on my committee and offering your perspectives from your respective fields. Dr. Weinschenk, thank you for introducing me to the rigorous field of organic chemistry, as I would have never fallen in love with ethnobotany without this strong background. Dr. Campbell, thank you for not only teaching me about the exciting field of microbiology but on how to read scientific literature and proper lab techniques in your discussion and laboratory classes.

To my mother and sisters – Thank you for being so supportive of my science career ever since day one. Despite the stresses that come with the honors program (and college in general), I always had you all to lean on, just a phone call away. I feel honored to call you my family.

To my friends and fraternity brothers – Thank you for your continual support and encouragement, cheering for me during the highs and lows of my research experience.

Research support and funding – Thank you to the Garden Club of America for awarding me with the 2022 Zeller Summer Scholarship in Medicinal Botany to support my project "Inhibition of Drug-Metabolizing Cytochrome P450 Enzymes by Common Dietary Supplements". I could have never completed this project without this scholarship, and I am incredibly thankful. This study was supported by the National Institutes of Health (R21AT011105 to CLQ).

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Chapter 1: Introduction

1.1 Botanical Medicine and Herbal Supplements

While seldom considered in medical discussions, herbal supplements play a major role in human health throughout the world. Throughout the course of human history, medicinal herbs have been an integral part of treating illness and are still prevalent in certain traditional medicines and even allopathic medicine. However, the terms “dietary supplement”, “herbal supplement”, “herbal medicine”, and “traditional medicine” all share key components of their definitions but are often mistakenly used interchangeably. The Food and Drug Association is the agency that regulates drugs and biological products in the United States, and it defines a dietary supplement as “a vitamin; mineral; herb or other botanical; amino acid; dietary substance for use by man to supplement the diet by increasing the total dietary intake; or a concentrate, metabolite, constituent, extract, or combination of the preceding substances. Unlike drugs, supplements are not intended to treat, diagnose, prevent, or cure diseases” (FDA).

Herbal supplements are dietary supplements that are derived from plant (and sometimes fungal) material, including fruits, flowers, inflorescence, berries, leaves, stems, or the whole plant. While there is a need to conduct more research on the efficacy of herbal supplements, research shows that many are indeed helpful, such as the ability of echinacea (*Echinacea purpurea* (L.) Moench, Asteraceae*)* to reduce one’s risk of catching a cold, the ability of St. John’s Wort (*Hypericum perforatum* L., Hypericaceae*)* to improve depression symptoms, and the ability of cranberry (*Vaccinium macrocarpon* Aiton, Ericaceae*)* to prevent UTIs in people at high risk for contracting them (U.S. Department of Health and Human Services). Additionally, there have even been two botanicals that have been approved for FDA use, sinecatechins (derived from *Camellia sinensis* (L.) Kuntze, Theaceae*)* in 2006 and Crofelemer (derived from *Croton lechleri* Müll.Arg.,

Euphorbiaceae) in 2013, which further cements the importance of botanical medicine in health (Sun & Qian, 2021).

Pharmaceutical drugs, unlike herbal medicines, tend to be composed of one or a few compounds. Herbal medicines instead are the product of years of evolution that plants and fungi have undergone to protect themselves from diseases and predators via secondary metabolites. This has resulted in the development of "pleiotropic, multi-targeted molecules" (Carmona & Soares Pereira, 2013) such that one herbal medicine can be used to treat multiple ailments. Furthermore, while pharmaceutical drugs are screened for their ability to modulate the activity of a protein of interest, plants have already evolved this ability, meaning that their herbal medicines contain many small molecules that can modulate or inhibit the ability of multiple proteins (Koehn & Carter, 2005). This can be attributed to the fact that while pharmaceutical drugs are composed of many heteroatoms like nitrogen, sulfur, phosphorus, and halogens, natural products contain complex carbon chains with many chiral centers and a much higher abundance of oxygen heteroatoms relative to pharmaceuticals (Koehn & Carter, 2005). One example can be seen in the differences between synthesized antifungals and antifungals isolated from plants (Figure 1.1). Miconazole is an antifungal that was synthesized in the 1960s, and its structure includes an imidazole moiety (two non-adjacent nitrogen atoms in a five-membered ring) and four chlorine atoms (Fothergill, 2006). On the other hand, micafungin, an antifungal derived from the fungus *Coleophoma empetri* (Rostr.) Petr. (Helotiales *incertae sedis*), is an echinocandin, a class of large water-soluble lipoproteins that can bind to many different proteins responsible for cell-wall synthesis (Carver, 2004).

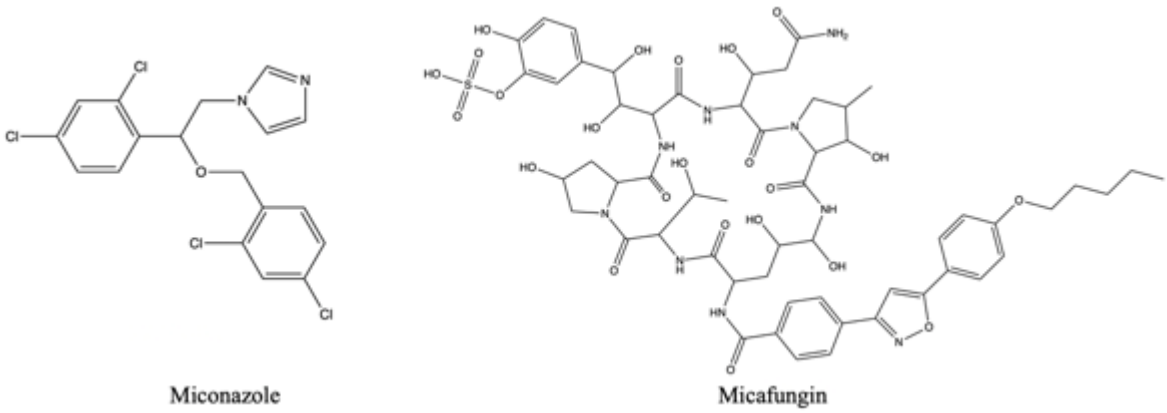


Figure 1.1. Comparison of synthesized and natural antifungals. Miconazole (left), a synthesized antifungal, is much simpler and contains more non-oxygen heteroatoms as compared to micafungin (right), a fungal secondary metabolite with antifungal properties.

In a 2002 nationwide study conducted by the National Center for Health Statistics, it was found that 18.9% of the U.S. used natural herbs or supplements in the past 12 months, which was double the rate in 2002 (Kennedy, 2005). In this study, the top ten most commonly used products were *Echinacea*, Ginseng (*Panax* L., Araliaceae)*, Gingko biloba,* garlic (*Allium sativum* L., Amaryllidaceae*)* supplements, glucosamine, St. John's Wort (*Hypericum perforatum*), peppermint (*Mentha* × *piperita* L., Lamiaceae), fish oils/omega fatty acids, ginger (*Zingiber officinale* Roscoe, Zingiberaceae) supplements, and soy (*Glycine max* (L.) Merr., Fabaceae) supplements (Kennedy, 2005).

Usage of herbal supplements was not equivalent across socioeconomic status, as usage was higher in females, individuals with greater than a high school education, and individuals whose annual family income was greater than or equal to $20,000. The most common conditions that were treated using herbal and natural products were head or chest cold; stomach or intestinal illness; arthritis, gout, lupus, or fibromyalgia; joint pain or stiffness; and anxiety/depression (Kennedy, 2005).

Interestingly, however, 57.6% of respondents did not inform their conventional medical professional that they were using herbal or natural products (Kennedy, 2005). Compounded with the fact that 20.6% of respondents used prescription and over-the-counter (OTC) medication in the past year, the unmonitored use of herbal medicines presents the potential risk for complications and unwanted side effects (Kennedy, 2005).

Reasons behind the usage of herbal supplements vary greatly, though can be divided into two broad categories: the belief that herbal medicine is superior to allopathic medicine and the patient's concerns about the modern healthcare system. Herbal supplements are significantly cheaper than pharmaceuticals, and many patients believe that herbal supplements are both more effective and safer than their allopathic alternatives. Similarly, in recent years more and more patients are losing their trust in the healthcare system, feeling as if their physicians are not truly addressing their concerns, and/or simply do not have time to visit a physician, which has culminated in a self-medication movement where patients take healthcare into their own hands via dietary supplements and botanicals (Ekor, 2014).

During the COVID-19 pandemic, sales of herbal supplements in the United States increased by 17.3% in 2020 from the previous year (Tyler Smith, 2021). The herbs with the largest increase in sales were elderberry (*Sambucus* spp. L., Viburnaceae) with a 150.3% increase, ashwagandha (*Withania somnifera* (L.) Dunal, Solanaceae) with a 185.2% increase, and apple cider vinegar (*Malus* spp. Mill., Rosaceae) with a 133.8% increase. Elderberry and apple cider vinegar both have been used to boost the immune system, with the former also having reported anti-viral activity, while ashwagandha was reported to be primarily used to help with stress and anxiety (Tyler Smith, 2021).

As previously stated, herbal supplements are not regulated by the U.S. Food and Drug Administration like prescription drugs. Instead, the Dietary Supplement Health and Education Act of 1994 was passed to amend the Federal Food, Drug, and Cosmetic Act. In doing so, the act provided guidelines to determine whether a dietary supplement should be considered "adulterated," and provided guidelines on what types of nutritional claims can be made about a supplement. Specifically, manufacturers are allowed to describe the classical structure function of the active ingredient and diseases that arise from a deficiency of the ingredient. However, the manufacturer is only allowed to promote truthful, non-misleading information and additionally include the following warning: "This statement has not been evaluated by the Food and Drug Administration. This product is not intended to diagnose, treat, cure, or prevent any disease." (*S.784 - Dietary Supplement Health and Education Act of 1994*, 1994).

While manufacturers are not legally allowed to market dietary supplements as specific cures, they are allowed to sell supplements that circumvent FDA approval and offer little benefit to the consumer. One such example is green tea extract. Green tea is a beverage that is consumed all throughout the world, primarily by preparing an infusion of unfermented *Camellia sinensis* leaves. However, green tea extract can be prepared via many different methods and extracting solvents. While some extracts may be beneficial, others may be ineffective at treating the same ailments *C. sinensis* infusions treat, and some may be linked to liver injury (Thakkar et al., 2020).

The danger of herbal supplements is apparent when examining data from the National Poison Control Data System. The National Poison Control Data System, which collects data from US Poison Control Centers, reported that between 2000 and 2012, there were 274,998 dietary supplement exposures. 31.9% of these exposures were botanicals, and of the botanical exposures, 10.1% had serious outcomes (Rao et al., 2017). 12.1% of all exposures were attributed to Ma Huang,

a tea produced from the plant *Ephedra sinica* Stapf, Ephedraceae according to Chinese Traditional Medicine. Ma Huang has traditionally been used to treat allergies, bronchial asthma, colds, and fevers, and pharmacologically has anti-inflammatory, anti-bacterial, anti-fungal, anti-viral, and anti-cancer activity (Zhang et al., 2018). Despite this, *E. sinica* has high levels of amphetamine-type alkaloids, ephedrine and pseudoephedrine, both of which are central nervous system stimulants and bronchodilators (Zhang et al., 2018). When these alkaloids are combined with other stimulants like caffeine (a methylxanthine alkaloid), the combination is cardiotoxic in rodent models (Dunnick et al., 2007).

1.2 Drug Metabolism

Drug metabolism is a cornerstone of modern medicine and pharmacology. It is imperative to understand the process of drug metabolism when investigating the pharmacokinetics, potency, and toxicity of a xenobiotic. A xenobiotic is defined as a "non-essential chemical that enters the body from the environment. The term includes many secondary natural products (alkaloids, terpenes, etc.), drugs, carcinogens, and various synthetic chemicals" (Guengerich et al., 2005). Resultingly, one of the major hurdles researchers encounter during drug development is the investigation of the metabolism of the drug, as a drug that produces strong results but is also highly toxic to the body is not safe to distribute.

Drug metabolism is a series of biotransformations undergone by the xenobiotic. This type of metabolism usually occurs in two phases: Phase I, modification, and Phase II, conjugation. Some drugs undergo a third phase, Phase III, which assists in the excretion of the drug from cells.

Phase I is known as the modification phase. This is the phase in which the drug's chemical structure is altered via different chemical modifications, such as oxidation, reduction, hydrolysis, cyclization, or dehydrogenation (Susa & Preuss, 2017). The enzyme most responsible for

phase I metabolism are enzymes of the cytochrome P450 system (CYP450), though other enzymes are important as well, such as flavin-containing monooxygenases, monoamine oxidases, prostaglandin H synthase, alcohol dehydrogenase, and aldehyde dehydrogenase (Strolin Benedetti et al., 2006).

Phase I modifications is also the phase in which prodrugs are converted to active metabolites (Susa & Preuss, 2017). While most drugs are the active metabolite, about 10% of drugs on the market are instead prodrugs, "inactive precursors of an active drug designed to be bio-converted (activated) post-administration with the main aim of improving the pharmacokinetic properties of the parent drug" (Najjar & Karaman, 2019). One such example is floxuridine, a chemotherapy drug designed to treat colon and hepatic cancers. By converting the active metabolite to a prodrug via an ester-linked amino acid, the prodrug is able to cross into hepatic and colonic cells much more efficiently than the active metabolite via the protein (PEPT1) transporter, allowing the drug to be selective for the tumor and minimize the damage to undesired cell types (Landowski et al., 2005).

Phase II is known as the conjugation phase, as this phase involves the addition, or "conjugation" of the xenobiotic with chemical moieties with the purpose to convert the xenobiotic to an inert and/or water-soluble form to reduce toxicity and aid in excretion. Types of conjugation include methylation, acetylation, sulfonation, glucuronidation, and glutathione conjugation (Susa & Preuss, 2017) (Figure 1.2). These processes are mitigated by the phase II transferases – methyltransferase, acetyltransferase, sulfotransferase, UDP-glucuronosyltransferase, and glutathione S-transferase, respectively (Jancova et al., 2010).

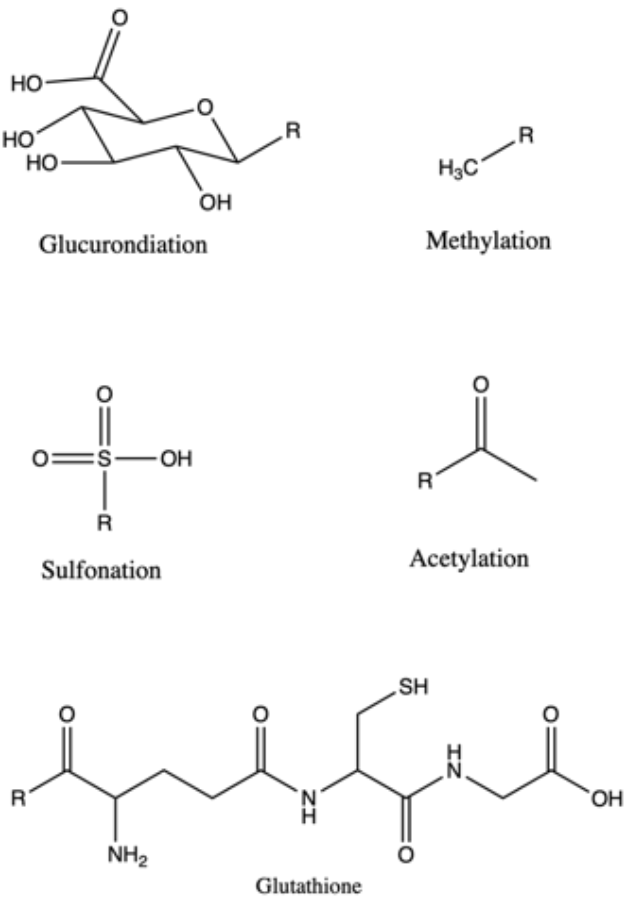


Figure 1.2. Types of conjugation xenobiotics undergo during phase II of drug metabolism.

One such example of Phase II metabolism is the metabolism of acetaminophen, the active ingredient in Tylenol. Acetaminophen is either glucuronidated or sulfonated, converting the drug into an inactive compound that reduces toxicity in the body and assists with excretion through urine (Mazaleuskaya et al., 2015) (Figure 1.3).

