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Cytochromes P450s are present in all forms of life and play an important role in the oxidative transformation of endogeneous and exogeneous molecules. P450 2E1 is a major isoform of cytochrome. The consumption of herbal products in the world is increasing greatly during last decade. The concurrent use of herbal products with prescription carries a risk for unanticipated adverse herb-drug pharmacokinetic interaction, particularly as a result of cytochrome P450 2E1 inhibition.

In this research, the preparation and purification of extract of Green Tea, Goldenseal, Echinacea and Spilanthes was performed. In addition, P450 2E1 related activities were to be examined with respect to inhibition by four potential different inhibitors, including GTE, Goldenseal, Echinacea and Spilanthes. The objective of this research is to determine whether herbal products, such as GTE, Goldenseal, Echinacea and Spilanthes, inhibit the activity of the human P450 2E1 enzymes.

EFFECTS OF HERBAL PRODUCTS ON HUMAN P450 2E1 ACTIVITY

by

Yingqing Wang

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Approved by

_____
Committee Chair

To my daughter Crystal Fu Wang and my wife Yingli Fu

APPROVAL PAGE

This thesis has been approved by the following committee of the Faculty of The Graduate School at The University of North Carolina at Greensboro.

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CHAPTER I

INTRODUCTION

1.1 Cytochrome P450 and P450 2E1

1.1.1 Cytochrome P450: General overview

Cytochrome P450s were discovered in 1955 by Axelrod and Brodie[1]. In 1958 Garfinkel and Klingenberg detected a CO binding pigment in liver microsomes, which had a maximum at wavelength 450nm [2]. From electron spin resonance spectroscopy, we know that P450s are a low spin ferric hemoproteins [3] with a cysteine thiol as an axial ligand [4, 5, 6]. P450s have two major classes: bacterial /mitochondrial (type I), and microsomal (type II) [7]. Many members of this family play a key role in the oxidative transformation of endogeneous and exogeneous molecules [8, 9].

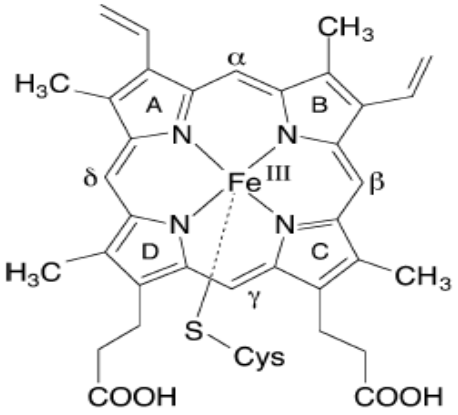

Figure 1. Chemical structure of iron-protoporphryn IX bound via cysteine

Most metabolizing P450s are membrane bound enzymes and are centered on an iron-protoporphryn IX prosthetic group, which is an active site for oxygen binding. The structure of this prosthetic group is shown in Fig. 1. The highest concentration of P450s is in liver in mammals, but the P450s have been reported in almost all tissue including lung, kidney, colon, intestine and brain [10].

P450 catalyzes a variety of reactions including epoxidation, N-dealkylation, O-dealkylation, S-oxidation and hydroxylation. A typical cytochrome P450 catalyzed reaction can be represented by the following chemical equation [11]:

$$NADPH + H^+ + O_2 + RH \Longrightarrow NADP^+ + H_2O + R\text{-}OH$$

In the above equation, RH is an organic substrate and the oxygen atom incorporated into the product R-OH is derived from molecular oxygen. NADPH, in the equation stands for nicotinamide adenine dinucleotide phosphate in its reduced form. This is the biological electron donor to P450 enzymes in the liver [12, 13].

The overall reaction occurs in stages that can be described as a cycle. The catalytic cycle of Cytochrome P450 can be summarized as shown in Figure 2. The steps of the cycle are as follows [14]:

1. Substrate binding

The binding of a substrate to a P450 causes a lowering of the redox potential of the heme, which makes the transfer of an electron favorable from its redox partner, cytochrome P450 reductase, which gets electrons from NADH or NADPH. [15].

2. The first reduction

The next stage in the cycle is the reduction of the Fe^{3+} ion by an electron transferred from NAD(P)H.

3. Oxygen binding

An O_2 molecule binds rapidly to the Fe^{2+} ion forming Fe^{2+} O^{2-} [16].

4. Second reduction

A second reduction is required by the stoichiometry of the reaction. This has been determined in many instances to be the rate-determining step of the reaction. In fact, there is very little direct evidence for the subsequent intermediates in the cycle, because relative to this intermediate they are very unstable [17, 18].

5. O_2 cleavage

The O_2 reacts with two protons from the surrounding solvent, breaking the O-O bond, forming water and leaving an $(Fe\text{-}O)^{3+}$ complex.

6. Product formation

The Fe-ligated O atom is transferred to the substrate forming a hydroxylated form of the substrate, through an “oxygen rebound” type mechanism.

7. Product release

The product is released from the active site of the enzyme which returns to its initial state.

Currently many members of the Cytochrome P450 super-family of hemo-proteins are known, and the numbers continue to grow as more genomes are sequenced. There are almost 4000 identified P450 genes today. In the broadest terms, there are two main

functional roles for these oxygenases. One role is to metabolize foreign chemicals, which serves a protective function for the host organism. A second broad functional role is in the biosynthesis of critical signaling molecules used for control of development and homeostasis. For example, the synthesis of a variety of steroid hormones is carried out by Cytochrome P450 family members [19].

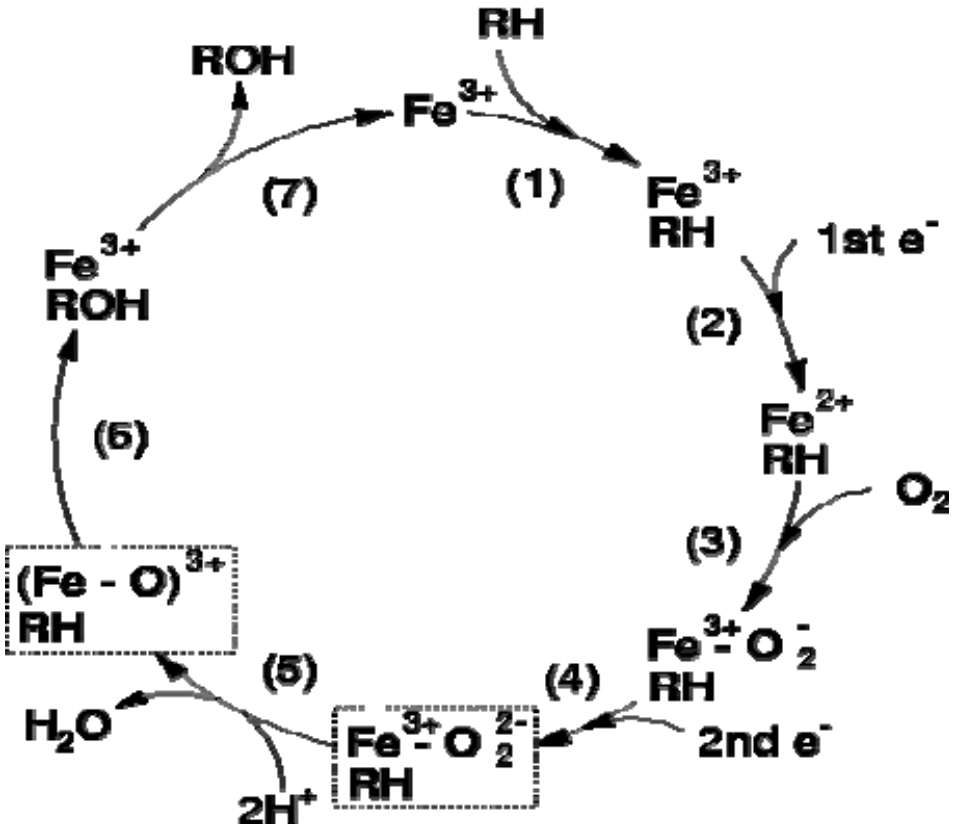

Figure 2. The catalytic cycle of P450

1.1.2 Active-Site Structure of P450 Enzymes

P450 enzymes have a common overall fold and topology despite less than 20% sequence identity across the gene super-family. In past years significant progress has been made in determining the crystal structures of mammalian P450s. The conserved P450 structural core is formed by a four-helix bundle composed of three parallel helices labeled D, L, and I and one anti-parallel helix E. The prosthetic heme group is confined between the distal I helix and proximal L helix and bound to the adjacent Cys heme-ligand loop containing the P450 signature amino acid sequence [20].

The fold of cytochrome P450s is highly conserved and shown in the ribbon representation in Figure 3. Substrate recognition sequence (SRS) regions are shown in black and labeled [20].

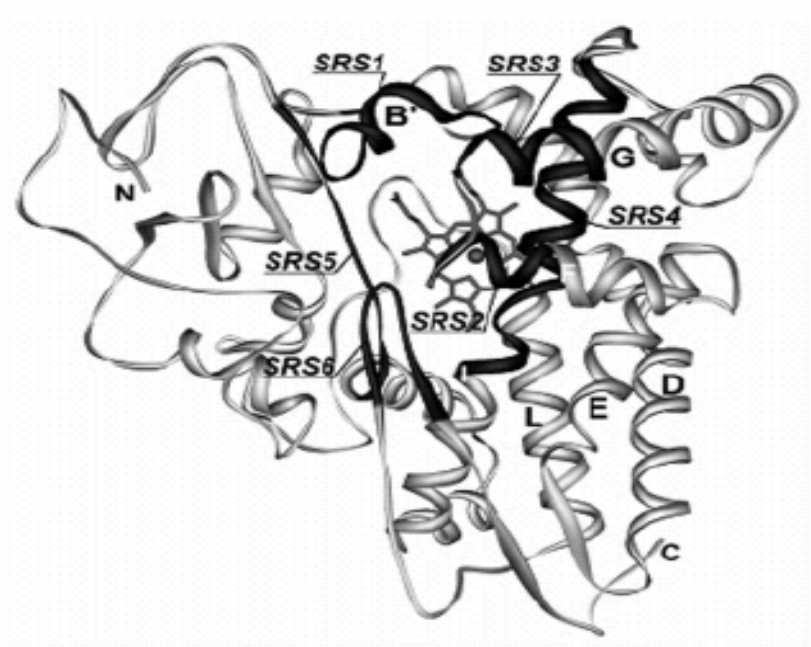

Figure 3. Chemical structure of P450

1.1.3 Cytochrome P450 2E1

Cytochrome P450 2E1 is a major cytochrome isoform in the liver and is an ethanol-inducible form with broad substrate specificity. It is inducible by a number of small organic molecules including therapeutic agents and toxicants. P450 2E1 is an enzyme responsible for the metabolic activation of many carcinogens. This enzyme is also believed to participate in the oxidation of other compounds, such as ethanol, to produce free radicals that may initiate lipid peroxidation and then carcinogenesis. Its inhibition effects could have important clinical applications. Table 1 summarizes many substrates that can be effectively metabolized by P450 2E1 [21].