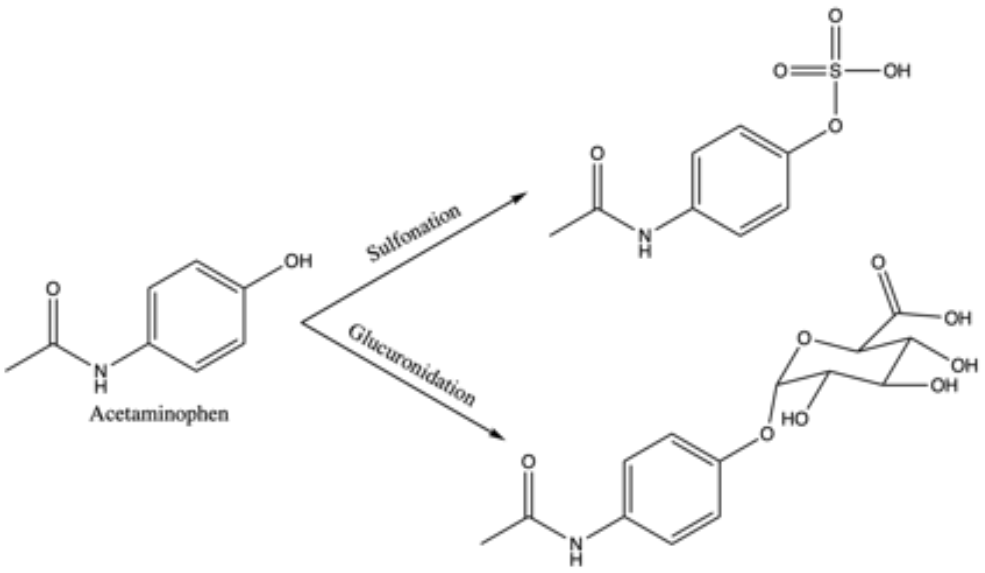


Figure 1.3. Metabolism of acetaminophen via sulfonation and glucuronidation in Phase II metabolism. Acetaminophen can be metabolized by sulfonyl transferases or UDP-glucuronosyltransferases to produce a conjugated metabolite that is more easily excreted by the body.

1.3 The Cytochrome P450 System

The cytochrome P450 system is the enzyme system that is most responsible for the phase I metabolism of xenobiotics. The cytochrome P450 system (CYP) is a superfamily of hemeproteins that metabolize drugs and other xenobiotics, such as plant and fungal secondary metabolites (Munro et al., 2018). In addition to xenobiotics, the cytochrome P450 system is responsible for the metabolism of sterols, fatty acids, eicosanoids, and vitamins A and D (F. Peter Guengerich, 2006).

There are 57 known cytochrome P450 enzymes in humans, of which one-quarter of them are responsible for xenobiotic metabolism (F. Peter Guengerich, 2006). Cytochrome P450 enzymes are monooxygenases, meaning that they use molecular oxygen (O_2) and a pyridine nucleotide (NADH or NADPH) to oxidize the substrate (Guengerich, 2018). The cytochrome P450 system tends to be specific in terms of drug metabolism, such that a drug of interest will be metabolized by a

specific CYP. Ninety percent of drug metabolism is concentrated amongst 6 specific CYPs: 3A4, 3A5, 1A2, 2C9, 2C19, and 2D6 (F. Peter Guengerich, 2006).

Like many enzymes, cytochrome P450 enzymes can be inhibited and induced. Inhibition of cytochrome P450 enzymes can be categorized by the mechanism in which the compound(s) inhibit the enzyme: competitive, uncompetitive, non-competitive, or irreversible. Additionally, inhibitors can be mixed inhibitors, meaning that they bind to both the active and allosteric sites, and some substrates can also be classified as inhibitors. Supplemental Table 1 is adapted from Indiana University School of Medicine's Clinical Pharmacology Department's "Drug Interactions Flockhart Table™," which includes a list of substrates, inhibitors, and inducers of the 6 CYPS aforementioned.

One of the most notorious inhibitors of the cytochrome P450 system is grapefruit juice, coming from the plant species *Citrus* × *paradisi*. Grapefruit contains the compound bergamottin, a furanocoumarin and plant secondary metabolite. Bergamottin is also one of the primary compounds in *Citrus* × *paradisi* that inhibits the cytochrome P450 system, specifically CYP3A4 (He et al., 1998). Bergamottin is thought to act as a competitive inhibitor of CYP3A4, which poses an issue when other xenobiotics are needed to be metabolized (He et al., 1998).

CYP2C9 is one of the approximately fifty CYPs found in humans. Like other CYPs, CYP2C9 is a phase I drug-metabolizing enzyme. An analysis of 248 clinical drugs with known CYP involvement revealed that CYP2C9 was responsible for the metabolism of 12.8% of those drugs, only behind CYP3A4/5 (30.2%) and CYP2D6 (20%) (Zanger & Schwab, 2013). The enzyme is responsible for the metabolism of multiple drug classes: non-steroidal anti-inflammatory drugs such as celecoxib, diclofenac, and ibuprofen; sulfonylureas such as glipizide and tolbutamide; angiotensin II receptor agonists including irbesartan and losartan; certain antidepressants including amitriptyline

and fluoxetine (Flockhart DA). CYP2C9 also is responsible for the metabolism of dietary flavonoids found in citrus fruits – naringenin, naringin, quercetin, and rutin (Sousa et al., 2013).

CYP2C9 can be inhibited and induced by both pharmaceutical drugs *and* secondary metabolites alike. Some of the most potent CYP2C9 drug inhibitors include amiodarone, fluconazole, fluvastatin, metronidazole, miconazole, sulfaphenazole, while potent CYP2C9 secondary metabolite inhibitors include various flavones and flavanols (Si et al., 2009), two classes of polyphenols. Potent CYP2C9 drug inducers include rifampicin and phenobarbital, while secondary metabolite inducers include hyperforin, a secondary metabolite derived from *Hypericum perforatum* (St. John's Wort) (Chen et al., 2004).

1.4 Drug-Herb Interactions

Drug interactions are when the effect of a drug is altered due to the presence of another compound(s). While drug-drug interactions tend to be the type of interaction that appears to get the most attention, drug-herb interactions are equally important when considering the safety of drugs. Drug-herb interactions are types of drug interactions that occur between conventional drugs and herbal medicines. This type of interaction is especially dangerous because while drug-drug interactions tend to occur between two compounds, herbal medicinal products contain mixtures of many pharmacologically active compounds (Fugh-Berman & Ernst, 2001).

This is further confounded when considering that certain herbal medicines may not be a single herb, but rather a mixture of many different herbs with a multitude of different phytochemical profiles. Additionally, herbal medicine, unlike pharmaceuticals, poses the risk of adulteration and contamination. DNA-based authentication of herbal products revealed that 27% of global herbal products were adulterated (Ichim, 2019). The inclusion of undeclared species and contaminants such as pollen, insects, fungi, mold, and pesticides in herbal medicines drastically increases the risk of

interaction because while doctors can advise patients to avoid certain herbs due to their potential for interaction, this can only be done for declared species.

Drug-herb interactions, like other drug interactions, may be antagonistic or synergistic or neutralize drug activity altogether. In many cases, this occurs because both the drug and the herb interact with the same enzyme. By altering the pharmacokinetics and pharmacodynamics of drugs, complications can range from no effect to treatment failure, or harm to the patient. As a result, the American Society of Anesthesiologists recommends that patients stop all herbal supplements at least two weeks before any procedures to minimize the risk of side effects caused by drug-herb interactions (DeMuro, 2023).

Citrus × *paradisi* poses a potential danger to many patients as the plant is a potent CYP3A4 inhibitor, primarily due to the furanocoumarin bergamottin. An example of a drug-herb interaction that arises from *Citrus* × *paradisi* is when grapefruit is consumed with the drug felodipine. Felodipine is a calcium channel blocker that vasodilates blood vessels to reduce hypertension. The drug is metabolized by CYP3A4 to be converted into dehydrofelodipine, a metabolite that lacks vasodilatory activity (Bailey et al., 1993). Studies show that when felodipine is consumed with either pure bergamottin or grapefruit juice, the peak plasma concentration of felodipine increases significantly (Lown et al., 1997). Grapefruit juice produced a higher peak plasma concentration of felodipine compared to the administration of pure bergamottin, indicating that multiple phytochemicals in *Citrus* × *paradisi* are responsible for inhibition (Lown et al., 1997). Clinically, this results in tachycardia and diastolic hypertension, though only when felodipine is taken orally, not intravenously (Lundahl et al., 1997).

Conversely, *Citrus* × *paradisi* is also able to affect the drug metabolism of prodrugs, such as with clopidogrel. Clopidogrel is an inactive metabolite but is bioactivated by CYP2C19 and

CYP3A4 to form an active thiol metabolite that functions as an antiplatelet drug used to treat coronary artery disease and stroke (Holmberg et al., 2014). Studies revealed that when *Citrus* × *paradisi* was consumed with clopidogrel, there was a significant reduction in the mean peak plasma concentration in the active metabolite of clopidogrel, indicating that the drug was not being bioactivated due to inhibition of the CYP enzymes via *Citrus* × *paradisi* (Holmberg et al., 2014). Clinically, this results in impaired anti-platelet activity and an increased risk for blood clotting.

St. John's Wort (*Hypericum perforatum* L., Hypericaceae) is another commonly consumed botanical that is notorious for its potential for drug-herb interactions. Unlike *Citrus* × *paradisi*, St. John's Wort is dangerous not because it inhibits enzymes, but rather induces them. St John's Wort is a potent inducer of CYP3A4. One of the major constituents of St. John's Wort, hyperforin, binds to the pregnane X receptor, the receptor the regulates expression of CYP3A4 (Moore et al., 2000). As a result of binding, CYP3A4 expression drastically increases, causing substrates of the enzyme to be metabolized much quicker than normal, which can lead to potentially dangerous side effects.

One such example is the interaction between drug-herb interaction involving St. John's Wort is the co-administration of the herb and the drug atorvastatin, a drug metabolized by CYP3A4 (Andrén et al., 2007). Atorvastatin belongs to the statin drug class, a class of drugs that hypolipidemic agents used to treat hyperlipidemia and cardiovascular disease by inhibiting 3-hydroxy-3-methylglutaryl-coenzyme A reductase, resulting in the reduction of cholesterol levels (Stancu & Sima, 2001). Studies have found that co-administration of St John's Wort and atorvastatin resulted in patients with significantly higher cholesterol levels compared to control patients, indicating that St. John's Wort alters the pharmacokinetics and pharmacodynamics of atorvastatin by

converting the active metabolite to an inactive metabolite quicker and preventing the drug from exerting its mechanism of action (Andrén et al., 2007).

1.5 Project Aims

The purpose of this project is to investigate the inhibitory effects different species of the *Cinnamomum* genus may have on the enzymes of the cytochrome P450 system, as well as the identification of the phytochemical(s) that are responsible for the inhibitory activity. This research helps to elucidate potential drug-herb interactions that may arise from the consumption of these species commonly found in cinnamon herbal supplements and pharmaceutical medications.

Chapter 2: Background

2.1 The Lauraceae family and *Cinnamomum*

Lauraceae, also known as the laurel family, is a botanical family of aromatic trees and shrubs that are cultivated in tropical and subtropical regions. The family consists of over 40 genera, such as *Persea, Laurus, Sassafras,* and *Cinnamomum* and 1,000 species. Notable species from the Lauraceae family include the avocado tree (*Persea americana* Mill.), bay laurel (*Laurus nobilis* L.), sassafras (*Sassafras albidum* (Nutt.) Ness), and the different cinnamon species (Bailey, 1949) (Figure 2.1).

Figure 2.1. Specimen vouchers of various Lauraceae species from Emory Herbarium. *Persea borbonia* Spreng. (left)*, Cinnamomum camphora* (L.) J.Presl (middle left)*, Damburneva coriacea* (Sw.) Trofimov & Rohwer (middle right)*, Laurus nobilis* L. (right). (Source: Emory University Herbarium).

The Lauraceae family first emerged in the Mid-Cretaceous period (around 100 million years ago), and are found in tropical and subtropical regions across the world, with the most biodiversity centered in the tropics of the Americas and Australasia (Chanderbali et al., 2001). The combination of its large geographic distribution and anciency has enabled the family to develop a rich phytochemical profile, including various isoquinoline alkaloids, flavonoids, and cinnamic acid derivatives (Oh et al., 2022).

The *Cinnamomum* genus consists of over 250-350 species of evergreen trees and shrubs, with at least 21 species originating in the Malay peninsula alone (Abdelwahab et al., 2017). Species of the genus usually have simple, opposite leathery, and 3-nerved leaves and a cinnamon or camphor-like aroma (Bailey, 1949) (Figure 2.2).

This genus has historically been known for its uses as both an aromatic and medicinal spice. Medicinally, cinnamon has been used as a traditional medicine for thousands of years across numerous cultures. In traditional Chinese medicine, *Cinnamomum cassia* (L.) J.Presl (Chinese cinnamon/rou gui) was used to treat diarrhea, fever, and menstrual cramps and acted as a tonic (Heinrich et al., 2017). Similarly, in traditional Japanese herbal medicine (Kampo medicine), *C. cassia* bark was used as an antipyretic, in addition to a diaphoretic and stomachic. In Ayurvedic medicine cinnamon bark essential oil (CBEO) has been used as an anti-arthritic and to reduce pain (Han & Parker, 2017). The camphor tree (*Cinnamomum camphora* (L.) J.Presl) has been used in traditional medicines as an aromatic stimulant and decongestant (Heinrich et al., 2017).

In modern Western medicine, cinnamon has been marketed to help treat certain gastrointestinal conditions. For example, studies have shown that administration of oral *Cinnamomum cassia* capsules reduced the mean fasting serum glucose, triglycerides, LDL cholesterol, and total cholesterol (Khan et al., 2003). Additionally, The essential oil of some *Cinnamomum* species has also been shown to have repellant properties, such as *Cinnamomum verum* J.Presl (Jo et al., 2013).

For non-medicinal ethnobotanical uses, *Cinnamomum burmannii* (Ness & T. Ness) Blume (Indonesian cinnamon), *C. cassia* (Chinese cinnamon), *Cinnamomum loureiroi* Ness (Vietnamese cinnamon), and *Cinnamomum verum* J.Presl (True/Sri Lankan cinnamon) are the

four main species of cinnamon sold for culinary purposes (Iqbal et al., 1993), while *C. camphora* (camphor tree) is used as a plasticizer in the preparation to prepare explosives and disinfectants (Malabadi et al., 2021).

Figure 2.2. Specimen vouchers of different *Cinnamomum* species. *C. burmannii* (left, source: University of South Florida Herbarium), *C. cassia* (source: University of Florida Herbarium), *C. verum* (source: Arizona State University Vascular Plant Herbarium), and *C. camphora* (right, source: Emory University Herbarium).

2.2 Previous Screening of Quave Natural Products Library

The Quave Natural Product Library is a collection of over 2,500 botanical and fungal extracts. The library is unique as it utilizes ethnobotanical data by collecting plants and fungi that have documented usage in human medicine and food. The library is incredibly biodiverse, representing plants and fungi from 49 families and over 750 different species.

From April-July 2021, the Quave Research Group conducted CYP-inhibition assays of 652 different plant and fungal extracts against CYP3A4, CYP2C9, and CYP2C8. My research began by going through each of these plant extracts and identifying the extracts that had significant CYP inhibition (at least 50% inhibition relative to the positive control), of which 146

extracts demonstrated significant CYP inhibition. Next, each of the plants or fungi that the extract was made from was researched to determine if it has any food or medicinal usage and if there was any reported CYP inhibitory activity through a literature review on Google Scholar. This literature review revealed that only 30 extracts had reported inhibition and food usage, and four of these extracts were from *Cinnamomum* species. As such, the four species, *Cinnamomum burmannii* (Ness & T. Ness) Blume, *Cinnamomum verum* J.Presl*, Cinnamomum cassia* (L.) J.Presl*,* and *Cinnamomum camphora* (L.) J.Presl, were selected to be the botanicals of interest (Figure 2.3). To further validate the inhibitory activity of these species, a single-dose CYP2C9-inhibition assay at 8 µg/mL was performed with 27 different extracts from the Quave Natural Products Library, which included ethanolic and aqueous extracts of *C. burmannii* and *C. verum*, and two pure compounds found in *Cinnamomum* spp., eugenol, and cinnamaldehyde.

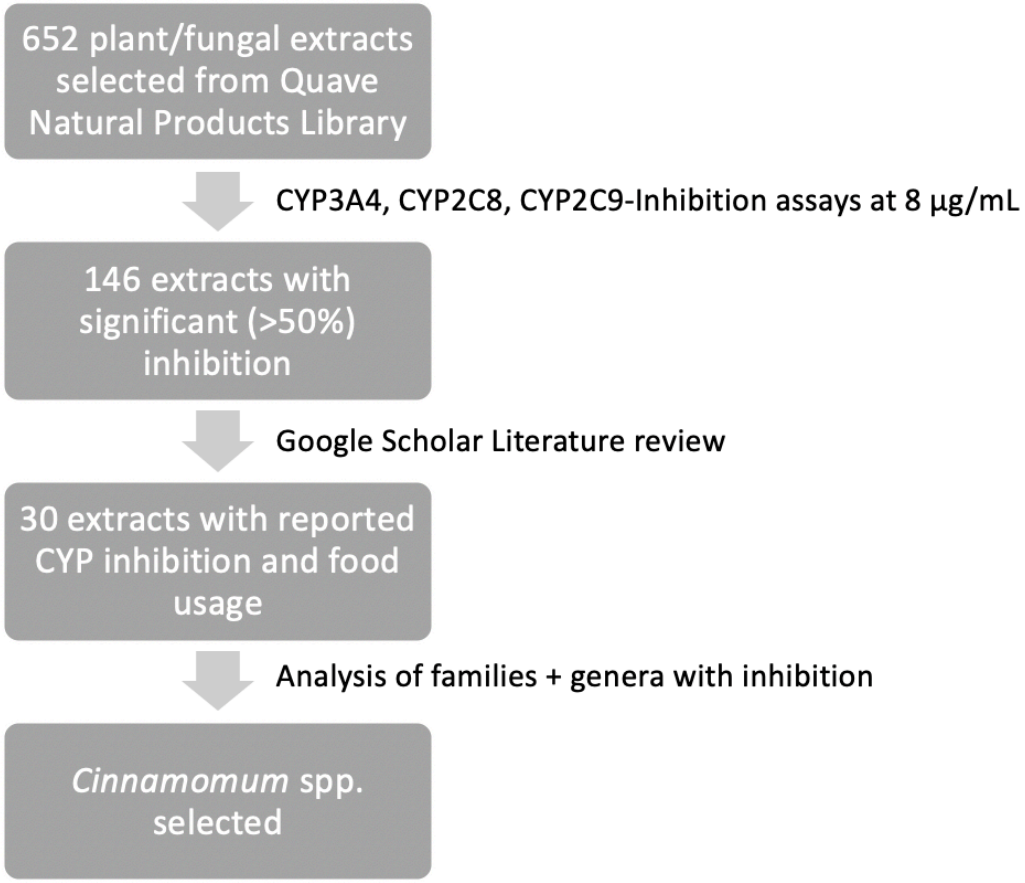


Figure 2.3. Selection of plant extracts of interest for further investigation for CYP inhibition.