Table 1. Substrates metabolized by P450 2E1

Substrate	Product Measured
Aromatic compounds	
Pyridine	Pyridine N-oxide
3-Hydroxypridine	2,5-Dihydroxypyridine
p-Nitrophenol	4-Nitrocatechol
Benzene	Phenol
Phenol	hydroquinone; catechol
Acetaminophen	Glutathione conjugates
Pyrazole	4-Hydroxypyrazole
Chlorzoxazone	6-Hydroxychlorzoxazone
Styrene	Glutathione conjugates
Anline	p-Aminophenol
Halogenated alkanes andalkenes/alkanes	
Chloroform	Glutathione conjugates
Pentane	product not measured
Chloromethane	Glutathione conjugates
Dibromoethane	Glutathione conjugates
Dibromomethane	Glutathione conjugates
1,2-Dichloropropane	1, N-Ethenoadenosine
1,1,1-Trichloroethane	1,1,1-Trichloro-2-hydroxyethane
Trichloroethylene	Chloral
Ethylene dibromide	1, N-Ethenoadenosine
Ethylene dichloride	1, N-Ethenoadenosine
Vinyl chloride	1, N-Ethenoadenosine
Vinyl bromide	1, N-Ethenoadenosine
Vinyl carbamate	1, N-Ethenoadenosine
Enflurane	Fluoride
Halothane	Trifluoroacetic acid
1,1,1,2-Tetrafluoroethane	Fluoride
Alcohols/ketones/nitriles	
Ethanol	Acetaldehyde
Propanol	Propionaldehyde
isopropanol	Acetone
Butanol	Butylaldehyde
Pentanol	Valeraldehyde
Glycerol	Formaldehyde
Acetol	Methyglyoxal
Acetone	Acetol
Acetonitrile	Cyanide
Ethers	
Diethyl ether	Acetaldehyde
Methly t-butyl ether	Formaldehyde/t-butanol
Reductive substrate	
Carbon tetrachloride	Lipid peroxidation/chloroform
Chromium	Product not measured
t-butylhydroperoxide	Methane/acetone
Oxygen	Superoxide/peroxide/water

1.2 Inhibition and Induction

1.2.1 Induction

Induction is an increased synthesis of enzyme that is associated with exposure to drugs. The induction can occur when a drug stimulates the biotransformation of drugs either through the same enzyme pathway or via an alternative pathway. However inducers are usually specific for a given Cytochrome P450 family [11].

1.2.2 Inhibition

1.2.2.1 Enzyme kinetics

The Michaelis-Menten model of enzyme kinetics is shown below:

$$E + S \underset{K_{-1}}{\overset{K_1}{\rightleftarrows}} ES \xrightarrow{K_2} E + P \qquad \text{(Eq. 1)}$$

Where, E = Enzyme S = Substrate P = Products

$$K_m = \frac{K_{-1} + K_2}{K_1} \qquad \text{(Eq. 2)}$$

K_m is the Michaelis constant.

The maximum velocity, V_{max}, is achieved when the enzyme is saturated with substrate. Therefore, the Michaelis-Menten equation can be shown in the following form:

$$V = \frac{V_{max} S}{K_m + S} \qquad \text{(Eq. 3)}$$

The Lineweaver-Burk equation can be derived from the Michaelis-Menten equation by taking the reciprocal of the Velocity and Substrate concentration. The equation then takes on the following form:

$$\frac{1}{V} = \frac{K_m}{V_{max}} \times \frac{1}{S} + \frac{1}{V_{max}} \quad \text{(Eq. 4)}$$

Where, K_m is the Michaelis constant; S is the substrate concentration and V_{max} is maximum velocity.

1.2.2.2 Types of enzyme inhibition

Many compounds can inhibit enzymes. Highly reactive chemicals can form covalent bonds with reactive groups on the enzyme and irreversibly inhibit the enzyme. Others interact with the enzyme in reversible ways to produce inhibition. We will concentrate here on the reversible inhibitors of enzymes [22].

1) Competitive inhibition

Competitive inhibition occurs when a compound has a similar chemical structure to the enzyme substrate. The inhibitor interacts with the enzyme to form an unproductive enzyme-competitive inhibitor complex. This results in competitive inhibitors increasing the value of K_m but not affecting V_{max} [22].

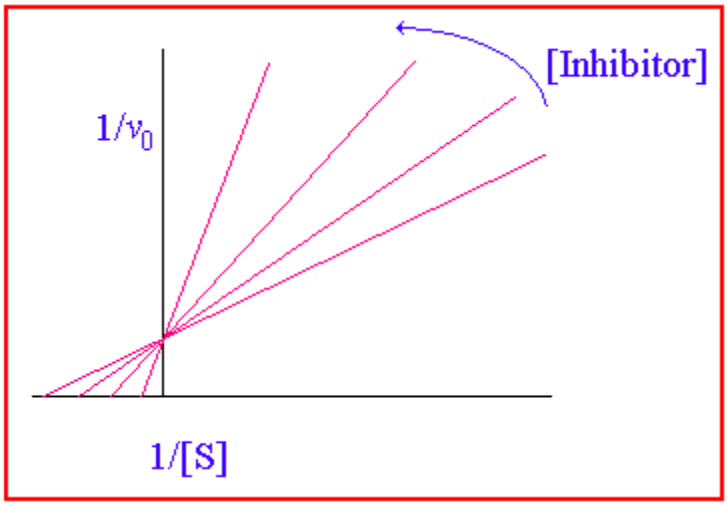

Figure 4. Lineweaver-Burk Plot of competitive inhibition

2) Noncompetitive inhibition

Noncompetitive inhibitors bind to the enzyme-substrate complex and block the catalytic step. They do not affect Km but decrease V_{max} [22].

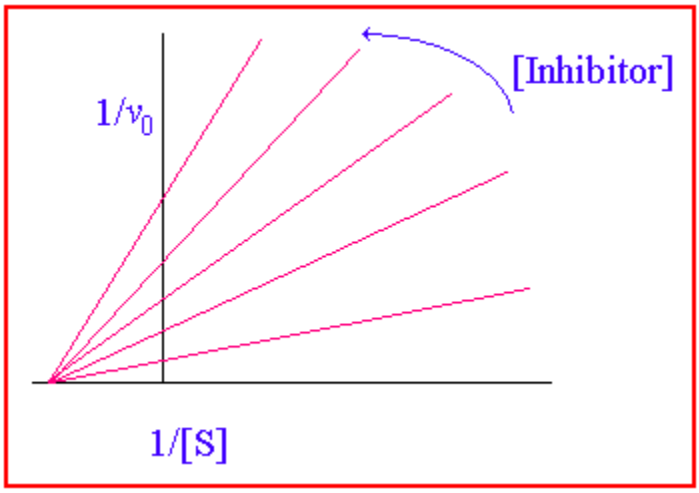

Figure 5. Lineweaver-Burk Plot of noncompetitive inhibition

3) Uncompetitive inhibition

Uncompetitive inhibitors bind to both free enzyme and enzyme-substrate complex. They consequently affect both K_m and V_{max} [22].

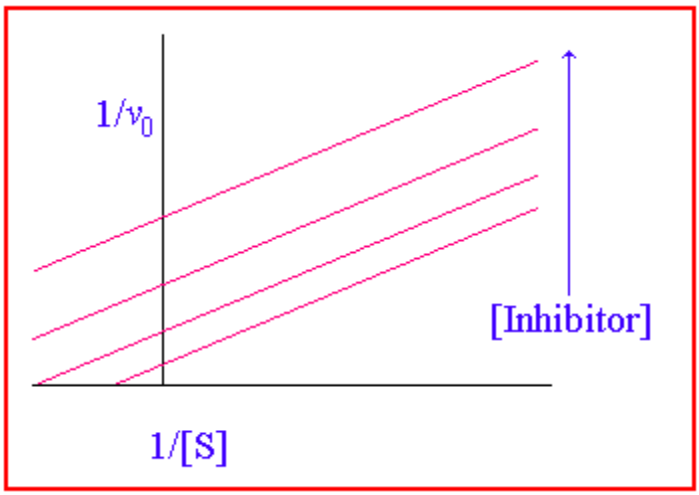

Figure 6. Lineweaver-Burk Plot of uncompetitive inhibition

1.3 Natural products with potential influence on P450 systems

1.3.1 Grapefruit juice

The grapefruit-drug interaction was discovered accidentally in 1989. Grapefruit can interact with a variety of prescription medications. As a widely available fruit source to meet daily nutritional requirements, grapefruit juice is consumed by many individuals everyday. For the above reasons, researchers need to understand grapefruit-drug interaction [23]. The inhibitory constituent of grapefruit juice is furanocoumarin, which

exists in various plants that are sometimes used to cure skin diseases. Previous studies have shown that furanocoumarins were both significant competitive and mechanism-based inhibitors of P450 3A4. This enzyme system in the liver is well known for its involvement with drug-drug interactions. In addition, different furano-coumarin derivatives have been reported to induce or inhibit P450 subtypes such as cytochrome P450 (CYP) 1A1, 1A2, 1B1, 2A5, 2A6 etc [24-30]. Grapefruit juice can also change oral drug pharmacokinetics by different possible mechanisms. Irreversible inactivation of CYP 3A4 is produced by commercial grapefruit juice with a normal amount (e.g. 200-300 ml) or by whole fresh fruit. As a result, P450-related metabolisms are reduced and oral drug bioavailability increased [31].

1.3.2 St John's Wort

For centuries, St John's Wort (*Hypericum perforatum*) has been used as a medicinal herb. It has been believed to possess sedative and astringent properties, and also has been used traditionally for the treatment of excitability, neuralgia, fibrositis, sciatica, anxiety, depression and nerve tonic. Currently, it is best known for its use in the treatment of mild-to-severe depressive disorders, but antiviral and antibacerial effects are also suggested. One reason for its popularity is that it is freely available over-the-counter and another reason is that it is presumed to be safe. However, it has been found to have herb-drug interaction with conventional medicines, including digoxin, cyclosporin, indinavir, amitriptyline, warfarin. Commercially available St. John's wort extracts have

been reported for the potential to inhibit human Cytochrome P450 enzyme activities, specifically of CYP 1A2, CYP 2C9, CYP 2C19, CYP 2D6 and CYP 3A4 [32-39].

1.3.3 Green Tea Extract

Green tea contains a variety of polyphenols including EGCG (epigallocatechin gallate), EGC (epigallocatechin) and EC (epicatechin). Most chemicals are not reactive themselves and require metabolism by a variety of enzymes responsible for drug metabolism. P450 1A enzymes are known to catalyze the metabolic activation of polycyclic aromatic hydrocarbons and aromatic amines. Interest in green tea as a cancer preventive agent has increased dramatically over the past 15 years. Animal studies have demonstrated that green tea popyphenols inhibit carcinogen-induced tumor in numerous tissues, inhibit tumor promotion and decrease tumor size. One mechanism for this may be through its ability to inhibit P450 1A enzyme activity or expression [40-48].

1.3.4 Goldenseal

Goldenseal (*Hydrastis canadensis*) is a popular immuno-stimulant and goldenseal-containing herbal supplements rank high among botanical products sold. Goldenseal extract contains isoquinoline alkaloids, including berberine, canadine and lesser amounts of hydrastine. These three goldenseal alkaloids possess a methylenedioxyphenyl (MDP) moiety, which frequently gives rise to inhibition. Goldenseal is a slow-growing plant and its root was used to treat diseases, such as inflammation and infection of skin. It is frequently combined with Echinacea in treatment

of colds and upper respiratory infections. Goldenseal's widespread popularity is primarily due to its antibiotic activity. Goldenseal was identified as a potent inhibitor of P450 3A4 in previous studies [49-53].