2.3 Literature Review and Selection of *Cinnamomum* Genus

A literature review was conducted to gain a better understanding of what is known about the potential effects *Cinnamomum* extracts, and the major constituents of *Cinnamomum* may have on the cytochrome P450 system. Through this review, a few things became apparent. First, many sources that discuss the inhibition of CYPs by *Cinnamomum* and *Cinnamomum* usage as a medicinal plant lack information regarding what species, extract method, or plant part was used. For example, one author reports that *C. verum* extract inhibits CYP2C9 and CYP3A4 (Brewer & Chen, 2017), but does not specify how the extract was prepared (maceration, decoction, infusion) nor what plant part was used for the extract (whole plant, bark, leaves), as each part contains different chemistry and has

different roles in traditional medicine. This is coupled with the fact that many studies also use different terminology. In the paper "Cinnamon and Cassia Nomenclature Confusion: A Challenge to the Applicability of Clinical Data," the authors found inconsistencies with cinnamon nomenclature in US and Canadian Food and Drug regulations and traditional pharmacopeias, which has spilled over into peer-reviewed articles. The authors examined one popularly cited review article that focused on 10 studies that used cinnamon and found that six of the ten studies claimed to use *C. cassia*, while only one of these studies actually reported that the specimen was botanically certified to be *C. cassia*. The authors then noted that the remaining four studies relied solely on manufacturer labels without any specimen authentication (Oketch-Rabah et al., 2018). Even worse, in a separate review titled *The Impact of cinnamon on anthropometric indices and glycemic status in patients with type 2 diabetes: A systematic review and meta-analysis of clinical trials* (Nazami et.al., 2019), the authors concluded that of the 18 studies in the meta-analysis, 10 of the papers did not even list what type of cinnamon was used, rendering their results meaningless (Namazi et al., 2019).

Secondly, while the literature on CYP inhibition does include *C. burmannii* and *C. verum*, there are gaps about the inhibitory effects of *C. cassia* and *C. camphora* and major constituents of all *Cinnamomum* species: cinnamic acid, cinnamyl alcohol, and cinnamyl acetate. Each *Cinnamomum* species has a different phytochemical profile, which then varies even more across plant parts and climate conditions (Oketch-Rabah et al., 2018). As such, there is a need for research on the inhibition of CYPs by *all* commonly traded *Cinnamomum* species, rather than just the most popular ones. Equally, there was little research available on the effects of *Cinnamomum* spp. against CYP2C19, the CYP responsible for the metabolism of many commonly used drugs such as escitalopram, proguanil, diazepam, and omeprazole (Li-Wan-Po et al., 2010).

2.4 *Cinnamomum burmannii*

Cinnamomum burmannii, known as Indonesian cinnamon, is a medicinal herb and spice, whose leaves, bark, and oil have been used for flavoring and treatment of various ailments.

The phytochemical profile of the leaves and bark is dominated by phenylpropanoids, shikimic-acid-derived natural products that contain a phenyl ring and an unsaturated propene group (Heinrich et al., 2017). Phenylpropanoids are secondary metabolites that not only make up the cell wall but also protect against UV radiation, are a deterrent against herbivores, and act as antioxidants (Agar & Cankaya, 2020). The essential oils of the leaves and bark are composed of over 70% trans-cinnamaldehyde, while the remainder contains other cinnamic acid derivatives such as trans-cinnamic acid, cinnamyl alcohol, and cinnamyl acetate (Figure 2.4) (Fajar et al., 2019). Other important compounds found in the leaf oil include eugenol, another phenylpropene used as a dental anesthetic and antiseptic (Heinrich et al., 2017).

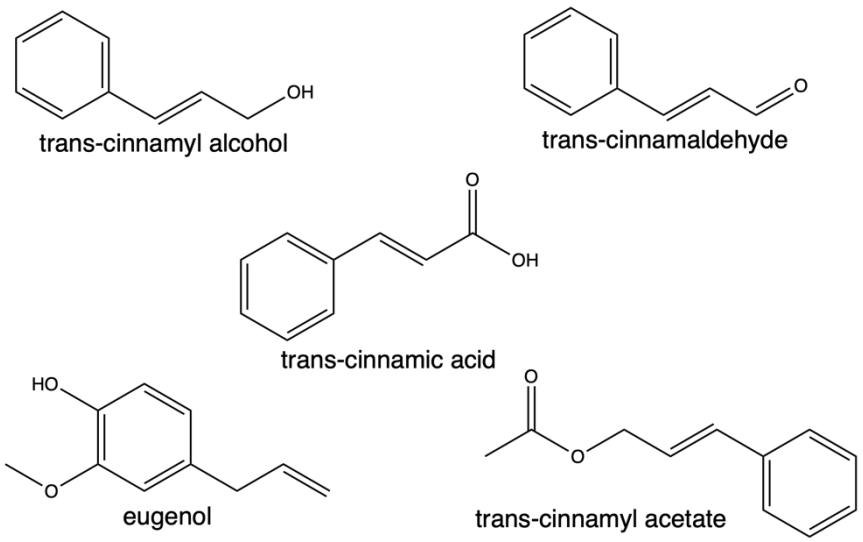


Figure 2.4. Chemical structures of most abundant compounds found in bark and leaf oil of *C. burmannii*.

The phytochemical composition of *C. verum* and *C. cassia* is similar to that of *C. burmannii*, with the most common constituents of the bark essential oil being cinnamaldehyde in both species - 71.5% in *C. verum* (Alizadeh Behbahani et al., 2020) and 69.146% in *C. cassia (Zachariah & Leela, 2006)*. The other constituents of *Cinnamomum* spp. essential oils mostly consist of other cinnamic acid derivatives and coumarin (Wang et al., 2013) and other terpenes.

However, other members of the *Cinnamomum* genus vary greatly in their composition. Essential oil from the bark from *C. camphora*, for example, contains little to no cinnamaldehyde, but rather is primarily composed of D-camphor (51.3%) (Figure 2.5) and other terpenes (Guo et al., 2016).

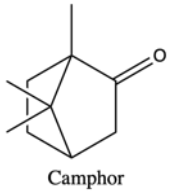


Figure 2.5. Chemical structure of camphor. Camphor is a terpenoid ketone, is the major constituent of *Cinnamomum camphora* unlike other *Cinnamomum* spp.

Chapter 3: Materials and Methods

3.1 Collection and processing

The authentication of plant and fungal specimens is imperative when conducting biochemical testing of botanicals to avoid issues with adulteration and misidentification of specimens. In this project, specimens were obtained both through a third-party vendor and wild specimens can be collected in the field. Organic dried bark chips of *Cinnamomum burmannii* ("Cassia Cinnamon Chips") and *Cinnamomum verum* ("Sweet Cinnamon Chips") were purchased from Mountain Rose Herbs in July 2020. The *C. burmannii* chips originated from Indonesia, while the *C. verum* chips originated from Sri Lanka. Mountain Rose Herbs authenticates its products via organoleptic, macroscopic, microscopic, high-performance thin-layer chromatography, and DNA analysis to ensure that the herbs sold are indeed the herb that is listed. The inner bark of *Cinnamomum cassia* was purchased from a commercial vendor. *Cinnamomum camphora* bark was collected in March 2016 in Desoto County, Florida, United States. The wild specimens were sent to the Emory Herbarium for authentication and to be deposited into their collection (Figure. 3.1).

Figure 3.1. Retention vouchers and authentication labels of *Cinnamomum* specimens. *Cinnamomum burmannii* (A), *Cinnamomum verum* (B), *Cinnamomum camphora* (source: Emory University Herbarium) (C).

3.2 Creation of the Extract Library

Fresh preparations of the selected extracts were prepared to ensure that all-natural products are intact and have not degraded during storage. At the laboratory, the plant specimens were ground into a fine powder using a Thomas Wiley Mill at a 2 mm mesh size (Thomas Scientific). The four *Cinnamomum* species were then prepared as fresh, crude extracts (Table 3.1). Each species was prepared via ethanolic macerations consisting of two 72-hour macerations in 80% ethanol in a 1:10 plant material in grams to ethanol in milliliters ratio. All extracts were vacuum filtered, concentrated *in vacuo* using a rotary evaporator, shell-frozen, and lyophilized. Dried extracts were then scraped from the bottles, collected, and stored at -20°C.

Table 3.1. Summary of Crude Extract Preparations.

Extract ID	Species (family)	Family	Part extracted	Extract type	Extract solvent	Extraction method
2017	*Cinnamomum burmannii*	*Lauraceae*	Bark	Crude	80% EtOH	2 x 72-hour maceration
2018	*Cinnamomum burmannii*	*Lauraceae*	Bark	Crude	dH_2O	20-minute maceration
2019	*Cinnamomum verum*	*Lauraceae*	Bark	Crude	80% EtOH	2 x 72-hour maceration
2020	*Cinnamomum verum*	*Lauraceae*	Bark	Crude	dH_2O	20-minute maceration
2728	*Cinnamomum cassia*	*Lauraceae*	Inner bark	Crude	80% EtOH	2 x 72-hour maceration
2729	*Cinnamomum camphora*	*Lauraceae*	Bark	Crude	80% EtOH	2 x 72-hour maceration

3.3 Initial Screening and CYP-inhibition assay

Dried extracts from the Quave Natural Products Library were tested for CYP inhibition against 5 CYP isoforms – CYP2B6, CYP2C9, CYP2C19, CYP2D6, and CYP3A5 – using Vivid™ CYP450 Screening Kits.

Extracts were dissolved in either DMSO for organic extractions (ethanolic, methanolic) or water for decoctions at a concentration of 10 mg/mL, and then transferred to a sister plate at 800 µg/mL. 2 µL of extract from the daughter plate were transferred into an intermediate plate containing 78 µL of buffer for a testing concentration of 8 µg/mL and a final vehicle

concentration of 1% DMSO or water. Thirty-two microliters of 8 µg/mL extract were then pipetted into a black 96-well plate containing 40 µL of a "master pre-mix," which contains CYP450 BACULOSOMES® Plus Reagents and the Regeneration System (glucose-6-phosphate and glucose-6-phosphate dehydrogenase). The CYP450 BACULOSOMES® Plus Reagents are microsomes derived from insect cells that express different isozymes of human cytochrome P450. The regeneration system converts $NADP^+$ to NADPH, a cofactor required by cytochrome P450s to function. The master pre-mix and extracts were incubated for 10 minutes and then read using a Cytation 3 Cell Imaging Multimode Reader.

Following incubation, 8 µL of the "master mix," which contains $NADP^+$ and the Vivid® substrate was added. Vivid™ substrates are blocked dyes that only yield fluorescent signals once cleaved or hydroxylated by the CYP enzyme. Thus, CYPs that have been inhibited by natural products will emit low to no fluorescence (Figure 3.2). This reaction was performed in the dark and in a black 96-well plate because $NADP^+$ and the Vivid® substrates are light-sensitive. Immediately following the addition of the master-mix, the plate was read in the fluorescence plate reader for 26 minutes in "kinetic mode," meaning that the fluorescence is measured at multiple time points throughout the duration of the run. For each experiment, all concentrations and extracts were tested in triplicate, and experiments were performed at least twice on different days to account for two biological replicates.

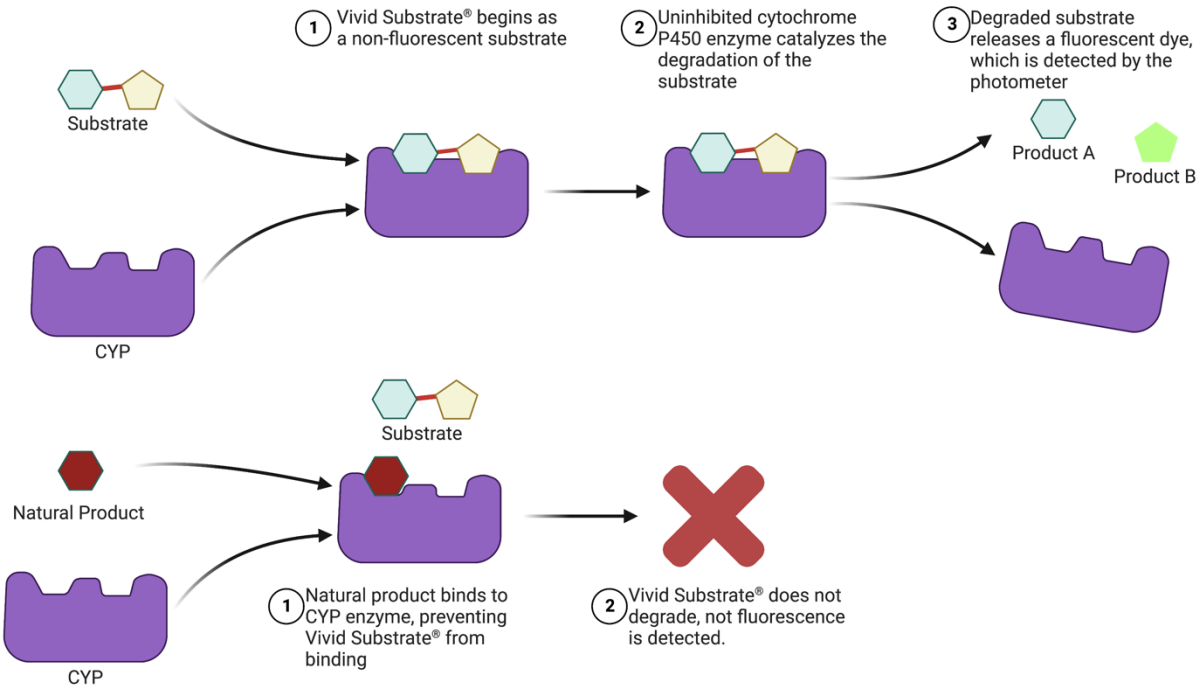


Figure 3.2. Assay theory of Cytochrome P450-inhibition assay. Inhibition of the CYP enzyme via the natural product results in no fluorescence.

3.4 Bioassay guided fractionation

Bioassay-guided fractionation is a technique commonly used in natural products when screening plant and fungal extracts for bioactivity . Bioassay-guided fractionation begins with the identification of a plant or fungal extract with bioactivity. Next, the extract is fractionated, and the fractions of the extract are tested again for bioactivity, and for the purposes of this project, bioactivity was defined as the inhibition of cytochrome P450 enzymes. Fractions with significant bioactivity are then analyzed and further fractionated. The process of fractionation and analysis is repeated until the fractions are single, isolated compounds (Figure 3.3). It is important to note that plant and fungal extracts have polyvalent effects, so the most potent fraction may not be the isolated molecule but rather a combination of compounds.

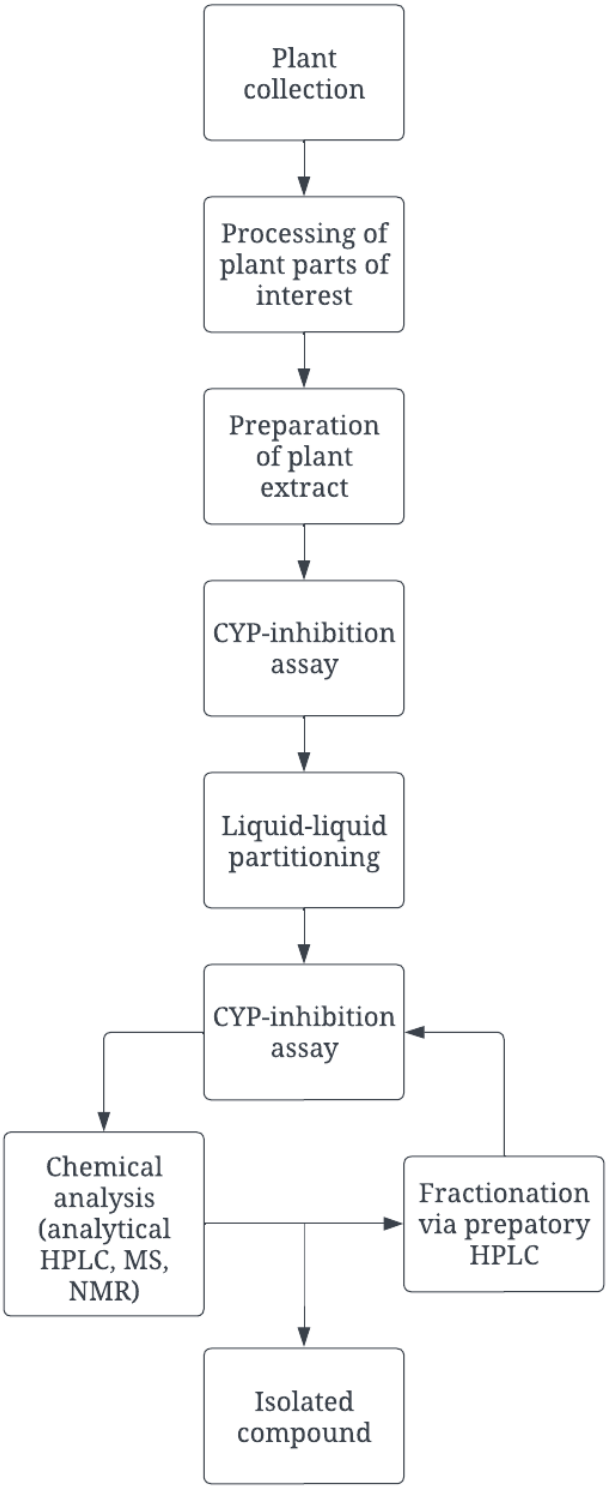


Figure 3.3. Overview of bioassay-guided fractionation of *Cinnamomum* spp. extracts for inhibitory activity against CYP isoforms.

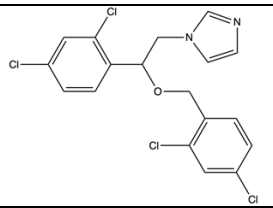
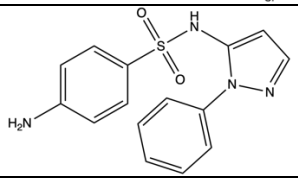
3.5 Dose-Response CYP-inhibition assay

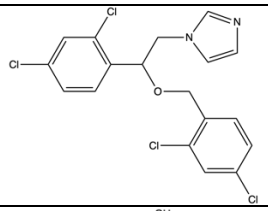
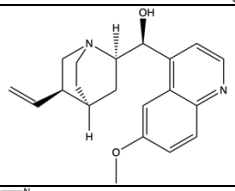
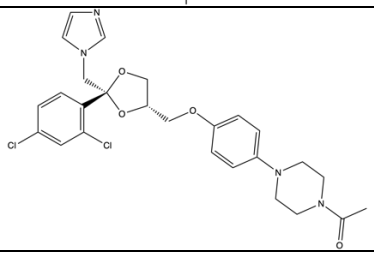
Dried crude extracts were dissolved in dimethyl sulfoxide (DMSO) at seven concentrations – 6400 µg/mL, 3200 µg/mL, 1600 µg/mL, 1200 µg/mL, 800 µg/mL, 400 µg/mL, and 200 µg/mL. These extracts were then tested against four CYP isoforms, CYP2B6, CYP2C9, CYP2D6, and CYP3A5, using Vivid™ CYP450 Screening Kits. The procedure for the assay was the same as described in the initial screening. Table 3.2 lists the positive controls used for each isoform.

The data from the reader was analyzed using PRISM GraphPad via a nonlinear regression to determine the half-maximal inhibitory concentration (IC_{50}), which quantifies the inhibitory activity of the extract (Figure 3.4).

Nine pure compounds found in the bark extract of *Cinnamomum* spp. according to the literature were additionally tested for CYP inhibition to determine whether these pure compounds produced similar levels of CYP inhibition as compared to the entire extract. These compounds were eugenol, cinnamaldehyde, cinnamyl acetate, cinnamyl alcohol, cinnamic acid, coumarin, camphor, limonene, and linalool.

Table 3.2. Positive Controls for Cytochrome P450 Inhibition Assays

Isoform	Positive control (concentration)	Structure
CYP2B6	Miconazole (30 µM)	
CYP2C9	Sulfaphenazole (30 µM)	

CYP2C19	Miconazole (30 µM)	
CYP2D6	Quinidine (10 µM)	
CYP3A5	Ketoconazole (10 µM)	

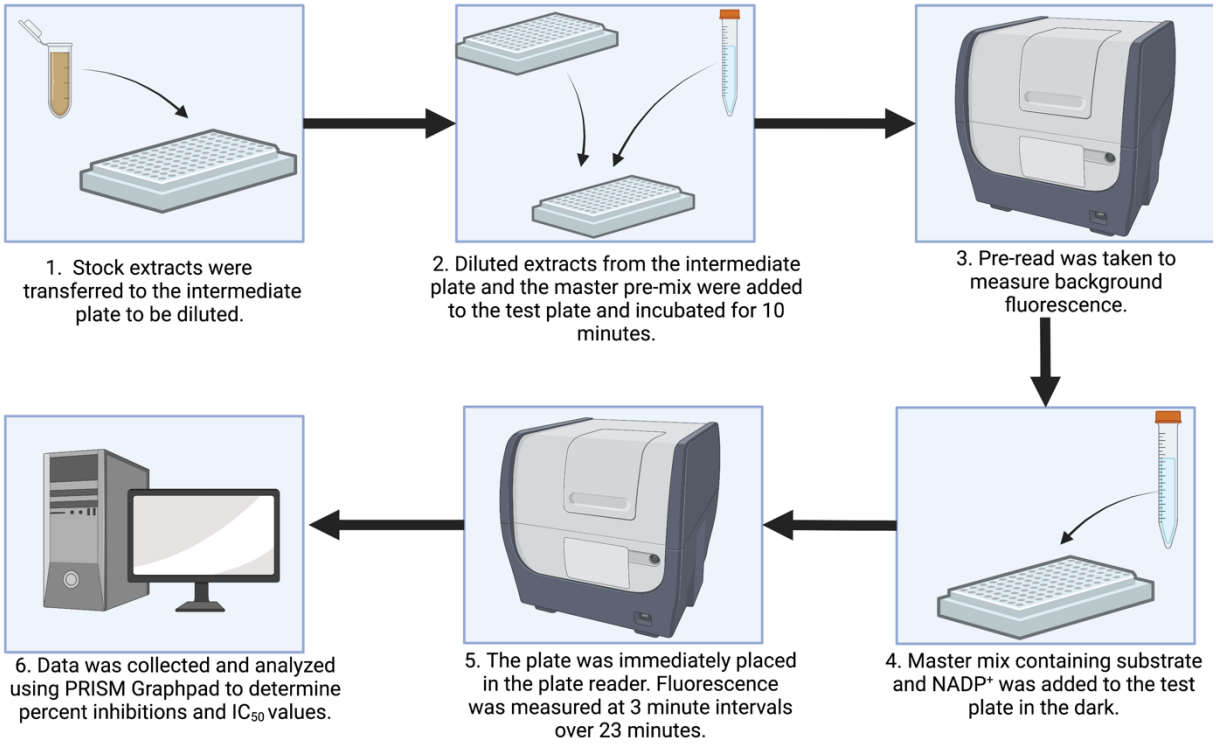


Figure 3.4. Methods of cytochrome P450 inhibition assay using Vivid™ CYP450 Screening Kits.

3.6 Preparation of partitions and single-dose CYP-inhibition assay testing

Liquid-liquid partitioning was performed on the crude extracts to separate the phytochemicals based on their polarity (Figure 3.5). A modified Kupchan partition scheme was performed on each active extract in water against hexanes, ethyl acetate, and *n*-butanol. Crude extracts were crushed in a mortar and pestle, dissolved in deionized water at a crude extract in grams to water in milliliters ratio of 1:30, and then sonicated until uniform.

Partitioning was performed in a large separatory funnel. Each organic partition consisted of 1/3 of the total aqueous volume. First, the hexane partition and crude extract were combined in the funnel, mixed, and left until two distinct layers formed, after which the hexane (top) layer was dried over anhydrous Na_2SO_4 into a round-bottom flask, while the aqueous partition was collected and returned back to the separatory funnel. The hexane partitioning was repeated until exhaustive (the hexane layer remained clear), indicating all the phytochemicals soluble in hexane had been extracted. These steps were repeated for ethyl acetate and *n*-butanol.

All partitions of the same solvent were combined into one flask and concentrated *in vacuo* using a rotary evaporator, shell-frozen, and lyophilized. This yielded four dried partition extracts: hexanes (B), ethyl acetate (C), *n*-butanol (D), and water (E).

Dried partitions were then dissolved in DMSO (for organic partitions) or water (for the aqueous partition) and tested for inhibition against the most active CYP isoform, CYP2C9, at a single concentration - 8 µg/mL – using the same methods as previously described for CYP inhibition assays. To achieve the desired testing concentration, the extract was initially prepared at 6400 µg/mL and serially diluted to 800 µg/mL.

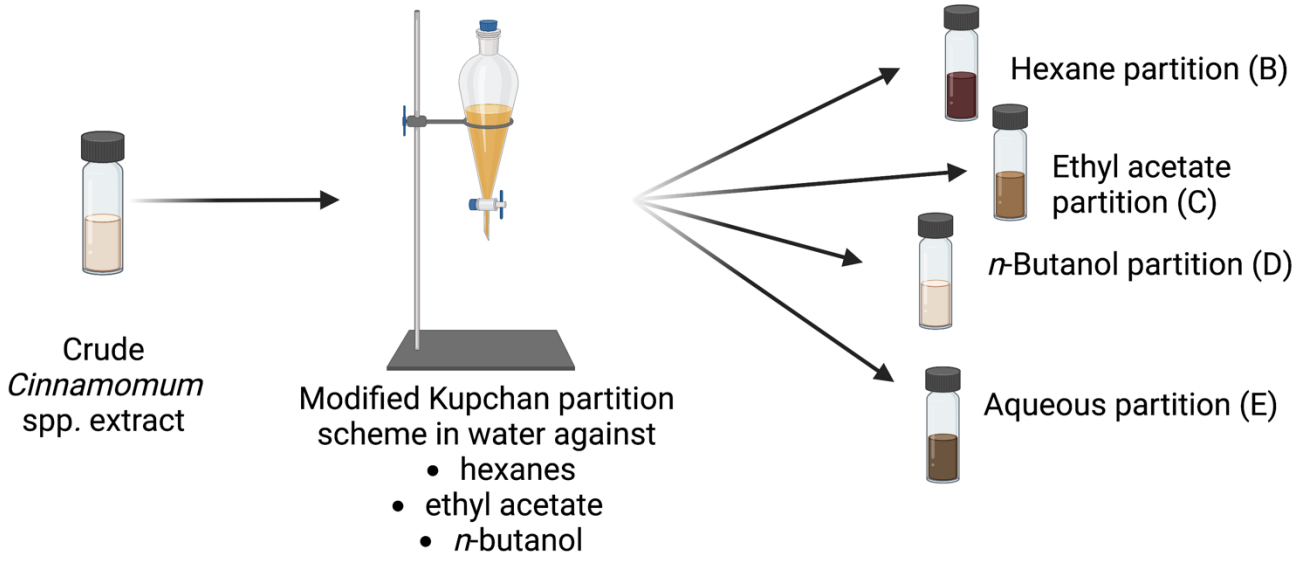


Figure 3.5. Modified Kupchan partitioning of crude *Cinnamomum* spp. extracts. Crude *Cinnamomum* spp. extracts were liquid-liquid partitioned using a modified Kupchan partitioning scheme in water against hexanes, ethyl acetate, and *n*-butanol

3.7 Analytical and Preparatory Reverse Phase-High Performance Liquid Chromatography

Extracts were subjected to analysis via reverse-phase high-performance liquid chromatography to separate and analyze the phytochemicals in the extracts based on polarity. The phytochemicals of the extracts were analyzed using analytical HPLC, while the extracts were fractionated via preparatory HPLC.

For analytical HPLC, extracts were dissolved in methanol at a concentration of 4 mg/mL. 200 µL of prepared extracts were injected into the HPLC via an autosampler. RP-HPLC was then performed using an Agilent 1260 Infinity system running OpenLab CDS ChemStation (Agilent Technologies, Santa Clara, CA, United States) with an Agilent XDB-C18 (250 mm x 4.6 mm, 5 µm) column at a column temperature of 25°C. The mobile phase reagents were 0.1% formic acid in HPLC-grade acetonitrile (A) and 0.1% formic acid in HPLC-grade water (B), at a

flow rate of 1 mL/min. The detector analyzed the sample at wavelengths 217, 221, 254, and 280 nm, with a reference wavelength of 4 nm.

For preparatory HPLC, extracts were dissolved in methanol at a concentration of 200 mg/mL. For each run, 180 µL of prepared extracts were then injected into the HPLC using a syringe. RP-HPLC was then performed using an Agilent 1260 Infinity system running OpenLab CDS ChemStation (Agilent Technologies, Santa Clara, CA, United States) with an Agilent XDB-C18 (250 mm x 30 mm, 5 µm) column with a compatible guard column at a column temperature of 25°C. The mobile phase reagents were a changing gradient of 0.1% formic acid in HPLC-grade acetonitrile (A) and 0.1% formic acid in HPLC-grade water (B), at a flow rate of 42.5 mL/min (Figure 3.6). The detector analyzed the sample at wavelengths 217, 221, 254, and 280 nm, with a reference wavelength of 360 nm.

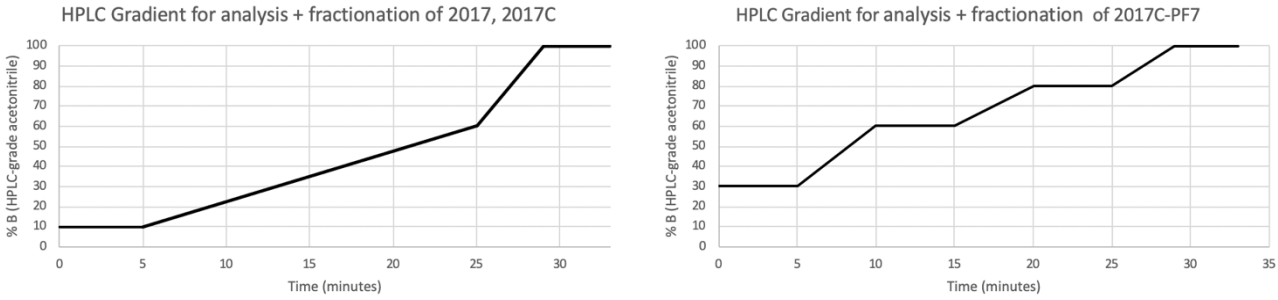


Figure 3.6. Mobile phase gradients used for analytical and preparatory high-performance liquid chromatography of 2017C (left) and 2017C-PF7 (right). The mobile phase begins at high ratios of 0.1% formic acid in water to 0.1% formic acid in acetonitrile but shifts to higher concentrations of 0.1% formic acid in acetonitrile over time.

Chapter 4: Results

4.1 Fractionation Scheme

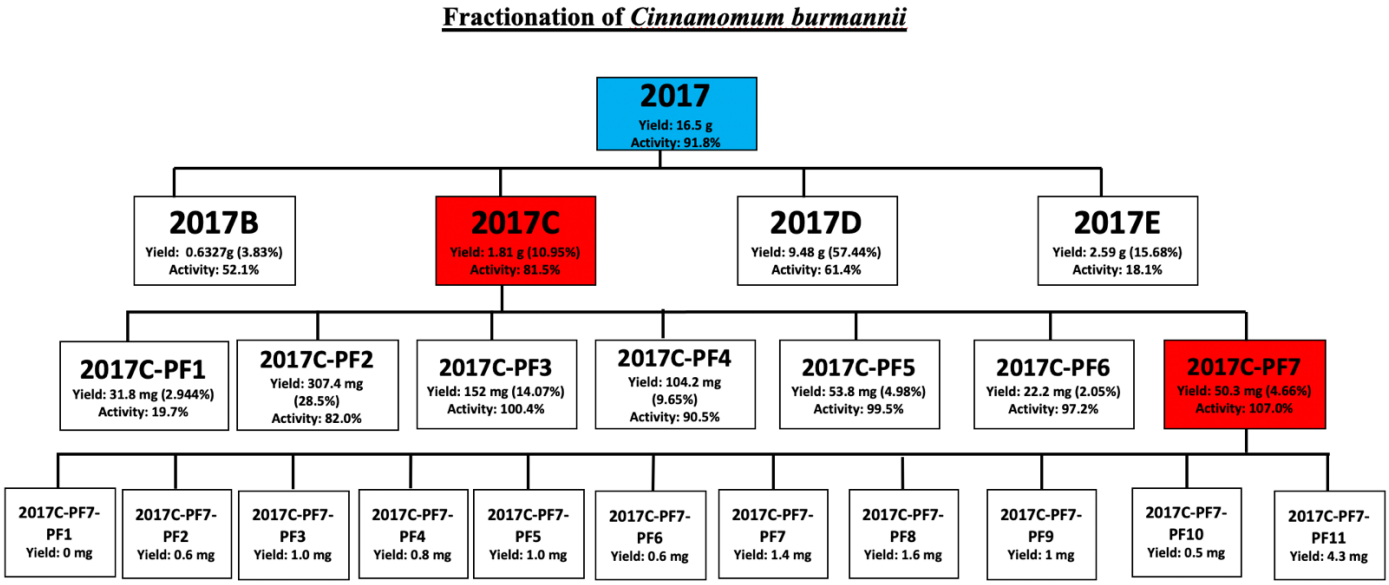


Figure 4.1. Fractionation tree of 2017 (*Cinnamomum burmannii*). Partitions (indicated by B, C, D, and E) were partitioned via liquid-liquid partitioning. Preparatory fractions are indicated by "PF".