1.3.5 Echinacea

Echinacea, also known as the American coneflower or purple coneflower, has a long history of traditional use by native North Americans for its immuno-stimulatory properties. Echinacea was considered as a panacea for a variety of diseases, including infections, trauma, inflammation, and fever, by the native people of North America [42]. In 1997 Echinacea was the number-one-selling herbal supplement in natural food stores in the United States. The three major species of Echinacea used medicinally are: *E. angustifolia*, *E. pallida*, and *E. purpurea* [50]. *E. purpurea* has been reported to demonstrated mild inhibition of P450 3A4 activity with7-benzyloxy-4-trifluoromethylcoumarin (BFC) as the model substrate, but it has mild inducing effect in the presence of substrate resorufin benzyl ether (Bz Res). Little effect on P450 2D6 and moderate inhibition of P450 2C9 was seen with *E. purpurea* from the report [54-59].

1.3.6 Spilanthes

Spilanthes is a member of the Asteraceae family with over 60 species and is a native of the tropics of Africa and South America. *Spilanthes acmella* is also known as the toothache plant. Spilanthes stimulates the flow of salvia which cleanses the mouth and enhances immune function. Spilanthes also improves digestion, eases flatulence and

helps to overcome nausea and vomiting by its stimulating effect on the salivary glands. There is no relative report about its inhibition of P450 super-family [60-63].

1.4 Herb-drug interaction

The consumption of herbal products in the world is increasing greatly during last decade. It has been found that many herbal medications were taken with drugs at the same time which can cause critical herb-drug interaction. The general public does not know that many health problems can be related with herbal medication. Among these herbal products, St. John's wort was the most common product reported in herb-drug interactions. Herb-drug interactions involving the human Cytochrome P450 systems are normal, and generally result from either enzyme inhibition or induction. Enzyme inhibition often involves competition with another drug for enzyme binding sites. Enzyme induction occurs when one drug stimulates production of a drug-metabolizing enzyme through one of a multitude of biochemical processes. The pharmacologic aspects of drug-herb interactions can provide useful information on the risk of interactions [64-70].

1.5 Proposed Research

Inhibition of cytochrome P450 2E1 enzymes is a clinical concern because drug interactions resulting in impaired drug metabolism can be detrimental and fatal. Inhibition of drug metabolism may cause drug overdose. The purpose of this research is to determine whether herbal products, such as GTE, Goldenseal, Echinacea and Spilanthes, inhibit the catalytic activity of the expressed human P450 2E1 enzymes and which fraction or components are responsible for that inhibition. It is very important because P450 2E1 is active in both detoxification and activation of many organic molecules, and the mechanism of how these herbs affect the activity of P450 2E1 will be useful for the scientific society to understand potential herb-drug interaction.

CHAPTER II

EXPERIMENTAL

2.1 Materials

Microsomes from human liver samples were purchased from Moltox Inc. (Boone, NC) and frozen at -70°C. P450 2E1, p-nitrophenol and NADPH were purchased from Sigma Chemical. Phosphate buffer (pH 7.4) was prepared with potassium phosphate, which was purchased from Sigma Chemical. The GTE catechins were purchased from sigma and GTE extract was manufactured by Spring Valley (Bohemia, NY) and purchased from a local store. The extract of Goldenseal, Echinacea and Spilanthes were prepared by the research group of Dr. Nadja B. Cech of UNCG, and the method for these preparations is described at chapter 2.3 and 2.4. HPLC-grade acetonitrile and acetic acid were purchased from Sigma-Aldrich.

2.2 Preparation and purification of Goldenseal extract and its alkaloid components

Goldenseal samples were purchased from Kentucky, Ohio and North Carolina, and the goldenseal extract was made by Dr. Cech's research group at UNCG. The goldenseal root was powdered using a pestle and weighed to 0.001g. Then a 10mL aliquot of 100% ethanol was added to each mixture. Samples were sonicated for one hour

and then cooled to room temperature. The solvent was evaporated by using a rotory evaporation and the concentrated extracts were filtered. Then a 10mL aliquot was added to the round bottom flask and it was sonicated for 15 minutes to dissolve residue. Finally the solution was transferred to a refrigerator for storage.

The samples were analyzed using HPLC-ESI-MS. The gradient elution of acetonitrile (from 15% to 25% over 25 minutes) and mixed (90% ammonium acetate buffer and 10% acetonitrile) buffer (from 85% to 75% over 25 minutes) were conducted. Samples were injected on a Prevail C18 column (50mm×2.1mm) with 3um packed silica, and the flow rate of solvent is 0.2mL/Min. For the MS, the sheath gas was at 45arb (unit for flow rate given by Xcalibur software) and auxiliary gas was at 20arb. The voltage of the capillary, ion spray source and tube lens was at 11.00V, 4.5KV, 55 kV respectively.

2.3 Preparation and Purification of Spilanthes and Echinacea extract

The extracts of Goldenseal, Spilanthes, and Echinacea were prepared by the research group of Dr. Nadja B. Cech of UNCG. For purification of the alkylamides, a C18 SPE column was first washed with 50mL 100% ethanol and then washed with 50mL 50% ethanol. Then a sample containing 100mL Echinacea extract was diluted to 200mL with DI water, and loaded onto the column using a peristaltic pump. Then the column was washed with 100mL 50% ethanol and eluent was collected in 4 Falcon tubes (50mL), each tube containing 25mL. Solutions containing 55% and 60% ethanol were then used in successive washing steps using the same procedure. The content of each fraction was

analyzed by HPLC, in order to locate the various isobutylamides. For the purification of Spilanthes, a similar procedure was used except the concentrations of ethanol were 40%, 50%, 55% and 60%.

2.4 Assay of P450 2E1 (Nitrophenol Hydroxylation Assay)

The nitrophenol hydroxylation assay was used for determining the inhibition for all four different herbal products, which are GTE, Goldenseal, Echinacea and Spilanthes.

For inhibition experiments using Green Tea Extract, reaction mixtures (total volume: 1000uL) containing 2uL expressed P450 2E1, 50uL NADPH, 100uL buffer and GTE (50-750uL of a 25mg/mL sample) were incubated at 37°C for 20 min before the reaction was stopped by adding 100uL 6% perchloric acid (v/v). After quenching, samples were then placed on ice for 10 min, followed by centrifugation for 10 min at 8,000 rpm. Supernatants were transferred to autosampler vials for HPLC analysis. A C18 HPLC column was used with a mobile phase consisting of 70% C (0.5% acetic acid in H2O) and 30% D (0.5% acetic acid in acetonitrile), with a column flow rate of 1mL/min. The product nitrocatechol was detected at 350 nm. In addition, experiments with GTE (Green Tea Extract), the individual catechins, EGCG, EGC and EC, were tested for their ability to inhibit P450 2E1 using an identical procedure.

For Goldenseal, Echinacea and Spilanthes, reaction mixtures (total volume: 250uL) containing 2.5uL expressed P450 2E1, 12.5uL NADPH, 25ul buffer and Inhibitor (0-50uL stock solution) were incubated at 37°C for 30 min before the reaction was

stopped by adding 50uL 6% perchloric acid (v/v). After quenching, samples were then placed on ice for 10 min, followed by centrifugation for 10 min at 8,000 rpm. Supernatants were transferred to autosampler vials for HPLC analysis. A C18 HPLC column was used with a mobile phase consisting of 70% C (0.5% acetic acid in H2O) and 30% D (0.5% acetic acid in acetonitrile), with a column flow rate of 1mL/min. The product nitrocatechol was detected at 350 nm.

The HPLC system used was Shimadzu and consisted of a personal computer interfaced to an SCL-10Avp controller. Samples were injected into the C18 column (4.6mm×250mm) at a flow rate of 1 ml/min by using the SIL-10ADvp autosampler. The Shimadzu SPD-10Avp UV/Visible detector was used for detection of product at 350nm. The designated peak areas were calculated and concentrations were determined by using the standard curve. Finally, the Vmax and Km were calculated by using the Slidewrite Plus software.

2.5 Data processing

The apparent kinetic constants (V_{max}, K_m) were determined by the non linear regression Enzyme Kinetics program from Slidewrite Plus.

When only a single concentration of substrate and inhibitor were examined, the K_i values for each of the competitive inhibitors were calculated from the following formula:

$$K_i = \frac{V * I * K_m}{V_{max} * S - V * (K_m + S)} \qquad \text{(Eq. 5)}$$

Where K_i is the inhibition constant, V is the rate of reaction in presence of inhibitor [I] at substrate [S], and K_m and the V_{max} are the Michaelis constants for a given reaction. The concentration of substrate is assumed to be constant since the high concentration of substrate is used compared with the concentration of inhibitor.

When a compound inhibits an enzyme competitively, it increases the Km for the reaction without decreasing the V_{max}. In other words, the effect of a competitive inhibitor can be overcome by increasing the substrate concentration.

The K_i values for noncompetitive inhibitors were calculated from the following formula:

$$K_i = \frac{V * I * (K_m + S)}{V_{max} * S - V * (K_m + S)} \quad \text{(Eq. 6)}$$

CHAPTER III

RESULTS

3.1 Results of Green Tea Extract (GTE)

Table 2-5 shows the raw activity and inhibition data for the complete GTE and the catechins, EGCG (epigallocatechin gallate), EGC (epigallocatechin) and EC (epicatechin) with respect to P450 2E1 activity. Values represented in the tables and graphs are peak areas of the product in the reaction of p-nitrophenol with P450 2E1. The peak areas are directly proportional to the velocity of the reaction.

3.1.1 EGCG (100ug/mL)

Table 2. The values of concentration of product (nitrocatechol) with and without potential inhibitor of EGCG

Substrate (uM)	Product w/ inhibitor (ug/mL)	Product w/o inhibitor (ug/mL)
10	0.478122	0.473755
20	0.604760	0.674629
40	0.652795	0.844934
60	0.766332	0.967205
80	0.665895	0.971572
100	0.980306	1.172445

3.1.2. EGC (100ug/mL)

Table 3. The values of concentration of product (nitrocatechol) with and without potential inhibitor of EGC

Substrate (uM)	Product w/ inhibitor (ug/mL)	Product w/o inhibitor (ug/mL)
10	0.657162	0.709563
20	0.779432	1.032707
40	0.989039	1.089476
60	0.805633	0.884236
80	0.853668	0.954105
100	0.853668	0.975939

3.1.3 EC: (100ug/mL)

Table 4.The values of concentration of product (nitrocatechol) with and without potential inhibitor of EC

Substrate (uM)	Product w/ inhibitor (ug/mL)	Product w/o inhibitor (ug/mL)
10	3.355852	3.783799
20	4.526157	4.792533
40	5.412620	5.661528
60	5.465022	6.303450
80	5.451921	6.220480
100	5.840568	6.661528

3.1.4. GTE (100ug/mL)

Table 5. The values of concentration of product (nitrocatechol) with and without potential inhibitor of GTE

Substrate (uM)	Product w/ inhibitor (ug/mL)	Product w/o inhibitor (ug/mL)
10	1.971572	3.971572
20	2.390786	4.015240
40	3.478122	4.670262
60	3.814367	4.980306
80	3.858035	6.054541
100	3.884236	6.141878

Four different inhibitors were examined by using the nitrophenol assay, which was described in the previous chapter. From the above data, all four inhibitor clearly didn't inhibit the activities of P450 2E1 very much. Only GTE showed some inhibition of enzyme P450 2E1, but it is still not potent. Therefore we could draw a conclusion that the GTE and its components don't inhibit the activities of P450 2E1 and the kinetic parameters such as K_m, V_{max} and K_i don't need to be calculated.