The overall fractionation of the *Cinnamomum* species followed a bioassay guided fractionation scheme (Figure 4.1). In summary, double ethanolic macerations of the dried bark of four *Cinnamomum* species were performed, resulting in the crude extracts, 2017, 2019, 2728, and 2729. 2017, 2019, and 2728 were partitioned in water against hexanes, ethyl acetate, and *n*-butanol. The ethyl acetate partition of *Cinnamomum burmannii* (2017C), was fractionated using reverse-phase high-performance liquid chromatography with water and acetonitrile as solvents, which yielded seven preparatory fractions. The last preparatory fraction, 2017C-PF7, was further fractionated using reverse-phase high-performance liquid chromatography, which yielded eleven subfractions. The fractionation scheme of *Cinnamomum burmannii* (2017) is shown in Figure

4.1, while the fractionation scheme of *Cinnamomum verum* (2019) and *Cinnamomum cassia* (2728) can be seen in Supplemental Figure 6 and Supplemental Figure 7.

4.2 Inhibitory Activity of Crude *Cinnamomum* Extracts

Twenty-seven different crude ethanolic and aqueous extracts and two pure compounds known to be found in Cinnamomum species, eugenol and cinnamaldehyde, were examined for their ability to inhibit CYP2C9 based on the previous screenings conducted by Quave Research Group (Figure 4.2). Of these extracts, 2017 (*Cinnamomum burmannii*, 80% ethanolic maceration) and 2019 (*Cinnamomum verum*, 80% ethanolic maceration) demonstrated close to 100% inhibition relative to the control (30 µM sulfaphenazole).

However, 2018 (*Cinnamomum burmannii*, aqueous decoction) and 2020 (*Cinnamomum verum*, aqueous decoction) demonstrated significantly less inhibitory activity. This suggested that the compounds responsible for inhibition were found in much higher abundance in the ethanolic extracts. Accordingly, further studies only examined ethanolic preparations.

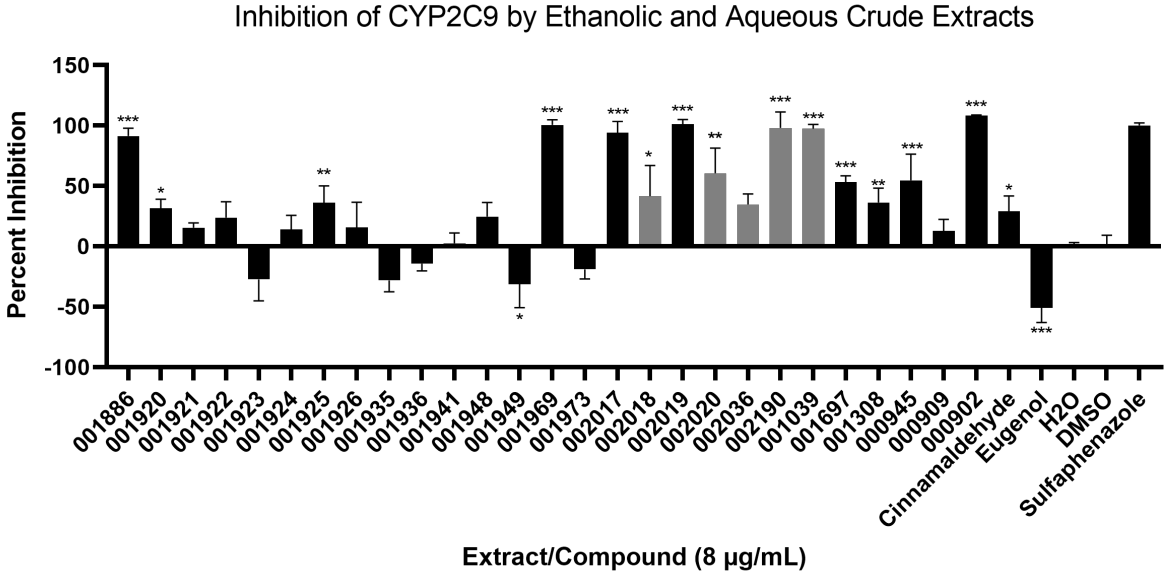


Figure 4.2. Inhibition of CYP2C9 by organic and aqueous extracts. Organic extracts are shown in black, while aqueous extracts are shown in gray. Ethanolic *Cinnamomum* extracts (2017,

2019) demonstrated some of the highest levels of inhibition and were significantly higher than aqueous *Cinnamomum* extracts (2018, 2020). One-way ANOVA with Dunnett's multiple comparison test was performed comparing the mean of each extract to the mean of the respective control. A p-value < 0.05 was considered significant ($*p \leq 0.05$, $**p \leq 0.01$, $***p \leq 0.0001$).

To determine which *Cinnamomum* species has the greatest ability to inhibit CYPs and to determine which CYPs were most sensitive to inhibition, dose-response inhibition assays were performed against CYP2B6, CYP2C9, CYP2C19, CYP2D6, and CYP3A5 (Table 4.1). CYP2C9 was the most sensitive to inhibition while CYP2D6 was the least sensitive. *C. burmannii* and *C. cassia* tended to be the most inhibitory, while *C. camphora* tended to be significantly less inhibitory compared to the other extracts.

Table 4.1 Inhibition of CYP450 isoforms by *Cinnamomum* spp.

	IC_{50} (µg/mL)			
Isoform	*C. burmannii*	*C. verum*	*C. cassia*	*C. camphora*
CYP2B6	14.69 ± 0.692	13.67 ± 10.94	9.309 ± 1.775	30.37 ± 10.26
CYP2C9	7.166 ± 0.214	7.434 ± 1.677	8.621 ± 0.349	12.515 ± 0.722
CYP2C19	20.63 ± 1.269	24.19 ± 2.361	19.61 ± 1.452	14.87 ± 1.247
CYP2D6	35.72 ± 2.856	4.324 ± 10.713	33.10 ± 3.687	62.38 ± 64.696
CYP3A5	9.591 ± 0.330	9.535 ± 0.264	14.67 ± 0.938	20.20 ± 8.970

4.3 Chemical Composition of Crude Extracts

Analytical HPLC was used to analyze the four crude extracts (2017, 2019, 2728, 2729) along with nine standard compounds known to be found in *Cinnamomum* species: eugenol, coumarin, cinnamyl acetate, camphor, cinnamic acid, limonene, cinnamyl alcohol, linalool, and cinnamaldehyde. Figure 4.3 shows the chromatogram of the analytical HPLC (at 254 nm) with peaks found in the standards and extracts highlighted together.

Figure 4.3. Chromatogram of crude *Cinnamomum* spp. extracts and pure compounds from analytical HPLC.

Table 4.2 summarizes the compounds identified in the crude extracts. It is important to note that fractions were collected according to UV/Vis spectroscopy. Wavelengths in the ultraviolet and visible light ranges are absorbed by organic compounds with high degrees of conjugation, so these fractions may additionally contain other compounds not shown in this chromatogram that lack this conjugation. Camphor and limonene are example of organic compounds that do not fluoresce under UV light.

Table 4.2. Chemical composition of crude *Cinnamomum* spp. extracts.

	Eugenol	Coumarin	Cinnamyl acetate	Cinnamyl alcohol	Cinnamic acid	Cinnamaldehyde	Linalool
2017	-	X	-	X	X	X	-
2019	-	-	X	X	X	X	-
2728	-	X	-	-	X	X	-
2729	-	-	-	-	-	-	-

4.4. Inhibitory Activity of *Cinnamomum* partitions and Analysis

Following liquid-liquid partitioning, single dose CYP-inhibition assays against CYP2C9 at 8 µg/mL were performed using the hexanes, ethyl acetate, *n*-butanol, and aqueous partitions of the *Cinnamomum* spp (Figure 4.4). The CYP2C9 isoform was selected for further study because the crude ethanolic *Cinnamomum* extracts were most inhibitory against this isoform. For this study, *Cinnamomum camphora* was not included as it was significantly less inhibitory than the other three *Cinnamomum* species.

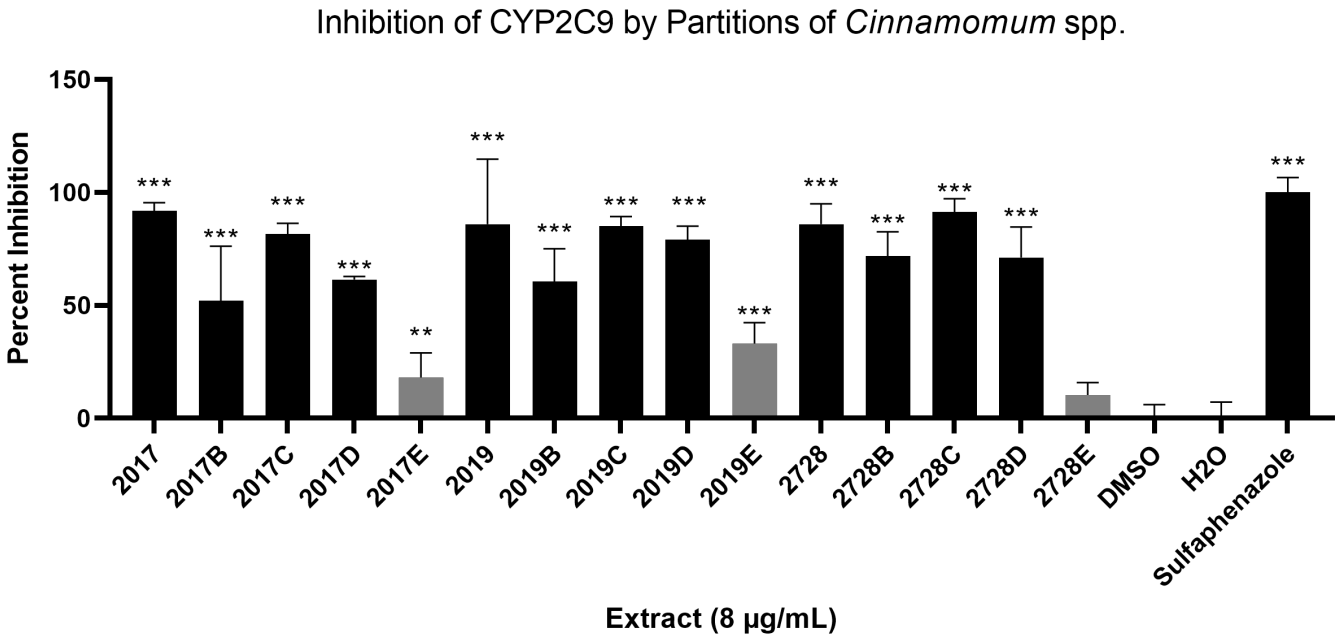


Figure 4.4. Inhibition of CYP2C9 by organic (black) and aqueous (gray) partitions of crude ethanolic *Cinnamomum* extracts. Hexanes (B), ethyl acetate (C), *n*-butanol (D), and aqueous (E) partitions were screened at a single-dose (8 µg/mL). One-way ANOVA with Dunnett's multiple comparison test was performed comparing the mean of each extract to the mean of the respective control. A p-value < 0.05 was considered significant ($*p \leq 0.05$, $**p \leq 0.01$, $***p \leq 0.0001$).

These CYP-inhibition assays showed that most of the organic partitions (hexanes, ethyl acetate, and *n*-butanol) showed similar levels of inhibition compared to their parent crude ethanolic extracts. However, the aqueous partitions of all species showed little to no inhibition against CYP2C9.

Due to its high level of inhibition relative to the control, other partitions, and its parent extract, and its ethnobotanical importance, the ethyl acetate partition of *Cinnamomum burmannii* (2017C) was selected for further biochemical fractionation and analysis.

The partitions of the crude ethanolic *Cinnamomum burmannii* extract (2017) were then analyzed using analytical reverse-phase high-performance liquid chromatography-UV/Vis spectroscopy (Figure 4.5). The same gradient used to analyze the crude extracts and standards shown in Figure 4.2 were used to analyze the partitions, so the retention times of the standards could be used to identify the peaks in the partition analytical HPLC. When comparing the peaks and retention times in Figures 4.2 and 4.3, the major, identifiable peaks are coumarin, cinnamyl alcohol, cinnamic acid, and cinnamaldehyde.

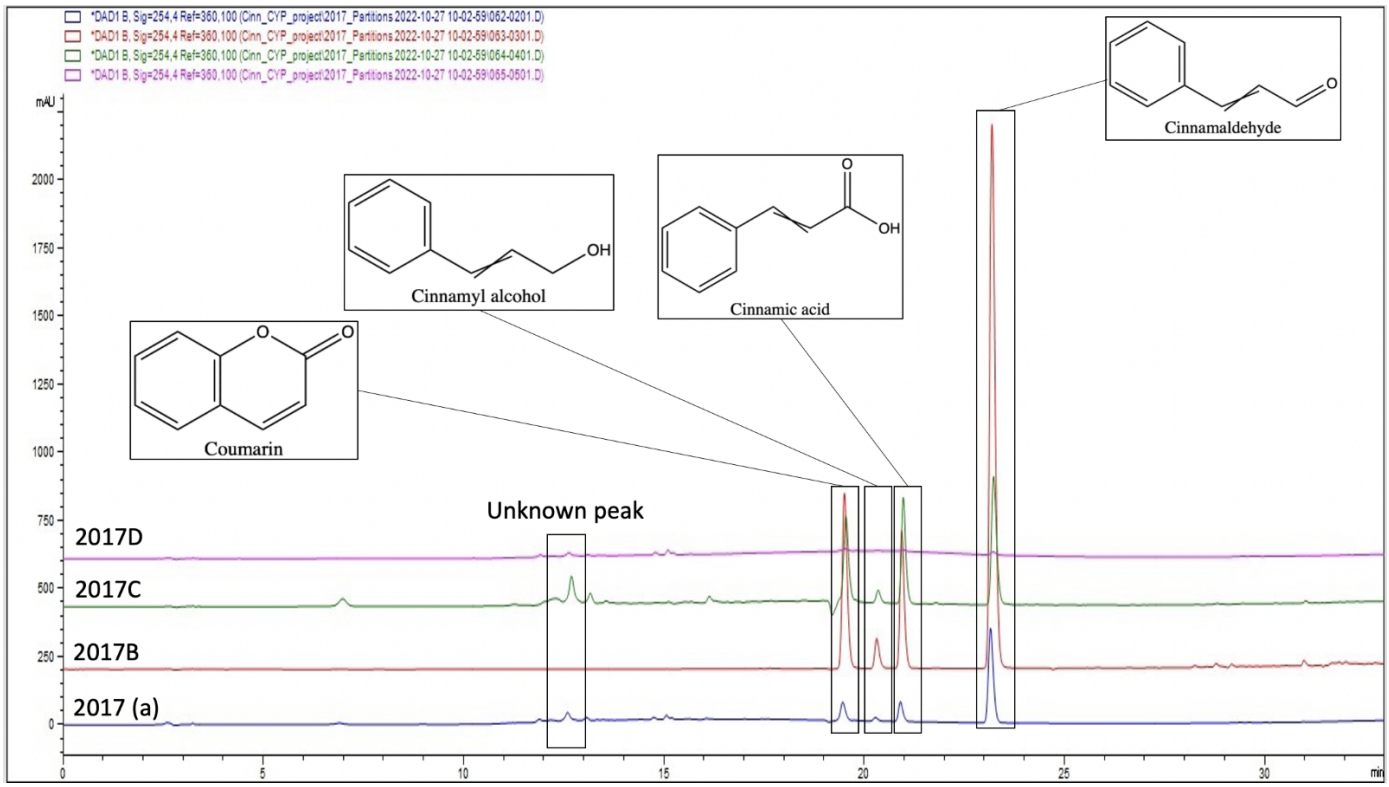


Figure 4.5. Chromatogram of partitions of *Cinnamomum burmannii* from analytical RP-HPLC. Compounds were identified by comparing retention times with the chromatogram in figure 4.2, which used the same gradient.

4.5. Fractionation of 2017C and Inhibitory Activity

2017C was further fractionated via preparatory reverse-phase high-performance liquid chromatography-UV/Vis spectroscopy using the gradient shown in Figure 3.6. Fractions were collected with the intention of isolating individual peaks in the chromatogram, shown above at 254 nm, the wavelength where the peaks had the highest intensity (Figure 4.6). Collected fractions were concentrated *in vacuo* using a rotary evaporator, shell-frozen, and lyophilized. The yields of each fraction are shown in Figure 4.1, with 2017C-PF2 having the highest yield. When compared against the retention times from the analytical HPLC that use the same methods

(Figures 4.3 and 4,5), 2017C-PF3 appears to be cinnamyl alcohol, 2017C-PF4 appears to be cinnamic acid, and 2017C-PF5 appears to be cinnamaldehyde.