3.2 Results of Goldenseal

Figure 7-10 shows the inhibition by goldenseal and the individual alkaloids Canadine, Hydrastine and Berberine with respect to P450 2E1 activity. Values represented in graphs are velocities of the product in the reaction of p-nitrophenol with P450 2E1. Experiments were run at two different inhibitor concentrations, 100 μM and 4 μM. The standard curve of nitrocatechol was prepared in which nitrocatechol was plotted versus peak area. The resulting linear equation was used to correlate area to product. y=23900x+1551, where x means concentration (μM) and y means peak area.

3.2.1 Inhibition effect on P450 2E1 with the potential inhibitor (100μM)

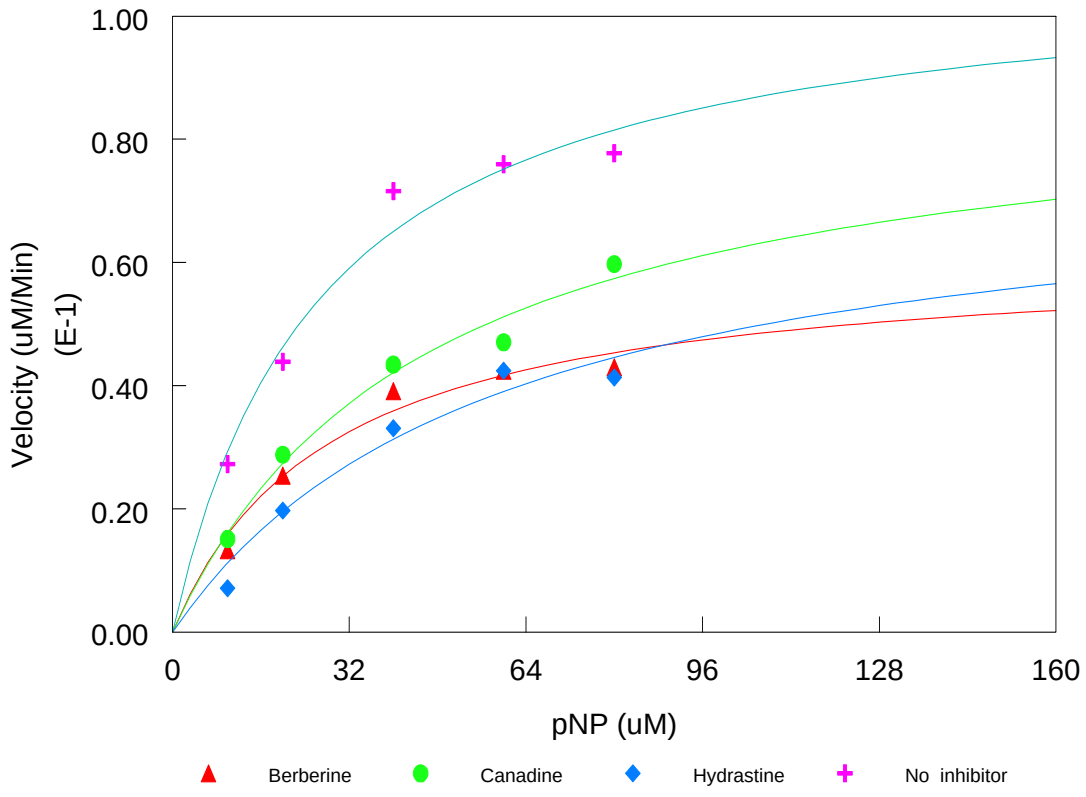

Figure 7. Michaelis-Menten plot of nitrophenol oxidation by 2E1 and inhibition by goldenseal components. Each data point is the average from two samples.

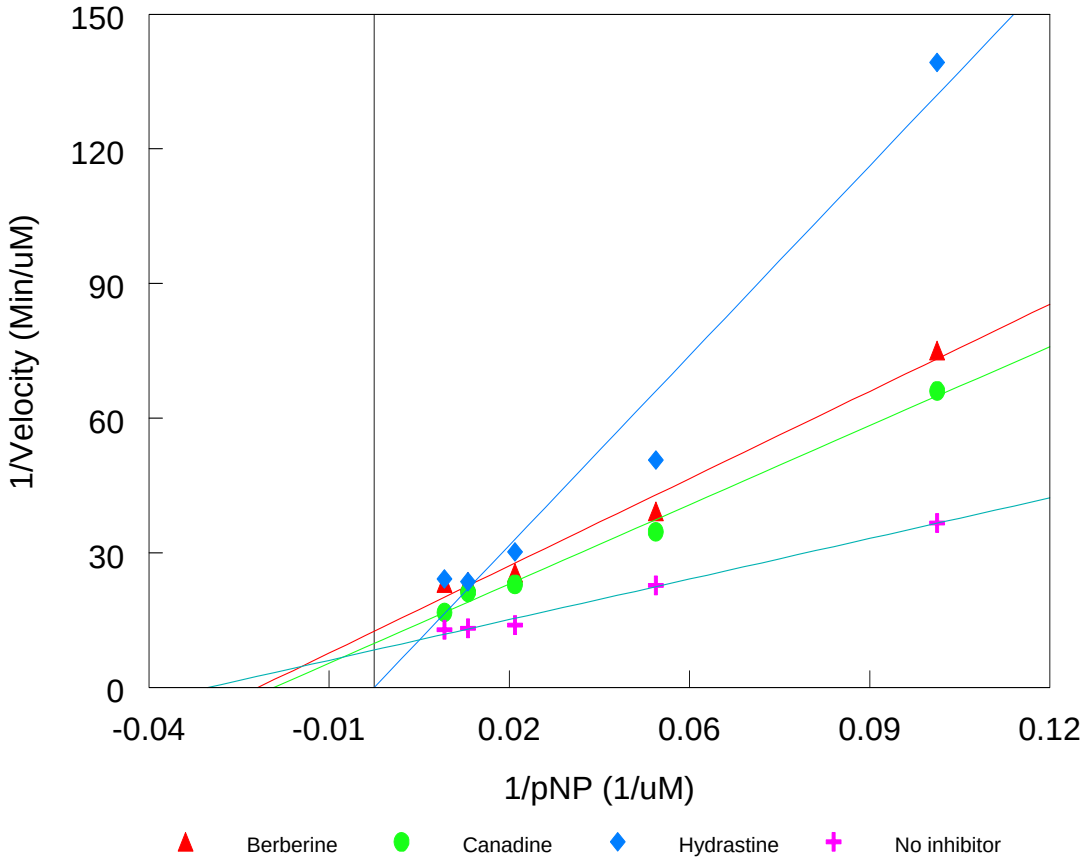

Figure 8. Lineweaver Burk plot of nitrophenol oxidation by 2E1 and inhibition by goldenseal components. Each data point is the average from two samples.

Figure 8 and 9 sumarizes the results of inhibition studies carried out using each of the main alkoloid components of a goldenseal extract, such as hydrastine, canadine and berberine, each at a concentration of 100 μM. As indicated by the Michaelis-Menten curve, each component was capable of inhibiting P4502E1 activity in human liver microsomes significantly. Hydrastine appeared to be the most potent inhibitor among these three components of Goldenseal. The data displayed in this figure was analyzed using the Michaelis-Menten model for enzyme inhibition, and the kinetic parameters such as K_m, V_{max} are summarized in table 6. From this data table, berberine appears to be noncompetitive inhibitor because it has very similar K_m with that of the control. Canadine and hydrastine appear to be competitive inhibitors with different K_i value. The Ki value of 86uM of hydrastine indicates that hydrastine is the most potent inhibitor on P450 2E1's activities.

K_i can be calculated from the following equations:

Competitive Inhibitor: $K_m' = K_m * (1 + [I]/K_i)$

Non-Competitive Inhibitor: $V_{max}' = V_{max}/(1 + [I]/K_i)$

3.2.2 Inhibition effect on P450 2E1 with the potential inhibitor (4uM)

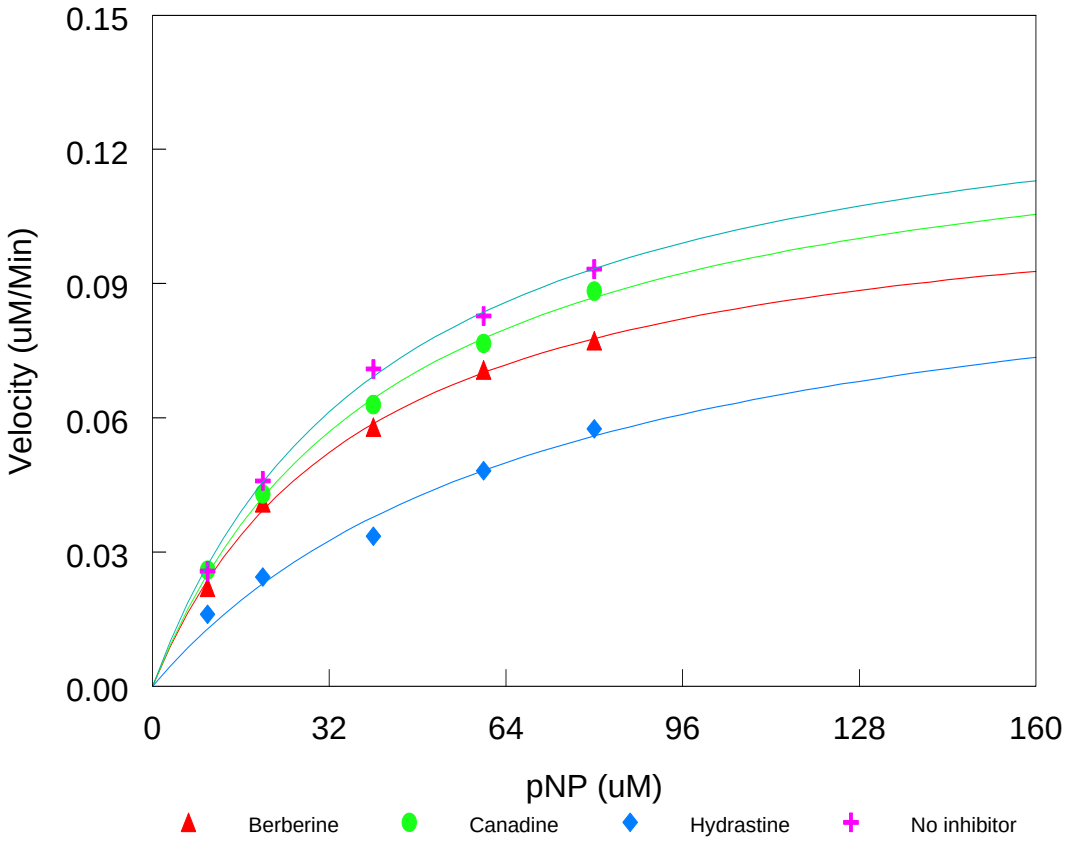

Figure 9. Michaelis-Menten plot of nitrophenol oxidation by 2E1 and inhibition by goldenseal components. Each data point is the average from two samples.

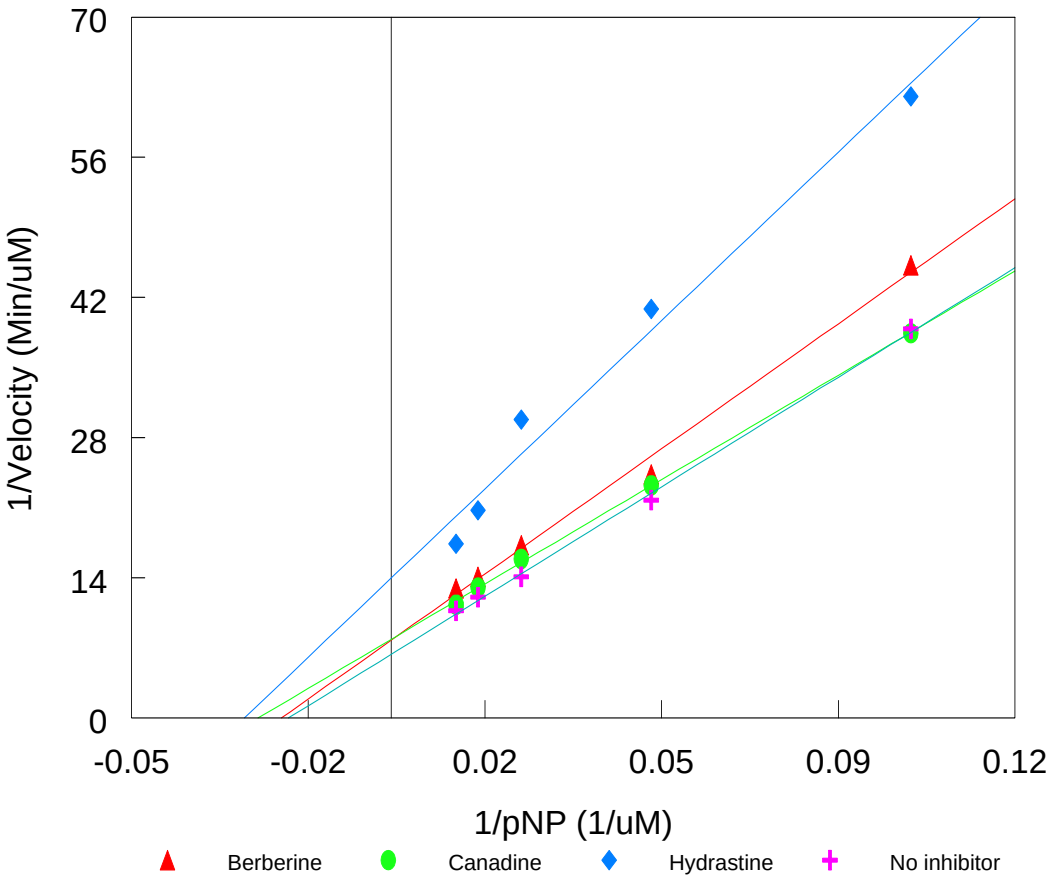

Figure 10. Lineweaver Burk plot of nitrophenol oxidation by 2E1 and inhibition by goldenseal components. Each data point is the average from two samples.

Figure 10 and 11 summarize the results of inhibition studies carried out using each of the main alkaloid components of a goldenseal extract (hydrastine, canadine and berberine), each at a concentration of 4uM. As indicated by the Michaelis-Menten curve, each component was capable of inhibiting P450 2E1 activity in human liver microsomes significantly. Of the three compounds tested, hydrastine appeared to be the most potent. The data displayed in this figure was analyzed using the Michaelis-Menten model for enzyme inhibition, and K_i values for each of the compounds were determined. These values are summarized in table 6. As seen from this table of goldenseal, Berberine and Canadine appear to be noncompetitive inhibitors since they have similar K_m with that of the control and the K_i are 18.4uM and 16.9 uM respectively. For hydrastine, it could have mixed inhibition. A K_i value of 3.2 uM for hydrastine indicates that this compound is the most potent inhibitor of P450 2E1.

Table 6. The values of inhibition constant (K_i), Maximum Velocity (V_{max}) and Michaelis Constant (K_m) for P450 2E1 in the presence of Goldenseal components (4µM and 100µM)

	V_{max} (µM/Min)	K_m (µM)	K_i (µM)
Berberine (4µM)	0.129±0.092	47.4±10.8	18.4
Canadine (4µM)	0.127±0.089	39.0±6.51	16.9
Hydrastine (4µM)	0.070±0.004	35.4±5.83	3.20
Control	0.157±0.097	50.5±11.6	N/A
Berberine (100µM)	0.062±0.003	28.5±4.79	132
Canadine (100µM)	0.090±0.005	45.9±10.2	144
Hydrastine (100µM)	0.077±0.004	58.8±11.9	86
Control	0.109±0.077	27.1±5.72	N/A

3.3 Results of Echinacea

Figure 11-17 represents inhibition data for Echinacea and the individual fraction with respect to P4502E1 activity. Values represented in the graphs are velocities of the product in the reaction of p-nitrophenol with P450 2E1. The standard curve used to calculate alkylamide concentrations in Echinacea gave the following linear equation y=2120x-30883, where x means concentration (μM) and y means peak area (Table 7). The fractions 1 to 4 are the eluent by using 50% ethanol washing (method was described in chapter 2.3). The fractions 5 to 6 are the eluent by using 55% ethanol washing, and the method also was described in chapter 2.3.

The standard curve of isobutylamide can be calculated from the following table.

Table 7. The peak area of different concentration of isobutylamide

Number	Concentration (μM)	Peak Area
1	1000	2099800
2	500	1010700
3	250	481800
4	100	205600

3.3.1 Fraction 1

The results of fraction 1 show that the activities of P450 2E1 are almost completely inhibited because the concentrations of product are very low, therefore Michaelis-Menten plots could not be prepared.

3.3.2 Fraction 2

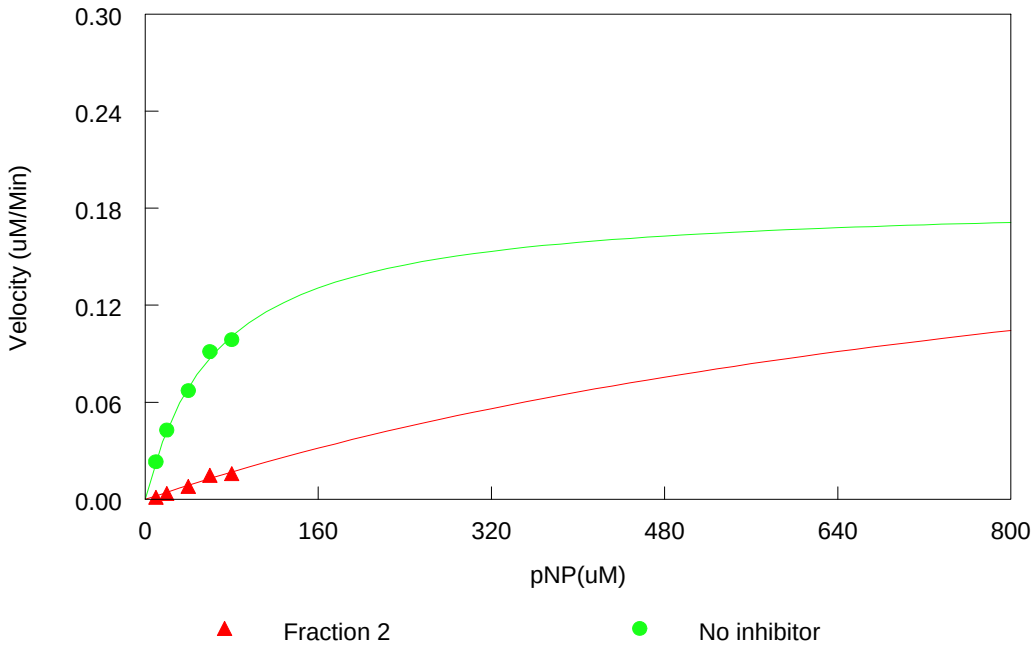

Figure 11. Michaelis-Menten plot of nitrophenol oxidation by 2E1 and inhibition by Echinacea fraction 2. Each data point is the average from two samples.

The ability of Echinacea fraction 2 to alter the 2E1 enzymatic activities is shown in Figure 11. From this Michaelis-Menten curve, fraction 2 of Echinacea was capable of inhibiting the P450 2E1 activities greatly. Five pNP (substrate) (10-80μM) concentrations were used.

3.3.3 Fraction 3

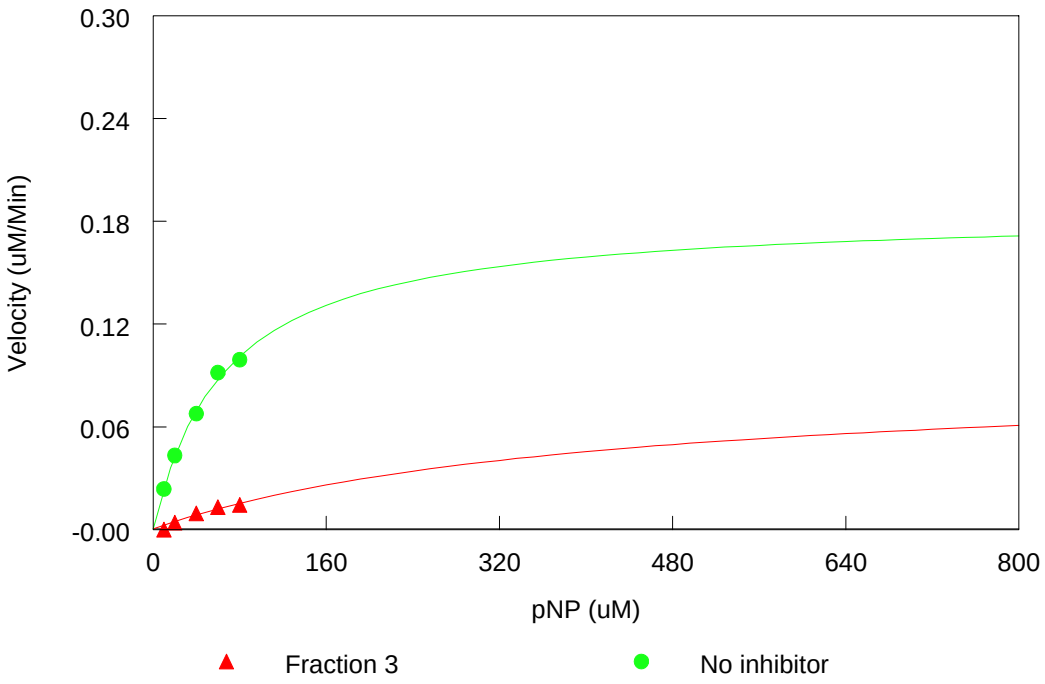

Figure 12. Michaelis-Menten plot of nitrophenol oxidation by 2E1 and inhibition by Echinacea fraction 3

Figure 12 indicates the inhibition effects of fraction 3 of Echinacea by using pNP assay, which is described in the previous chapter. From Figure 12, fraction 3 of Echinacea was also shown that it could greatly inhibit the P450 2E1 activities, and it has the similar inhibition effect on 2E1 with that of fraction 2.