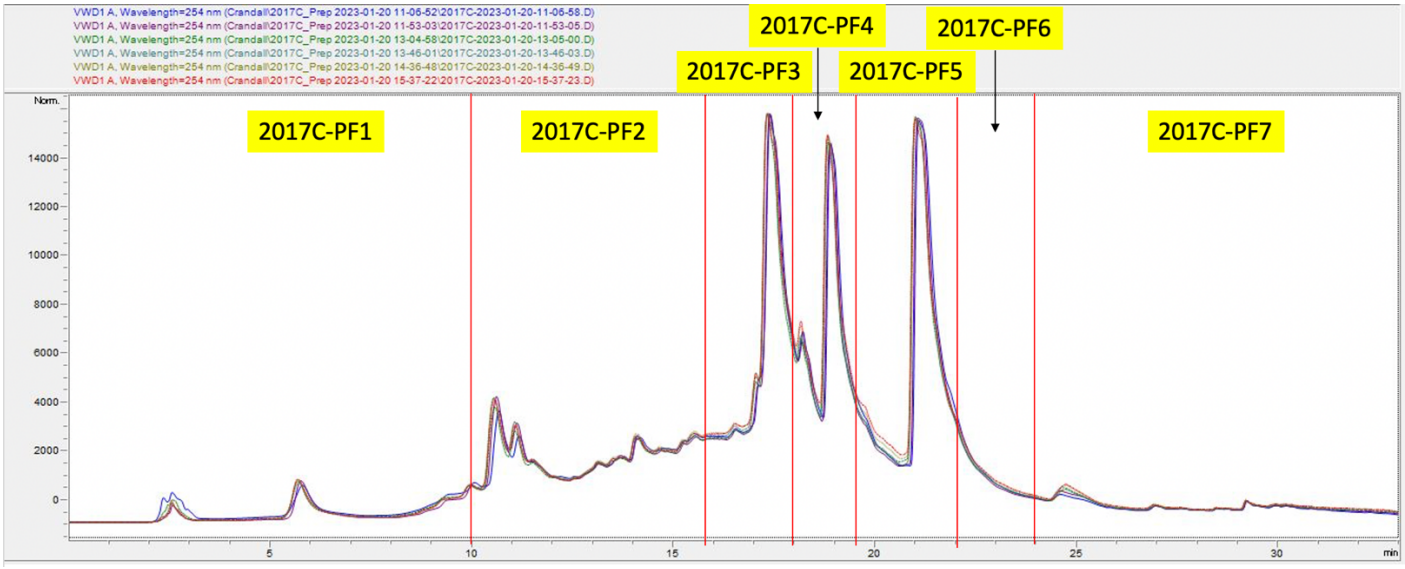


Figure 4.6. Chromatogram of fractions produced from the fractionation of 2017C via preparatory RP-HPLC.

These fractions were subsequently tested in CYP-inhibition assays against CYP2C9 at three concentrations – 8 µg/mL, 4 µg/mL, and 2 µg/mL (Figure 4.7). Unlike the previous study, three concentrations were tested instead of one because initial testing revealed that almost all fractions aside from 2017C-PF1 demonstrated almost 100% inhibition at 8 µg/mL.

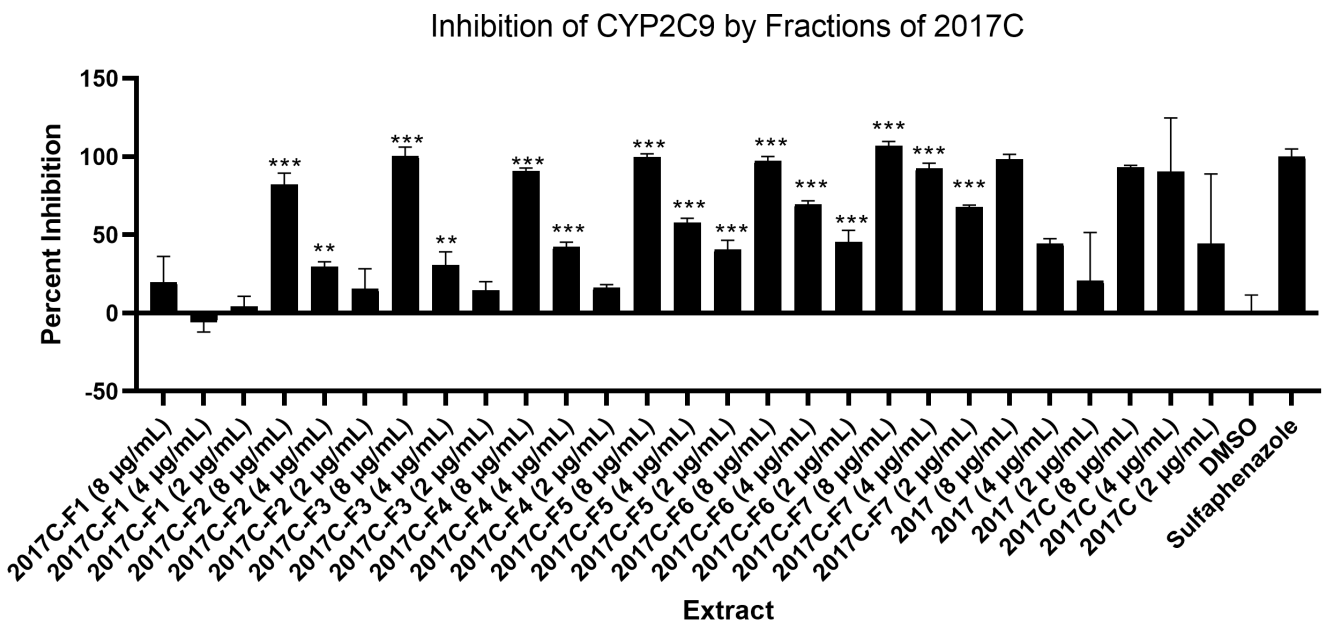


Figure 4.7. Inhibition of CYP2C9 by preparatory fractions of 2017C. Fractions were dissolved in DMSO at 8, 4, and 2 µg/mL. One-way ANOVA with Dunnett's multiple comparison test was performed comparing the mean of each extract to the mean of the respective control. A *p*-value < 0.05 was considered significant ($*p \leq 0.05$, $**p \leq 0.01$, $***p \leq 0.0001$).

This assay showed that of the seven fractions, 2017C-F7 was the most inhibitory. Additionally, this assay showed that fractions collected toward the end of the HPLC run when the solvent was higher percentages of acetonitrile to water had higher levels of inhibition. This suggests that the compounds responsible for inhibition are more non-polar.

With fraction 2017C-PF7 having the most inhibition activity, this fraction was selected for further fractionation, shown below, using the gradient shown in Figure 3.6b.

4.6. Fractionation of 2017C-PF7

Fraction 2017C-PF7 was fractionated into subfractions via preparatory reverse-phase high-performance liquid chromatography-UV/Vis spectroscopy (Figure 4.8) using the gradient

shown in Figure 3.6. The subfractions were collected and dried down and are awaiting bioactivity testing.

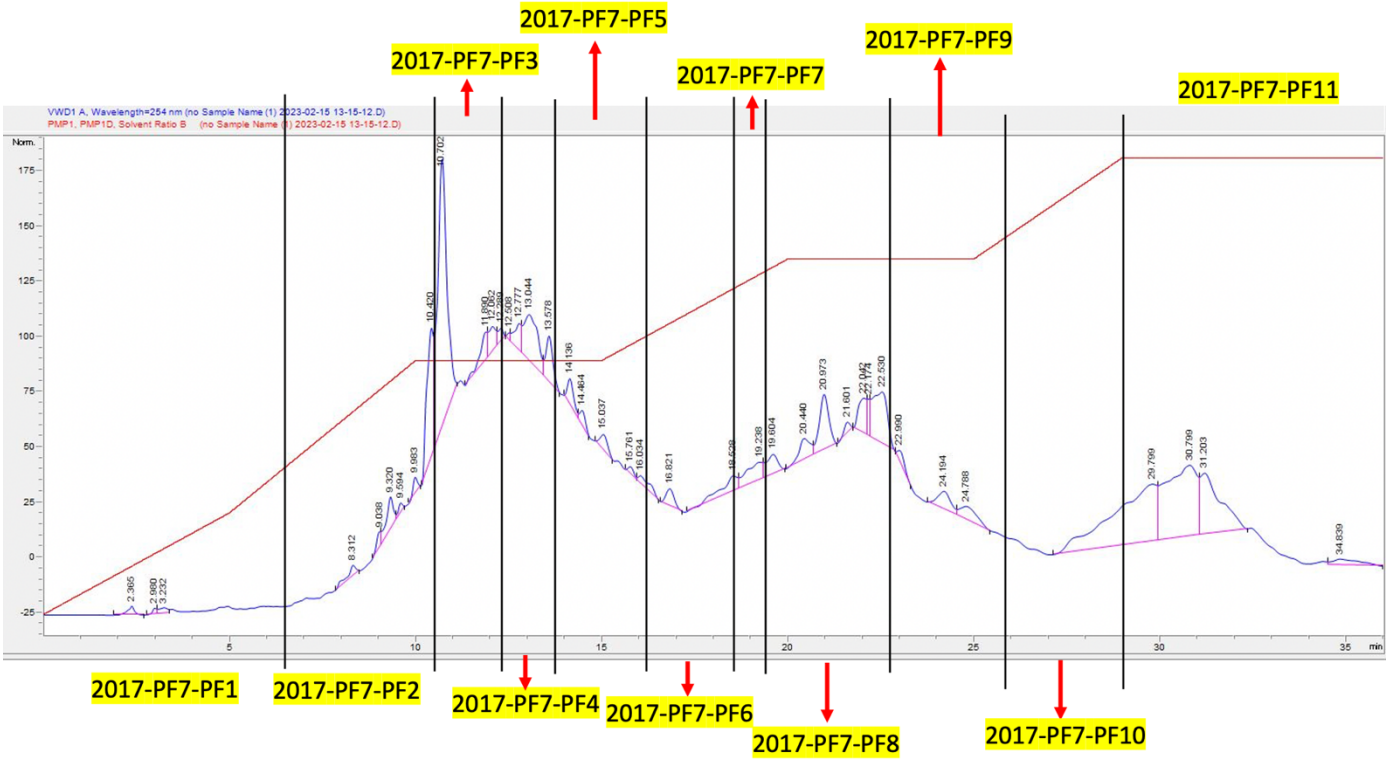


Figure 4.8. Chromatogram of fractions produced from the fractionation of 2017C-PF7 via preparatory RP-HPLC.

Chapter 5: Discussion

5.1 Conclusions

Cinnamon is an integral part of botanical medicine, with its usage found in Chinese and Indian traditional medicine, Egyptian religious and burial ceremonies, and even in Western medicine as an herbal supplement. Thus, the discovery of unreported drug-herb interactions between cinnamon and prescription medications is incredibly important towards the safety and efficacy of botanical medicine. This study is an on-going intragenus study using authenticated plant specimens that investigates the inhibitory activity of four different *Cinnamomum* species of the Lauraceae family, *Cinnamomum burmannii*, *Cinnamomum verum*, *Cinnamomum cassia*, and *Cinnamomum camphora*, against five isoforms of the cytochrome P450 system, a superfamily of enzymes primarily responsible for the metabolism of xenobiotics in the human body: CYP2B6, CYP2C9, CYP2C19, CYP2D6, and CYP3A5.

The study found that the crude ethanolic extracts of these four species demonstrated varying, though significant, levels of inhibition against the five isoforms. The greatest inhibition was observed in CYP2C9, with *C. burmannii* and *C. verum* being more inhibitory than *C. cassia* and *C. camphora*. This trend was seen across CYP2B6 and CYP3A5, while CYP2C19 was most inhibited by *C. camphora* and CYP2D6 showed negligible inhibition by the *Cinnamomum* extracts.

Bioassay-guided fractionation was used to identify which compounds in the *Cinnamomum* species were responsible for the inhibitory activity against the cytochrome P450 isoform CYP2C9, the isoform that was most inhibited. First, a modified Kupchan partitioning scheme was performed on each active crude extract in water against hexanes, ethyl acetate, and *n*-butanol to separate the phytochemicals based on their polarity. Next, the most active partition

was subjected to further fractionation via reverse-phase high-performance liquid chromatogram using a water-acetonitrile gradient. Based on this fractionation scheme, the most active species was *Cinnamomum burmannii*, the most active partition was the ethyl acetate partition, and the most active fraction was 2017C-PF7, the last fraction collected. These results indicate that the compounds responsible for inhibition appear to be non-polar compounds as the most active fraction was collected when the percent of acetonitrile during RP-HPLC was the highest.

5.2 Implications

The discovery of unreported drug-herb interactions is crucial to the safety of the practice of botanical medicine. This study demonstrated that ethanolic extracts of *Cinnamomum* spp., specifically *Cinnamomum* burmannii, inhibit CYP2C9 *in vitro*. As a result, the coadministration of *Cinnamomum* spp. and CYP2C9 substrates and/or inhibitors may lead to drug-herb interactions. Many important and commonly used drugs are metabolized by CYP2C9 (Supplemental Table 1), with one of the most important drug classes being non-steroidal anti-inflammatories, which include diclofenac, ibuprofen, meloxicam, and naproxen. Other important drugs metabolized by CYP2C9 are antidepressants such as fluoxetine and venlafaxine, antihypertensives such as losartan, and antithrombotics such as clopidogrel. However, this study only showed *in vitro* CYP2C9 inhibition due to *Cinnamomum* species extracts, and thus this merits further *in vivo* studies.

While this study examined the inhibitory effects of ethanolic extracts of *Cinnamomum* species against multiple isoforms, their inhibitory activity against many other isoforms still remains unaccounted for. Humans express 57 cytochrome P450 isoforms, and of these 57, there are 15 isoforms whose primary function is the metabolism of xenobiotics. The remainder of the cytochrome P450 isoforms are responsible for the metabolism of substrate classes (F. P.

Guengerich, 2006). Table 5.1 classifies each human cytochrome P450 by their major substrate class.

Table 5.1. Human Cytochrome P450s Classified by Primary Substrate Class

Sterols	Xenobiotic	Fatty acids	Eicosanoids	Vitamins	Unknown
• 1B1 • 7A1 • 7B1 • 8B1 • 11A1 • 11B1 • 11B2 • 17A1 • 19A1 • 21A2 • 27A1 • 39A1 • 46A1 • 51A1	• 1A1 • 1A2* • 2A6 • 2A13 • 2B6‡ • 2C8 • 2C9*‡ • 2C18 • 2C19*‡ • 2D6*‡ • 2E1 • 2F1 • 3A4* • 3A5*‡ • 3A7	• 2J2 • 4A11 • 4B1 • 4F12	• 4F2 • 4F3 • 4F8 • 5A1 • 8A1	• 2R1 • 24A1 • 26A1 • 26B1 • 26C1 • 27B1	• 2A7 • 2S1 • 2U1 • 2W1 • 3A43 • 4A22 • 4F11 • 4F22 • 4V2 • 4X1 • 4Z1 • 20A1 • 27C1

Six of these isoforms (3A4, 3A5, 1A2, 2C9, 2C19, and 2D6, denoted with * in Table 5.1) are responsible for 90% of xenobiotic metabolism, and while this study examined four of the six (CYP2C9, CYP2C19, CYP2D6, and CYP3A5, denoted with ‡ in Table 5.1), other isoforms require further investigation to elucidate the potential inhibitory effects of *Cinnamomum* spp. against them, specifically 1A2 and 3A4.

The cytochrome P450 system is undoubtedly an integral part of drug metabolism, but other enzymes/enzyme systems are additionally responsible for the metabolism of xenobiotics. While *in vitro* studies serve as a good starting point for identifying potential drug-herb interactions, these studies only study the cytochrome P450 system and thus are not sufficient at determining whether this inhibition will translate into *in vivo* models. One study from 2009

found that while dalcetrapib (a cholesteryl ester transfer protein inhibitor) was an *in vitro* inhibitor of CYP1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A4, two follow-up *in vivo* studies showed no significant CYP inhibition in healthy human males (Derks et al., 2009).

This difference observed in CYP inhibition activity can be attributed to the pharmacokinetics and pharmacodynamics of drug metabolism via the Phase II enzymes. One of the most important phase II enzymes is UDP-glucuronosyltransferases, which make xenobiotics more hydrophilic by conjugating the substrate with glucuronic acid. This process occurs with 40-70% of all clinical drugs and many more dietary xenobiotics. UGT1A1, an isoform of the UDP-glucuronosyltransferases superfamily, is responsible for the metabolism of many plant secondary metabolites such as phenols, anthraquinones, flavanones, and coumarins (Jancova et al., 2010).