3.3.4 Fraction 4

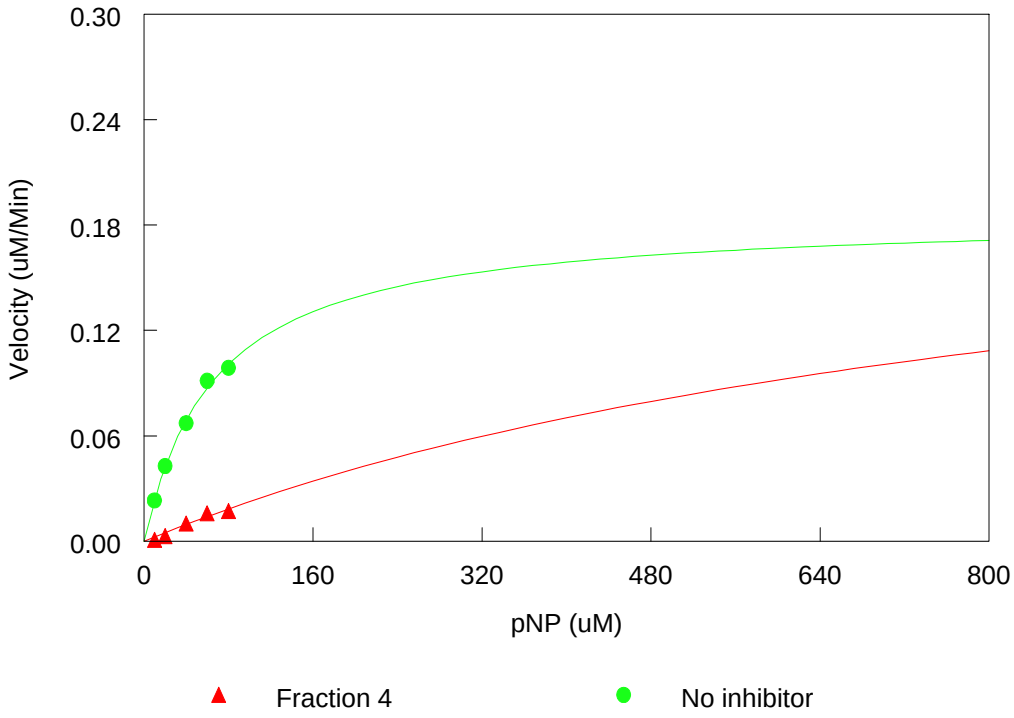

Figure 13. Michaelis-Menten plot of nitrophenol oxidation by 2E1 and inhibition by Echinacea fraction 4

The ability of Echinacea fraction 4 to alter the 2E1 enzymatic activities is shown in Figure 13. From this Michaelis-Menten plot, fraction 4 of Echinacea was able to inhibit the P450 2E1 activities greatly. The inhibition on P450 2E1 was similar to that of fraction 2 and fraction 3. The Lineweaver-Burk Plot of these three fractions will be plotted in the same graph to determine their relationship to control.

3.3.5 Fraction 5

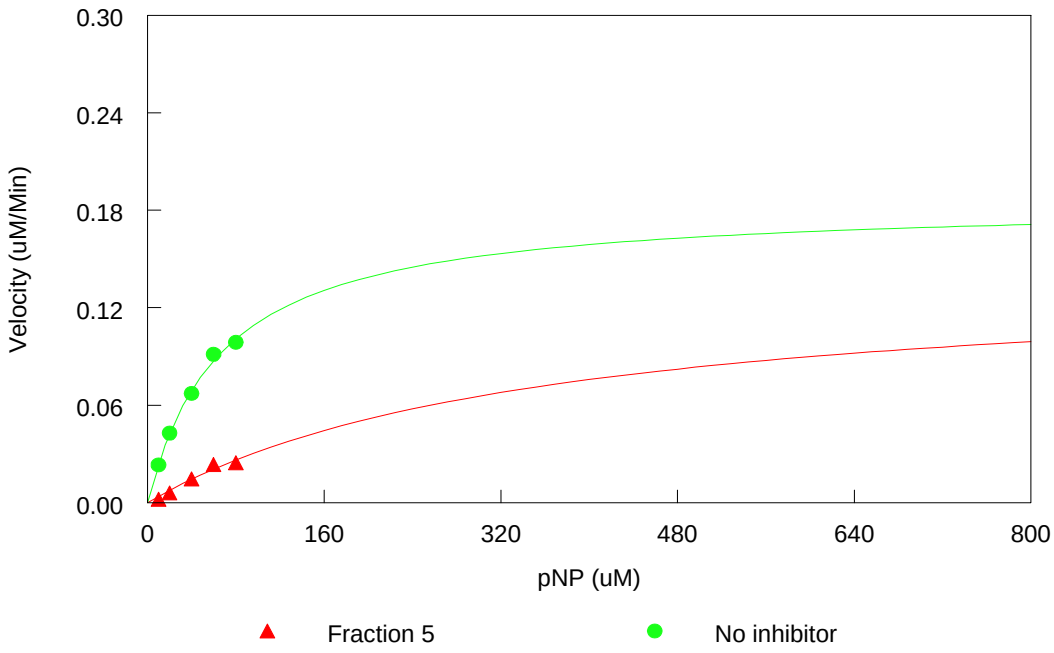

Figure 14. Michaelis-Menten plot of nitrophenol oxidation by 2E1 and inhibition by Echinacea fraction 5

Figure 14 indicates the inhibition effects of fraction 5 of Echinacea by using pNP assay. From this Michaelis-Menten curve, it shows that fraction 5 of Echinacea was capable of inhibiting the P450 2E1 activities to some extend, but is not as much as that of above three fractions 2, 3, 4. It can also be seen that the concentration of P450 2E1 inhibitor in fraction 5 is decreased.

3.3.6 Fraction 6

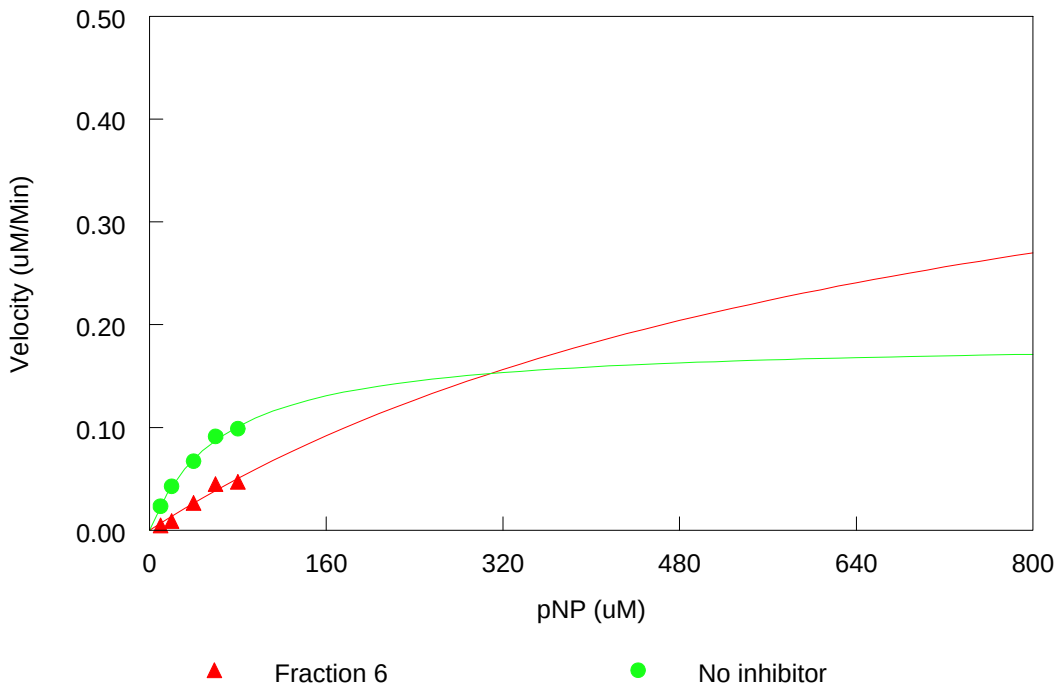

Figure 15. Michaelis-Menten plot of nitrophenol oxidation by 2E1 and inhibition by Echinacea fraction 6

The ability of Echinacea fraction 6 to alter the 2E1 enzymatic activities is shown in Figure 15. From this Michaelis-Menten plot, fraction 6 of Echinacea could inhibit the P450 2E1 activities to some extend. It has the least inhibition effect on P450 2E1 among these 5 fractions of Echinacea. The Lineweaver Burk Plot of fraction 4, 5, 6 will be plotted in the same graph to determine their relationship to control experiment.

3.3.7 Control

Table 8. The values of V(Velocity), Substrate(S), 1/V, and 1/S of control of Echinacea

Peak Area control	X (uM)	Velocity (uM/Min)	1/V (Min/uM)	S (uM)	1/S (1/uM)
18300	23.19953	4.342042	0.230306	10	0.1000
32300	29.8033	5.578009	0.179275	20	0.0500
49850	38.0816	7.127382	0.140304	40	0.0250
67050	46.19481	8.645857	0.115662	60	0.0167
72400	48.7184	9.118173	0.109671	80	0.0125

3.3.8 Comparison of fraction 2 3 4 with control

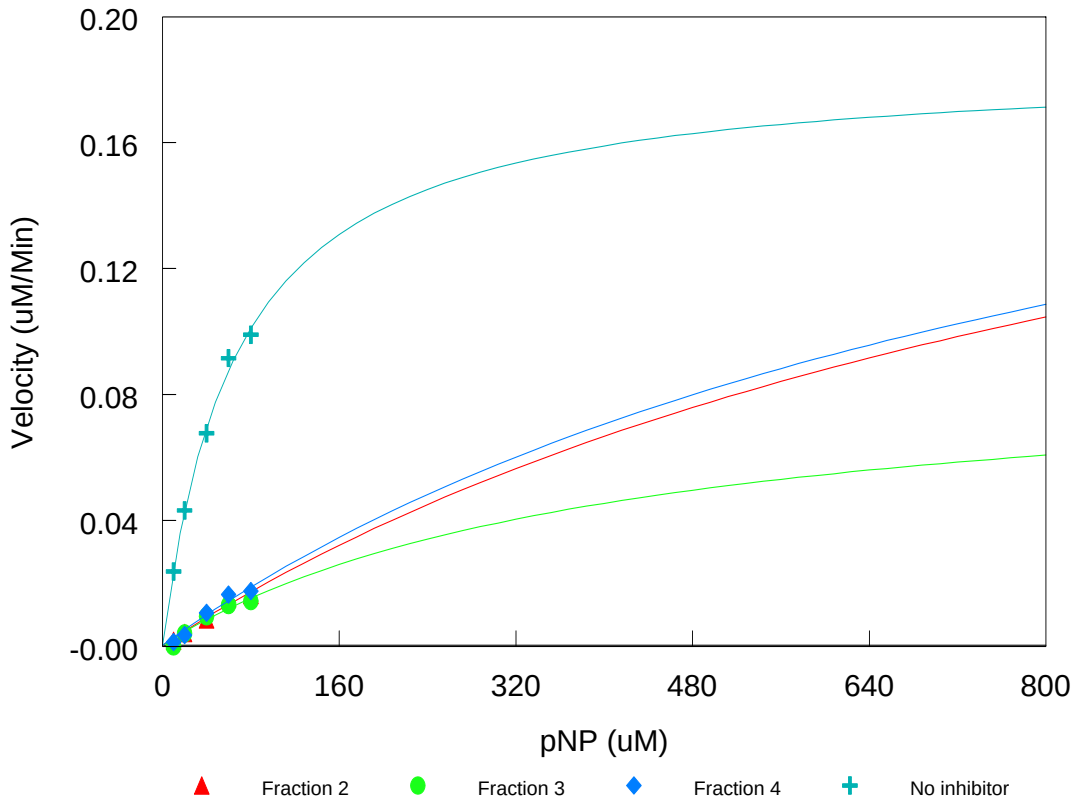

Figure 16. Michaelis-Menten plot of nitrophenol oxidation by 2E1 and inhibition by Echinacea fraction 2 3 4

The comparison of the ability of Echinacea fraction 2, 3, 4 to alter the 2E1 enzymatic activities is shown in Figure 16. Figure 16 is the Michaelis-Menten plot of these three fractions of Echinacea. The pNP assay was used for all three factions experiments. From this Michaelis-Menten curve, it appears that fraction 2, 3, 4 of Echinacea were capable of inhibiting the P450 2E1 activities greatly and it also can be seen that the fraction 2, 3, 4 have very similar inhibition effect on P450 2E1. From table 9, it appears that it is the mixed inhibition on P450 2E1 for fraction 3 of Echinacea because each of these fractions has different V_{max} and K_i from that of control. The fraction 2 and 4 appears to show the competitive inhibition on P450 2E1 activity since they have similar V_{max} with that of control.

3.3.9 Comparison of 4 5 6 with control

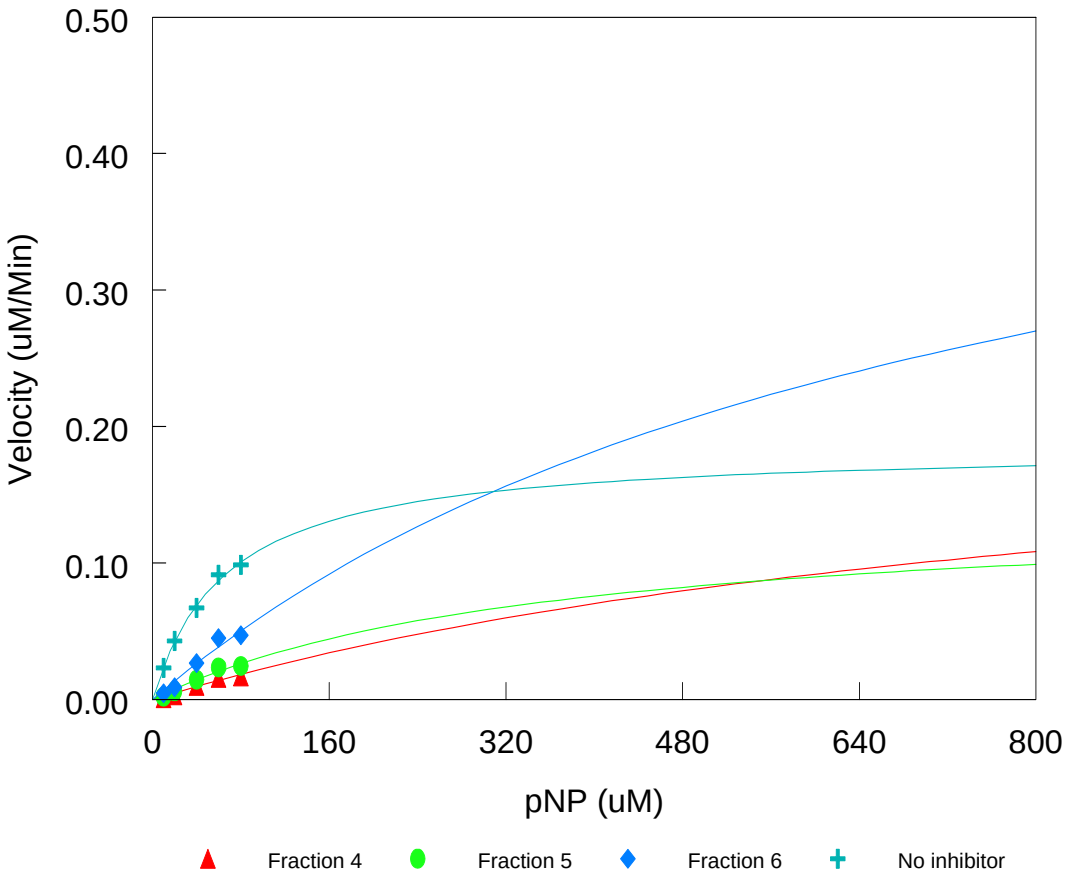

Figure 17. Michaelis Menten plot of nitrophenol oxidation by 2E1 and inhibition by Echinacea fraction 4, 5, 6

Figure 17 indicates the results of the comparison of inhibition effects of fraction 4, 5, 6 of Echinacea by using pNP assay, which is described in the previous chapter. Figure 17 is the Michaelis-Menten plot of these three fractions of Echinacea. From Figure 17, it appears that fraction 4, 5, 6 of Echinacea were capable of inhibiting the P450 2E1 activities to some extend, while fraction 6 has the least inhibition effect on P450 2E1. From table 9, it appears that fraction 6 of Echinacea shows the mixed inhibition on P450 2E1, while fraction 5 shows the competitive inhibition on P450 2E1.

Table 9. Values of Maximum Velocity (V_{max}) and Michaelis Constant (K_m) of the fractions of Echinacea

Number (Echinacea)	V_{max} (uM/Min)	K_m (uM)
Fraction 1	N/A	N/A
Fraction 2	0.245±0.035	1076±479
Fraction 3	0.091±0.008	411±271
Fraction 4	0.237±0.033	947±454
Fraction 5	0.143±0.027	355±207
Fraction 6	0.523±0.064	751±363
Control	0.186±0.029	67.3±17.0

3.4 Results of Spilanthes

The experimental method of Spilanthes is pNP assay which was described in the previous chapter. The following tables and graphs represent inhibition data for Spilanthes and the individual fraction with respect to P4502E1 activity. Values represented in the tables and graphs are peak areas of the product in the reaction of p-nitrophenol with P450. The standard curve for nitrocatechol used in the Spilanthes experiments is described by the following linear equation: y=2120x-30883, where x means concentration (μM) and y means peak area. The fraction 1 and 2 are the eluent by using 40% ethanol washing, which method was described in chapter 2.4. The fraction 3 to 5 are the eluent by using 50% ethanol washing, the fraction 7 to 9 are the eluent by using 55% ethanol washing and the fraction 10 to 12 are the eluent by using 60% ethanol washing.

3.4.1 Fraction 1, 2, 3

The experiments of these fractions were carried out by using pNP assay, but the peak of products overlapped with other peaks. Several different techniques were used to separate these peaks, such as using longer HPLC column and changing the mobile composition. Unfortunately, these peaks were still overlapped, therefore the data of these experiments were blank.

3.4.2 Fraction 4

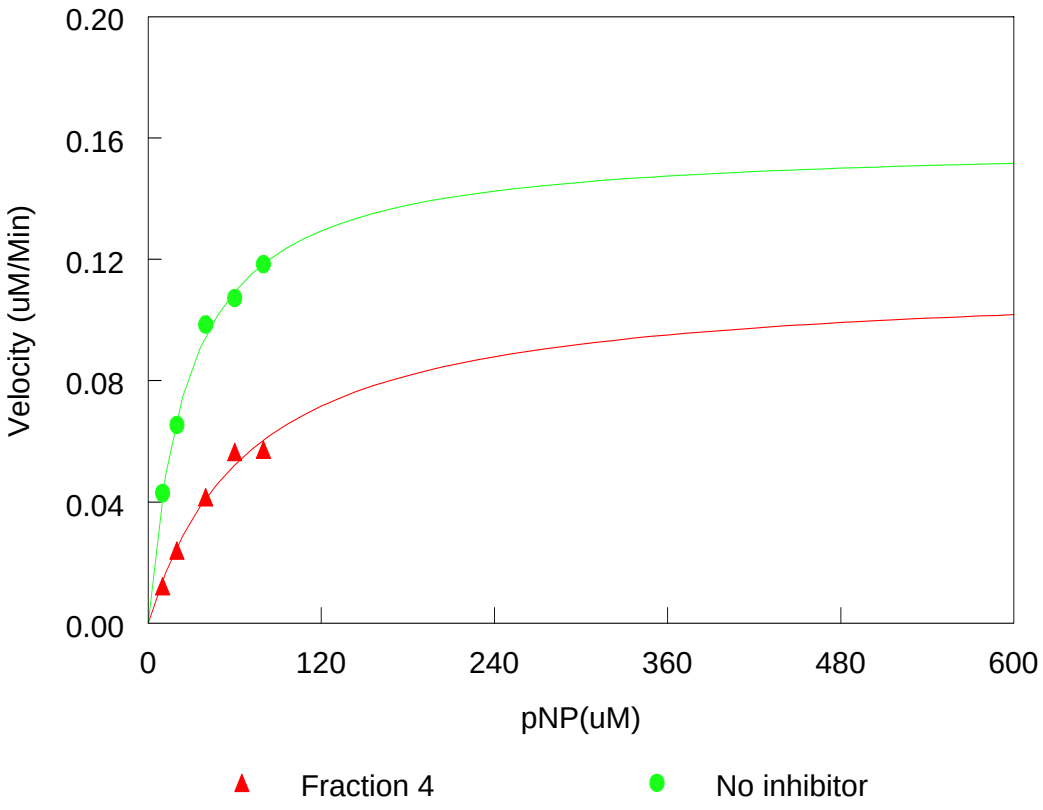

Figure 18. Michaelis Menten plot of nitrophenol oxidation by 2E1 and inhibition by Spilanthes fraction 4