Unlike the cytochrome P450 system, inhibitors of UDP-glucuronosyltransferases are not as well studied. However, certain analgesics, NSAIDs, antivirals, anticonvulsants and anxiolytics have been described as both inhibitors and inducers of the system (Jancova et al., 2010). As with the cytochrome P450 system, inhibition or induction of these enzymes alters the pharmacokinetics of the xenobiotic, leading to drug deactivation or increased levels of toxicity or drug clearance in the body.

Another confounding factor is the gut microbiota, a vast collection of obligate anaerobic bacteria including important genera like *Bacteroides, Clostridium, Lactobacillus,* and *Escherichia*, various species of yeast, and certain archaeal species such as *Methanobrevibacter*. These microbes benefit the host by aiding in the metabolism of foods to provide more energy to the host. However, this process also affects other xenobiotics, including pharmaceutical drugs and dietary supplements. The microbiota produces enzymes such as azoreductase and

nitroreductase, which aid in the metabolism of benzodiazepines and azole-containing drugs such as sulfasalazine.

The microbiota can also affect the metabolism of plant secondary metabolites. Digoxin, a cardiac glycoside derived from the foxglove plant (*Digitalis lanata* Ehrh., Plantaginaceae), has been found to be metabolized not only by the liver, but by the anaerobic bacterium *Eggerthella lenta* (Eggerthellaceae). The enzymes produced by this bacterium help to inactivate digoxin into a non-toxic metabolite (Mathan et al., 1989).

Altogether, Phase II xenobiotic metabolism enzymes and the gut microbiota are factors not considered in this study that may impact drug metabolism *in vivo* and explain why *in vitro* CYP inhibition does not always correspond to *in vivo* inhibition. Regardless, the discovery of *in vitro* inhibition of CYP2C9 via *Cinnamomum* species is important towards understanding the safety of dietary supplements and botanical medicines containing cinnamon.

As a part of this project, we have additionally been collaborating with the Natural Product-Drug Interactions Research Center (NaPDI). NaPDI was created by the National Institutes of Health National Center for Complementary and Integrative Health to "address the lack of consistency in design, implementation, and dissemination of studies to assess the clinical relevance of natural product-drug interactions" (Center, 2023). Through this collaboration, the inhibition potency and phytochemical composition data collected in this project are currently being deposited into the NaPDI Data Repository, an open-access repository that is publicly accessible internationally. This repository strives to provide reliable data for researchers and physicians interested in reported drug-herb interactions, pharmacokinetics, and their clinical relevance.

5.3 Future Studies

The goal of this study is to identify the natural products responsible for CYP inhibition. The immediate next steps are to perform mass spectroscopy and CYP-inhibition assays of 10 subfractions: 2017C-PF7-PF2 to 2017C-PF7-PF11. Mass spectroscopy data will be compared against the literature to assign a chemical structure to see if it is a previously discovered or novel compound. The subfraction(s) with the most CYP inhibition may be subjected to semi-preparatory HPLC if the mass spectroscopy contains many compounds, and then further analyzed using ^{1}H-NMR, followed by ^{13}C-NMR, 2-D and 3-D NMR if the data suggests the subfraction contains a novel compound.

Once isolated and identified, the natural product(s) responsible for inhibition will be tested in CYP-inhibition assays to obtain IC50s to quantify its potency and to compare its potency with the crude extract and its partitions and fractions. Extracts, partitions and fractions contain many compounds, so this data would reveal whether CYP inhibition by *Cinnamomum burmannii* is due to a single compound or a mixture of them.

Due to the high levels of inhibitory activity of the *Cinnamomum* species against CYP2C9, the inhibitory activity other *Cinnamomum* species merits study as well. One species, in particular, is *Cinnamomum loureirii* Ness. L. (Lauraceae). Like the other *Cinnamomum* species, this plant is used as a spice and medicine. However, it is unique phytochemically as it has higher contents of essential oils compared to other species (30% more than *C. cassia*), and much higher percentage of cinnamaldehyde in this oil. While the results indicate that cinnamaldehyde is not the compound responsible for the high levels of CYP inhibition by *Cinnamomum* spp. extracts (Figure 4.3), it is quite possible that due to the similarity of the phytochemistry across the *Cinnamomum* genus *Cinnamomum loureirii* will exhibit significant levels of CYP inhibition.

Enzyme inhibition assays serve as a good starting point for the safety of dietary supplements such as cinnamon and how they connect with human health. The next study that will be conducted will be a cytotoxicity study, which would investigate whether ethanolic extracts of *Cinnamomum* spp. and their fractions are damaging to cells, specifically HepG2 cells. HepG2 cells are a cell line derived from a human hepatoma, and many studies have shown that this cell line serves as one of the best *in vitro* models for drug toxicity and drug-drug interactions as opposed to using primary hepatocytes (Choi et al., 2015) (Bulutoglu et al., 2020).

Cytotoxicity will be measured via an MTT assay. MTT is a yellow tetrazole that is reduced to a purple formazan in living cells by various oxidoreductase enzymes. In this assay, HepG2 cells in culture medium will be incubated with different concentrations of *Cinnamomum* spp. extracts (crude, partitions, and fractions), and then MTT will be added. The plate will then be read using a plate reader at 590 nm, the wavelength associated with the color yellow. Lower absorbance of this wavelength indicates that MTT was not reduced and that the extracts cytotoxic to HepG2 cells.

Following the cytotoxicity study, an *in vivo* study will be conducted in animal models, most likely mice, to determine whether inhibition of CYP2C9 by *Cinnamomum* spp. extracts is only limited to *in vitro*. Many studies have investigated drug-herb and drug-drug interactions *in vivo* by administering the test drug/extract and a known substrate of the enzyme of interest and then measuring the levels of the substrate in the plasma (Parrish et al., 2016). For this project, mice would be orally administered a known substrate of CYP2C9 (preferably a substrate *only* metabolized by CYP2C9 and not other isoforms) and *Cinnamomum* spp. extracts. Plasma from the mice would be collected at various time intervals over 36 hours. These samples would be subjected to liquid chromatograph with tandem mass spectroscopy to measure substrate levels in

the plasma. Higher levels of substrate over time compared to the control would indicate the substrate is not being metabolized due to CYP inhibition by the *Cinnamomum* spp. extracts.

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Supporting Information

Table S1. Substrates, inhibitors, and inducers of the six CYP isoforms most responsible for drug metabolism.

CYP isoform	Drugs metabolized	Inhibitors	Inducers
CYP3A4/CYP3A5	**Antipsychotics** • Aripiprazole • Brexpiprazole • Haloperidol • Pimozide • Quetiapine • Risperidone **Anxiolytics/Hypnotics:** • Alprazolam • Diazepam • Midazolam • Trazodone • Zaleplon • Zolpidem **Anticonvulsants**: • Carbamazepine • Clobazam • Lamotrigine • Primidone • Phenytoin **Opiods/Opiates** • Alfentanil • Codeine • Fentanyl • Tramadol **Antihistamines** • Chlorpheniramine • Loratadine **Antibiotics** • Clarithromycin • Chloramphenicol • erythromycin **Anticoagulants** • apixaban • clopidogrel • ticagrelor **Corticosteroids** • Dexamethasone • Hydrocortisone	**Antibiotics** • Chloramphenicol • Ciprofloxacin • Clarithromycin • Erythromycin **Antifungals** • Fluconazole • Itraconazole • Ketoconazole • Voriconazole **Antivirals** • Boceprevir • Delaviridine • Indinavir • Nevirapine • Ritonavir • Saquinavir • Telaprevir • Telithromycin **Proton Pump Inhibitors** • Cimetidine • Esomeprazole • Omeprazole • Pantoprazole **Calcium channel blockers** • Diltiazem • Verapamil **HIV protease inhibitors** • Atazanavir • Indinavir • Ritonavir • Saquinavir **Tyrosine kinase inhibitors** • Ceritinib • Imatinib	**Barbiturates**: • Phenobarbital **Glucocorticoids** **HIV-antiretrovirals:** • Efavirenz • Letermovir • Nevirapine **Antiepileptics** • Carbamazepine • Clobazam • Eslicarbazepine • Oxcarbazepine **Anti-cancers** • Enzalutamide • Dabrafenibe • Vemurafenib **Antibiotics** • Rifabutin • Rifampin **Antidiabetics** • Pioglitazone • Troglitazone **Tyrosine Kinase Inhibitors** • Brigatinib • Lorlatinib **CNS Stimulant** • Modafinil **Botanicals** • St. John's wort

	Cardiovascular drugs: **Calcium-channel blockers** • Amlodipine • Diltiazem • Felodipine • Nifedipine • Verapamil **Beta-blockers** • Propranolol **Anti-cancers** • Docetaxel • Nilutamide • Taxol • Vincristine **Proton-pump inhibitors** • Esomeprazole • Lansoprazole • Omeprazole • Pantoprazole **Hormones** • Estradiol • Progesterone • Testosterone **Phosphodiesterase inhibitor** • Avanafil • Sildenafil **Antivirals** • Boceprevir • Daclatasvir • Indinavir • Nelfinavir • Nevirapine • Ritonavir • Saquinavir • Telithromycin • Velpatasvir **Antifungal** • Voriconazole **Tricyclic antidepressants** • Amitriptyline • Clomipramine	• Idelalisib • Tucatinib	

	• Doxepin • Imipramine **SSRI** • Citalopram • Escitalopram • vilazodone **Other** • Caffeine • Colchicine • Cocaine		
CYP1A2	**Antipsychotics** • Clozapine • Haloperidol • Olanzapine **Anti-inflammatory** • Naproxen • Nabumetone • Roflumilast **Beta-blockers** • Propranolol **Calcium Channel Blockers** • Verapamil **CNS Stimulant** • Caffeine • Theophylline **Estradiol** • Estradiol (Hormone) **Immunomodulatory** • Pomalidomide **Muscle Relaxant** • Cyclobenzaprine • Tizanidine **NSAIDS** • Acetaminophen • Naproxen • Nabumetone • Phenacetin **Neuroprotective** • Riluzole **Phosphodiesterase 4 Inhibitor** • Apremilast	**Adenosine receptor antagonist** • Furafylline **Antiarrhythmics** • Amiodarone **Antibiotics** • Ciprofloxacin **Anti-cancers** • Ribociclib • Rucaparib • Vemurafenib **Antiretroviral** • Efavirenz • Simeprevir **Flavonoid** • Quercetin **Furanocoumarin** • Methoxsalen **H2 blocker** • Cimetidine **SSRI** • Citalopram • Fluvoxamine	**Botanicals** • Broccoli • Brussel sprouts • Carbamazepine • Omeprazole • Tobacco

	Serotonin 5-HT3 Receptor Antagonist • Ondansetron **Sodium Channel Blocker** • Mexiletine • Ropivacaine **SSRIs** • Fluvoxamine **Tricyclic Antidepressant** • Amitriptyline • Clomipramine • Doxepin • Imipramine **Other** • Pirfenidone • Tasimelteon • Tacrine • Triamterene • Warfarin • Zileuton • Zolmitriptan		
CYP2C9	**Anticonvulsants**: • Phenytoin • Valproic acid **Antihypertensives** • Azilsartan • Irbesartan • Lesinurad • Losartan **Antidiabetics** • Glibenclamide • Rosiglitazone • Tolbutamide **NSAIDS** • Celecoxib • Diclofenac • Ibuprofen • Meloxicam • Piroxicam • S-naproxen • Suprofen **Antithrombotics**	**Antifungals** • Fluconazole • Voriconazole **Antivirals** • Efavirenz **Anticancers**: • Capecitabine • Ceritinib • Rucaparib **NSAIDs** • Phenylbutazone **Antibiotics** • Isoniazid • Metronidazole • Sulfamethoxazole • Sulfaphenazole **Antihyperlipidemics** • Amiodarone • Fenofibrate • Fluvastatin **SSRIs**	**Antibiotics** • Rifampin **Anti-convulsants** • Carbamazepine **Barbiturates** • Phenobarbital **Botanicals** • St. John's Wort

	• Clopidogrel **Antifungals** • Voriconazole **Anti-cancers** • Capecitabine • Tamoxifen **Antiemetics** • Olodaterol **Bisphosphonates** • Ospemifene **Beta blockers** • Torsemide **Corticosteroids** • Eliben **Statins** • Fluvastatin **Leukotriene receptor antagonist:** • Zafirlukast **JAK inhibitor:** • Ruxolitinib **Tricyclic antidepressants** • Amitriptyline • Doxepin **SSRIs** • Fluoxetine • Venlafaxine	• Fluvoxamine **Anti-asthmas** • Zafirlukast **Flavonoids** • Quercetin	
CYP2C19	**Anticonvulsants** • Brivaracetam • Carisoprodol • Clobazam • Hexobarbital • Phenobarbitone • Phenytoin • Primidone • r-Mephobarbital • s-Mephenytoin **Anxiolytics/Hypnotics:** • Diazepam **Attention-deficit/hyperactivity disorder drugs:** • Atomoxetine **Antibiotics**	**Anti-fungals** • Fluconazole • Ketoconazole **Anti-platelets** • Ticlopidine **Proton-pump inhibitors** • Esomeprazole • Omeprazole **SSRIs** • Fluoxetine • Fluvoxamine	**Antibiotic** • Rifampin **Anti-convulsant** • Carbamazepine **Anti-retrovirals** • Ritnavir **Glucocortisoid** • Prednisone **Botanicals** • St. John’s Wort

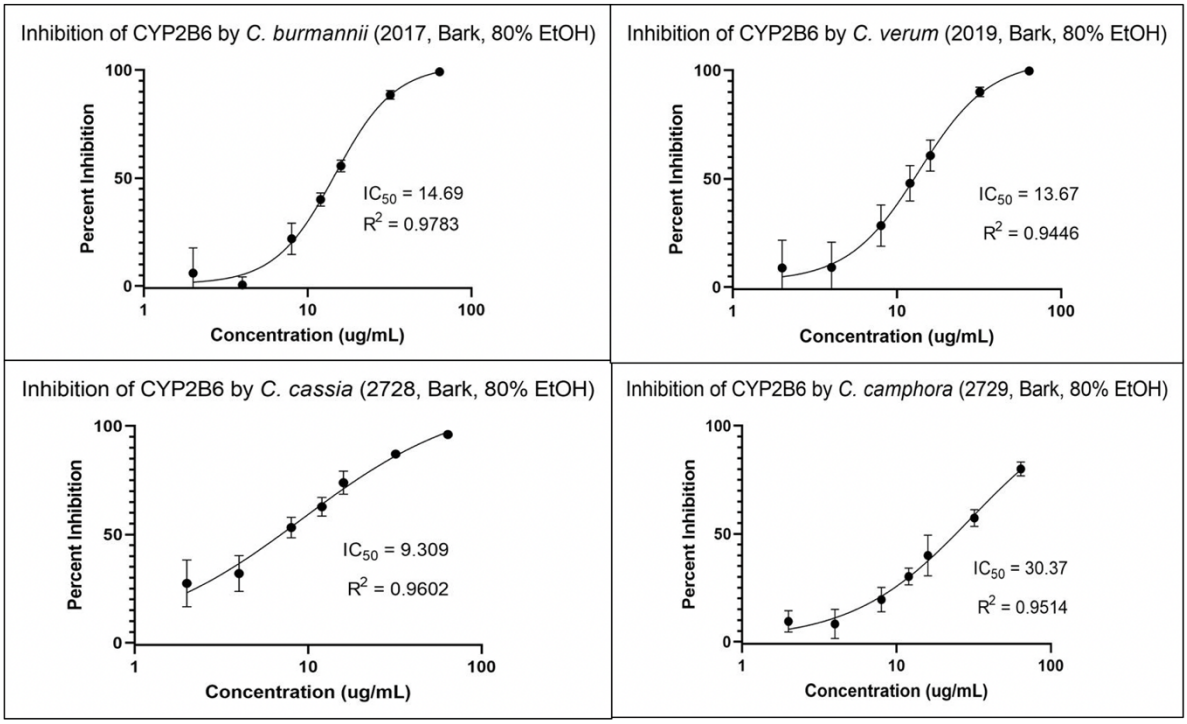
	• Chloramphenicol **Antithrombotics** • Clopidogrel **Chemotherapy drugs** • Cyclophosphamide • Teniposide **Progestins** • Progesterone **Antimalarials** • Proguanil **Beta blockers** • Labetalol • Propranolol **Proton pump inhibitors** • esomeprazole • lansoprazole • omeprazole • pantoprazole **Aromatase inhibitors:** • Nilutamide **Selective serotonin reuptake inhibitors:** • Citalopram • Escitalopram • Flibanserin • Vilazodone **Tricyclic antidepressants** • Amitriptyline • Clomipramine • Doxepin • imipramine **HIV protease inhibitors:** • Nelfinavir **JAK inhibitors:** • Tofacitinib **Antifungals**: • Voriconazole		
CYP2D6	**Beta Blockers** • Alprenolol • Bufuralol • Nebivolol • Propranolol • Timolol **Antipsychotics**	**Anti-androgen** • Abiraterone **Antiarrhythmics** • Amiodarone • Quinidine **Antihistamines** • Clemastine	None reported

	• Aripiprazole • Brexpiprazole • Cariprazine • Chlorpromazine • Haloperidol • Ibrutinib • Risperidone • Zuclopenthixol **CNS Stimulants** • Amphetamine • Atomoxetine • Methoxyamphetamine **Opioids** • Codeine • Oxycodone • Tramadol **Antihistamines** • Chlorpheniramine **Anticonvulsants** • Valproic acid **NSAID** • Acetaminophen • Phenacetin **Antiemetics**: • Metoclopramide • Ondansetron • Palonosetron **Anticoagulants** • Warfarin **Antiarrhythmics** • Encainide • Flecainide • Propafenone **Hormones**: Estradiol, Tamoxifen **Tricyclic antidepressants** • Amitriptyline • Clomipramine • Desipramine • Doxepin • Imipramine • Nortriptyline	• Diphenhydramine • Hydroxyzine • Promethazine • Tripelennamine **Antineoplastic drugs** panobinostat, vemurafenib **Antipsychotics** • Chlorpromazine • Haloperidol • Levomepromazine • Perphenazine **Anxiolytics** • Bupropion • Clobazam **MAOIs** • Moclobemide **NSAIDs** • Celecoxib **Opioids** • Methadone **Anti-platelets** • Ticlopidine **Antiretroviral** • Ritonavir **SNRIs** • Duloxetine **SSRIs** • Citalopram • Escitalopram • Fluoxetine • Paroxetine • Sertraline Stimulant: cocaine **Tricyclic antidepressants** • Clomipramine • Doxepin	

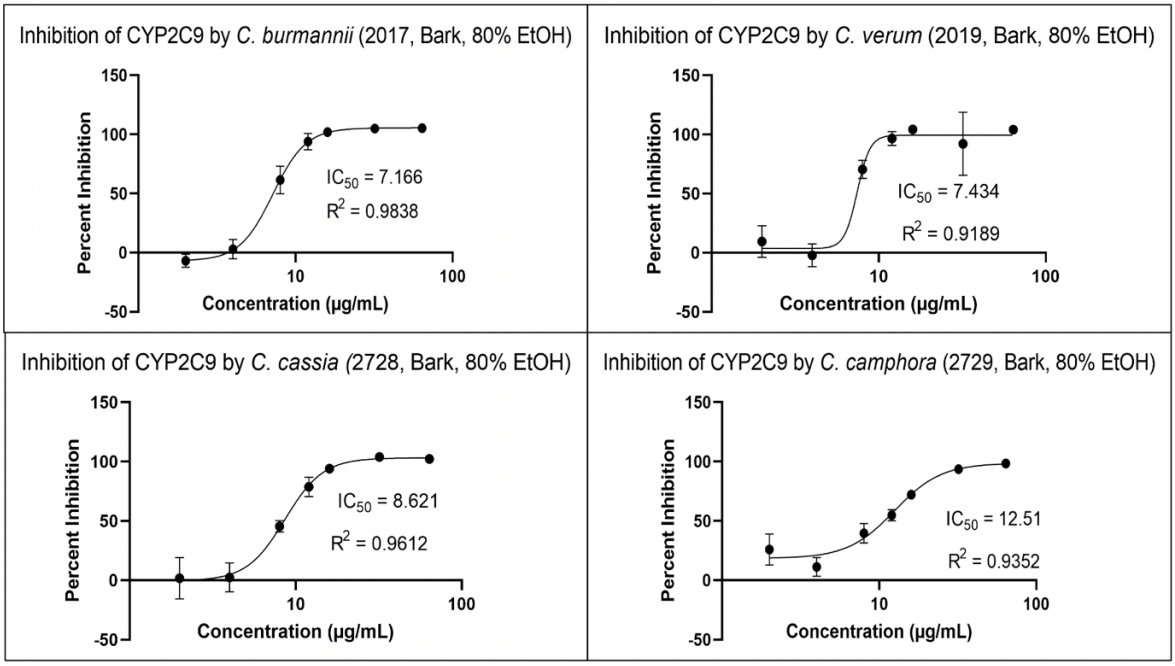
	Selective Serotonin Reuptake Inhibitors • Fluoxetine • Fluvoxamine • Paroxetine • Vilazodone **Serotonin-Norepinephrine uptake inhibitors** • Duloxetine • Venlafaxine, **Other**: • Aniline • Benzene • N, N-Dimethylformamide • Ethanol		

Supplemental Table 1. Cytochrome P450 interaction chart. This table is an adaptation of the Indiana University School of Medicine's Clinical Pharmacology Department's "Drug Interactions Flockhart Table™."

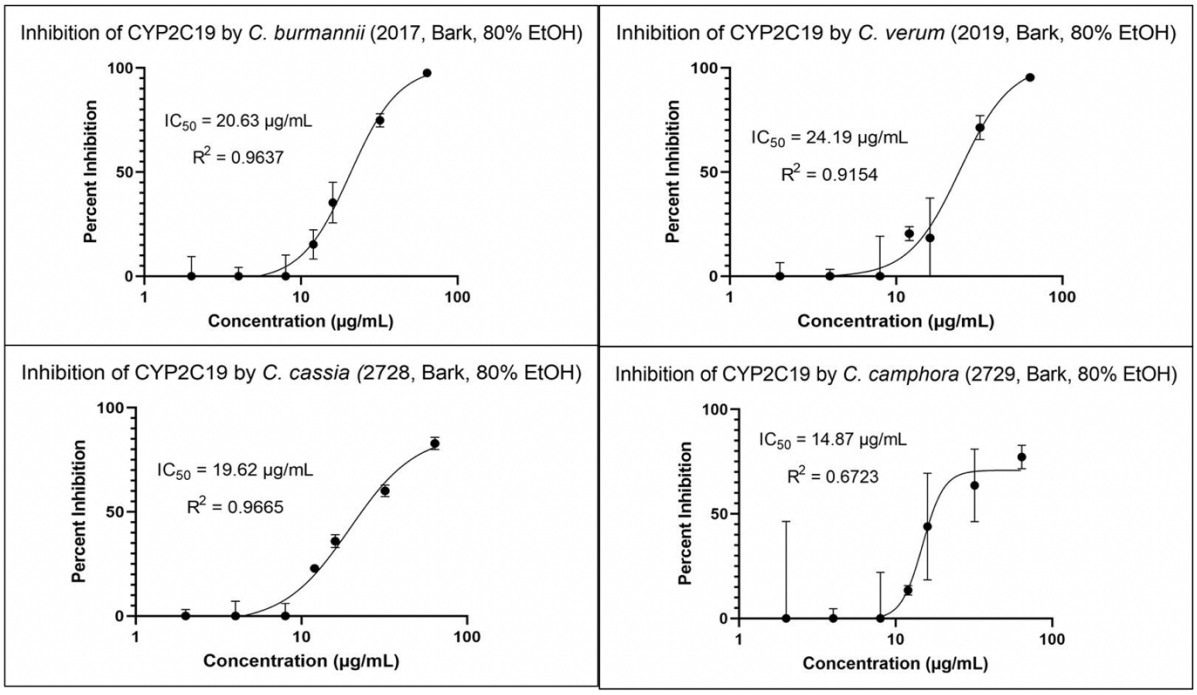
Supplemental Figure 1. Dose-response curve of inhibition of CYP2B6 by *Cinnamomum* spp.



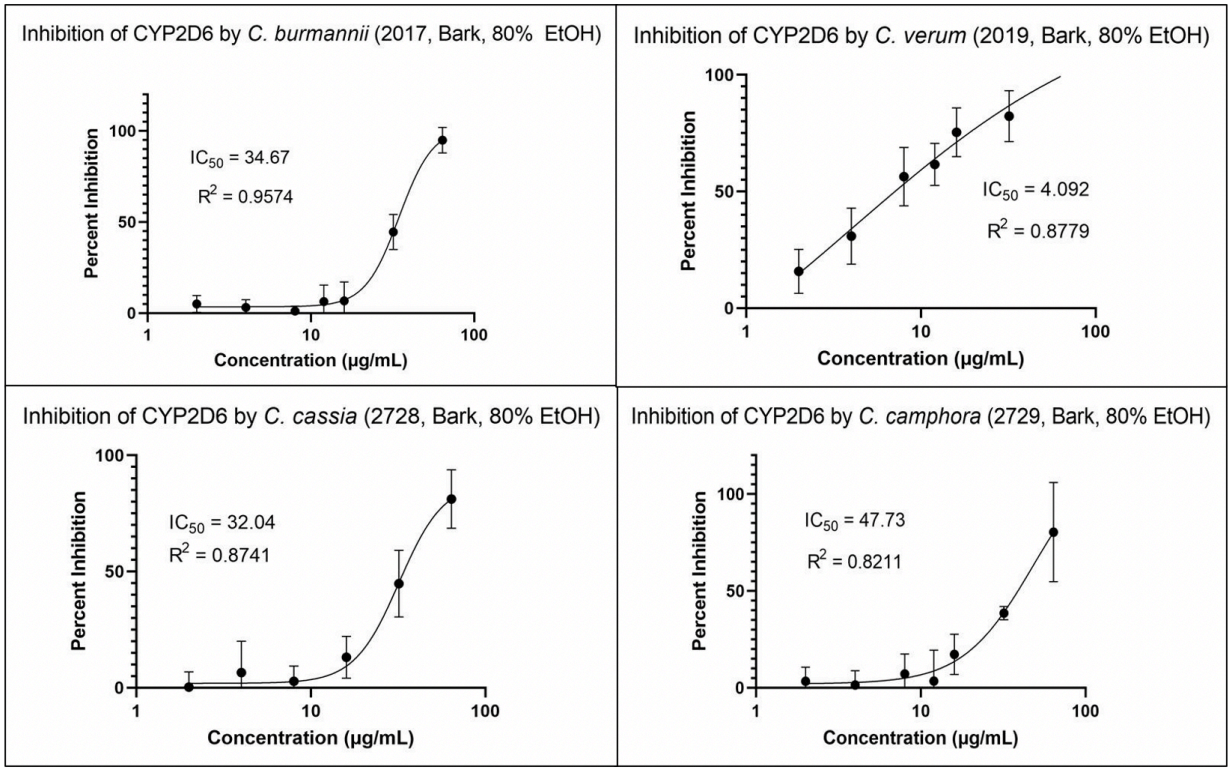
Supplemental Figure 2. Dose-response curve of inhibition of CYP2C9 by *Cinnamomum* spp.



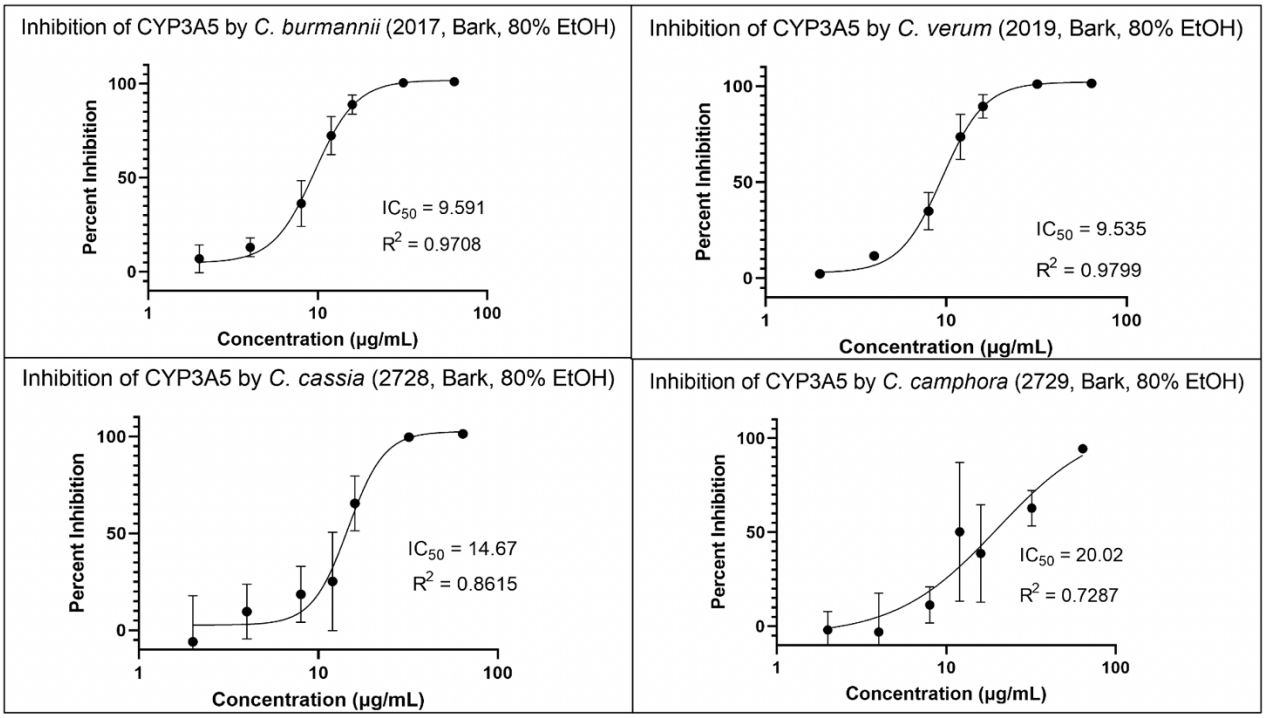
Supplemental Figure 3. Dose-response curve of inhibition of CYP2C19 by *Cinnamomum* spp.



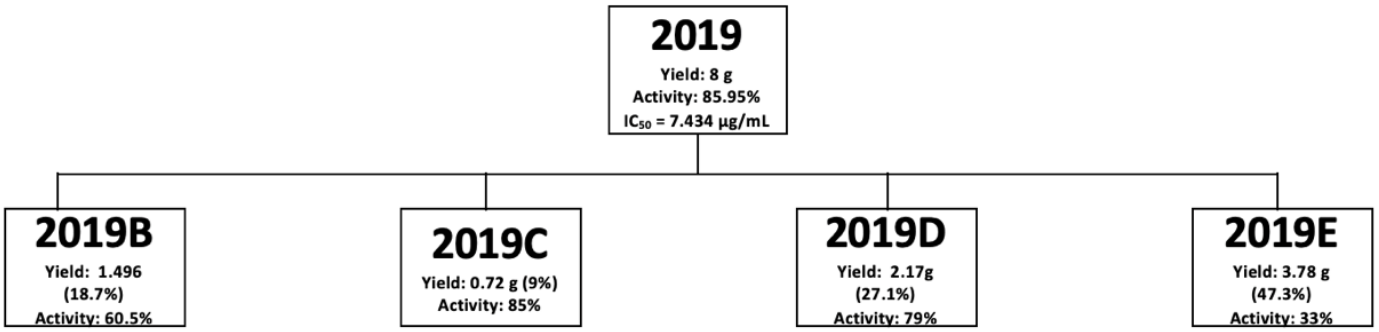
Supplemental Figure 4. Dose-response curve of inhibition of CYP2D6 by *Cinnamomum* spp.



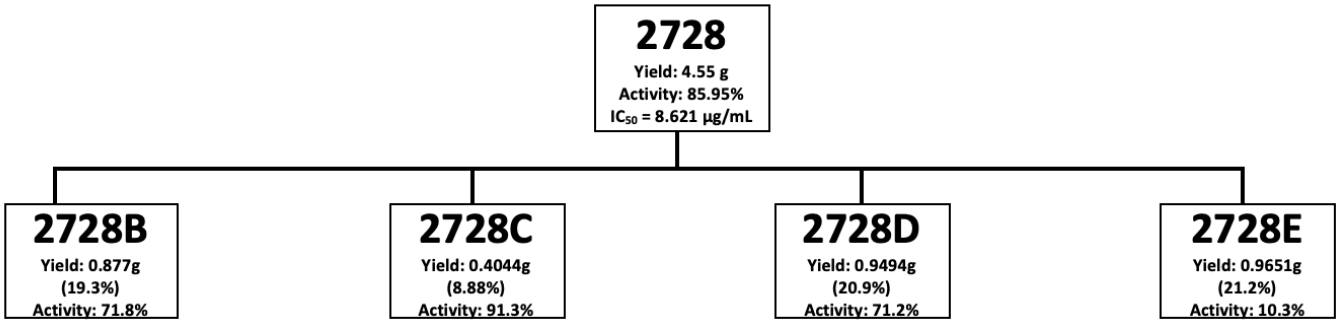
Supplemental Figure 5. Dose-response curve of inhibition of CYP3A5 by *Cinnamomum* spp.



Supplemental Figure 6. Fractionation scheme of *Cinnamomum verum* (2019). Activity is the percent inhibition of CYP2C9 at 8 µg/mL relative to positive control (30 µM sulfaphenazole).



Supplemental Figure 7. Fractionation scheme of *Cinnamomum cassia* (2728). Activity is the percent inhibition of CYP2C9 at 8 µg/mL relative to positive control (30 µM sulfaphenazole).


2728
Yield: 4.55 g
Activity: 85.95%
IC50 = 8.621 µg/mL
2728B
Yield: 0.877g
(19.3%)
Activity: 71.8%
2728C
Yield: 0.4044g
(8.88%)
Activity: 91.3%
2728D
Yield: 0.9494g
(20.9%)
Activity: 71.2%
2728E
Yield: 0.9651g
(21.2%)
Activity: 10.3%