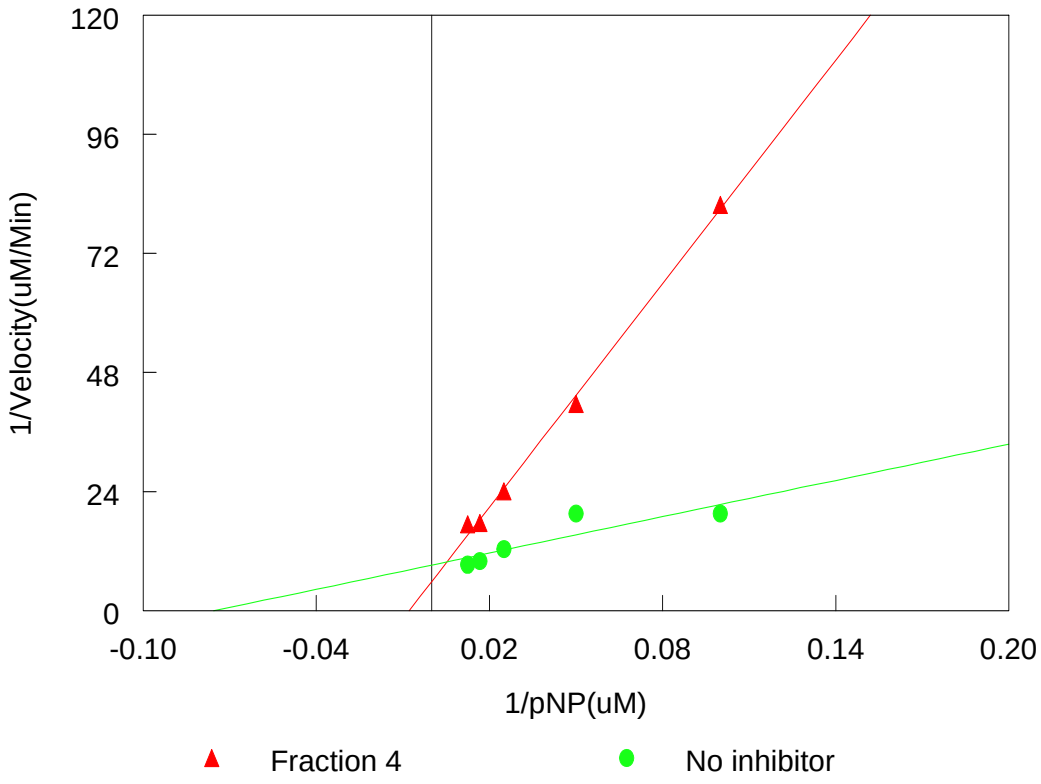

Figure 19. Lineweaver Burk plot of nitrophenol oxidation by 2E1 and inhibition by Spilanthes fraction 4

Figure 18, 19 indicates the inhibition effects of fraction 4 of Spilanthes on the pNP assay, which is described in the previous chapter. Figure 18 and Figure 19 are the Michaelis-Menten plot and the Lineweaver-Burk plot of fraction 4 of Spilanthes, respectively. From these two plots, it appears that fraction 4 of Spilanthes was capable of

inhibiting the P450 2E1 activities greatly. The competitive inhibition could be also concluded from the Figure 19 since fraction 4 has similar V_{max} with that of control, which are 0.114 μM/Min and 0.159μM/Min respectively.

3.4.3 Fraction 5

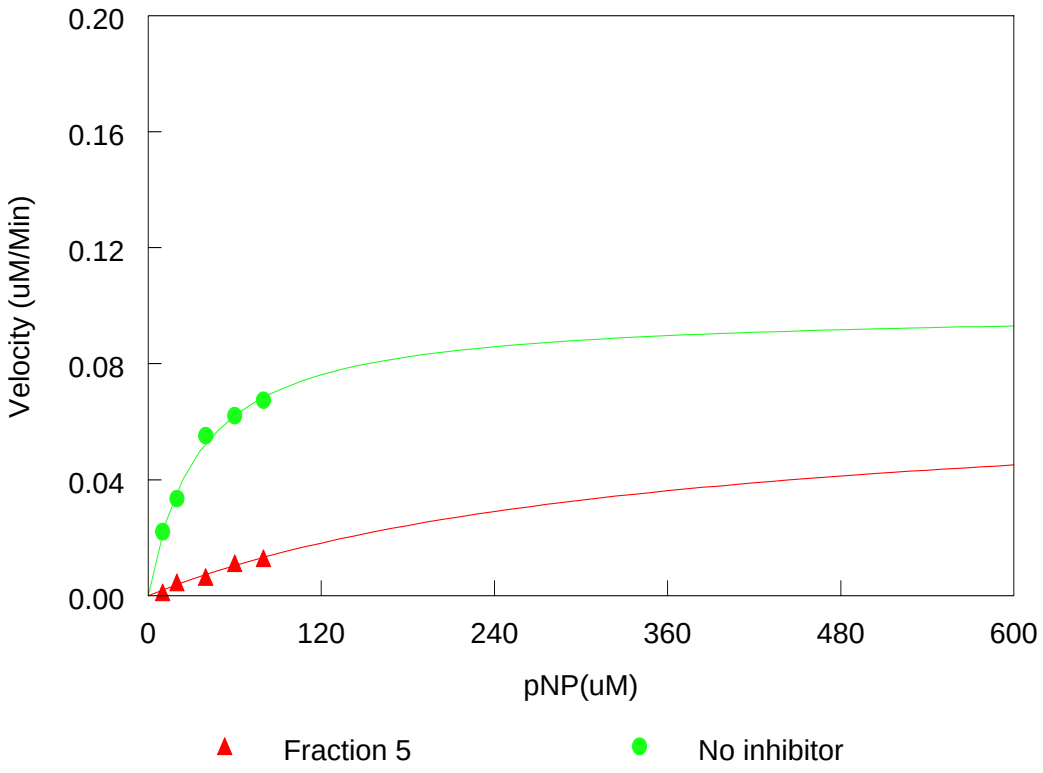

Figure 20. Michaelis Menten plot of nitrophenol oxidation by 2E1 and inhibition by Spilanthes fraction 5

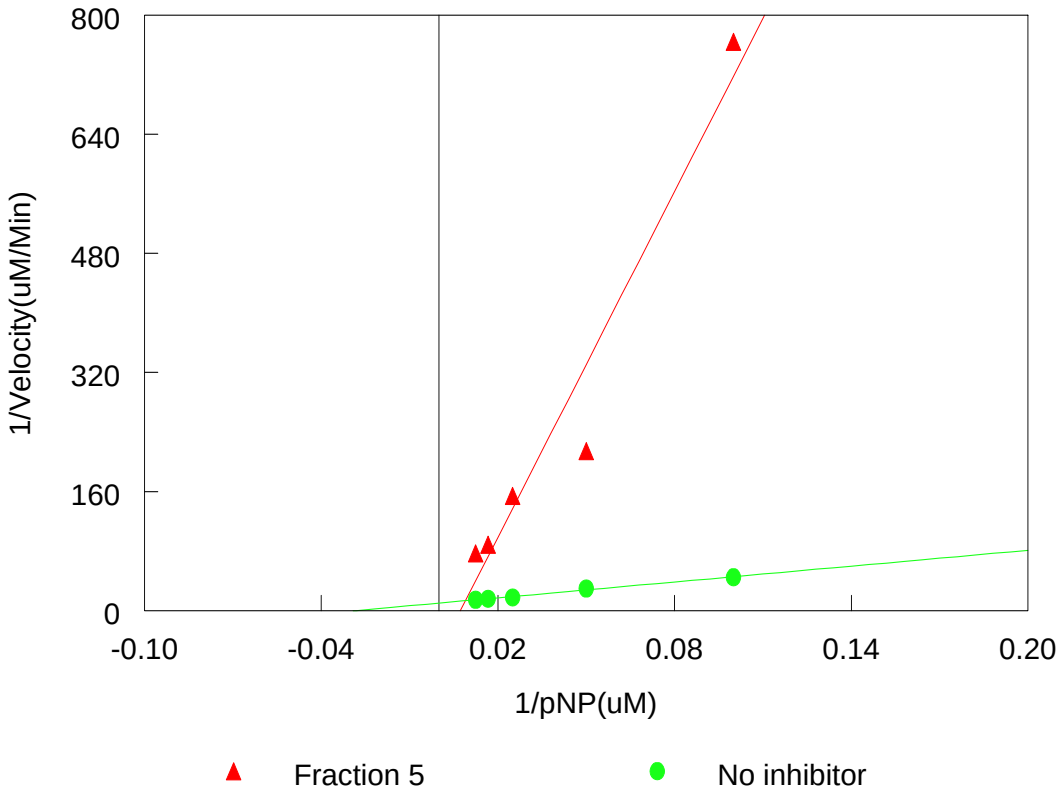

Figure 21. Lineweaver Burk plot of nitrophenol oxidation by 2E1 and inhibition by Spilanthes fraction 5

Similarly, Figure 20 and 21 indicates the results of inhibition effects of fraction 5 of Spilanthes by using pNP assay. From Figure 20, it can be seen that fraction 5 of Spilanthes could inhibit P450 2E1 activities greatly. From Figure 21 and Table 10, it appears that this fraction has competitive inhibition on P450 2E1's activities because they

have similar V_{max} value with that of control, 0.072uM/Min and 0.098uM/Min respectively.

3.4.4 Fraction 8

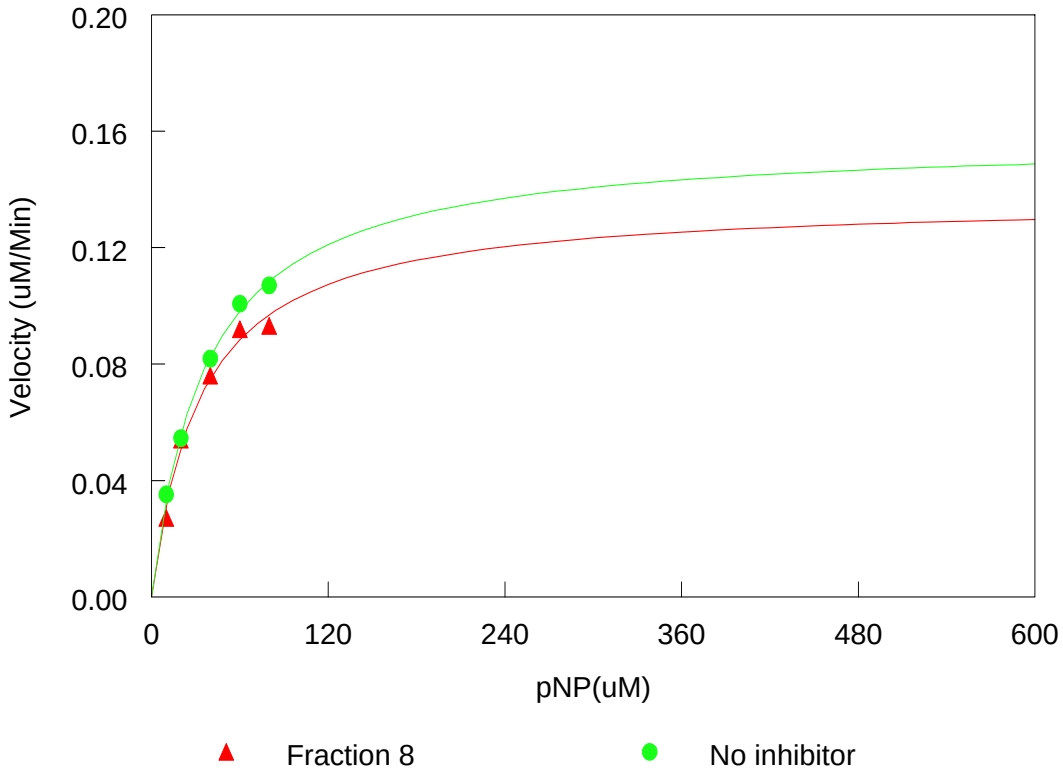

Figure 22. Michaelis Menten plot of nitrophenol oxidation by 2E1 and inhibition by Spilanthes fraction 8

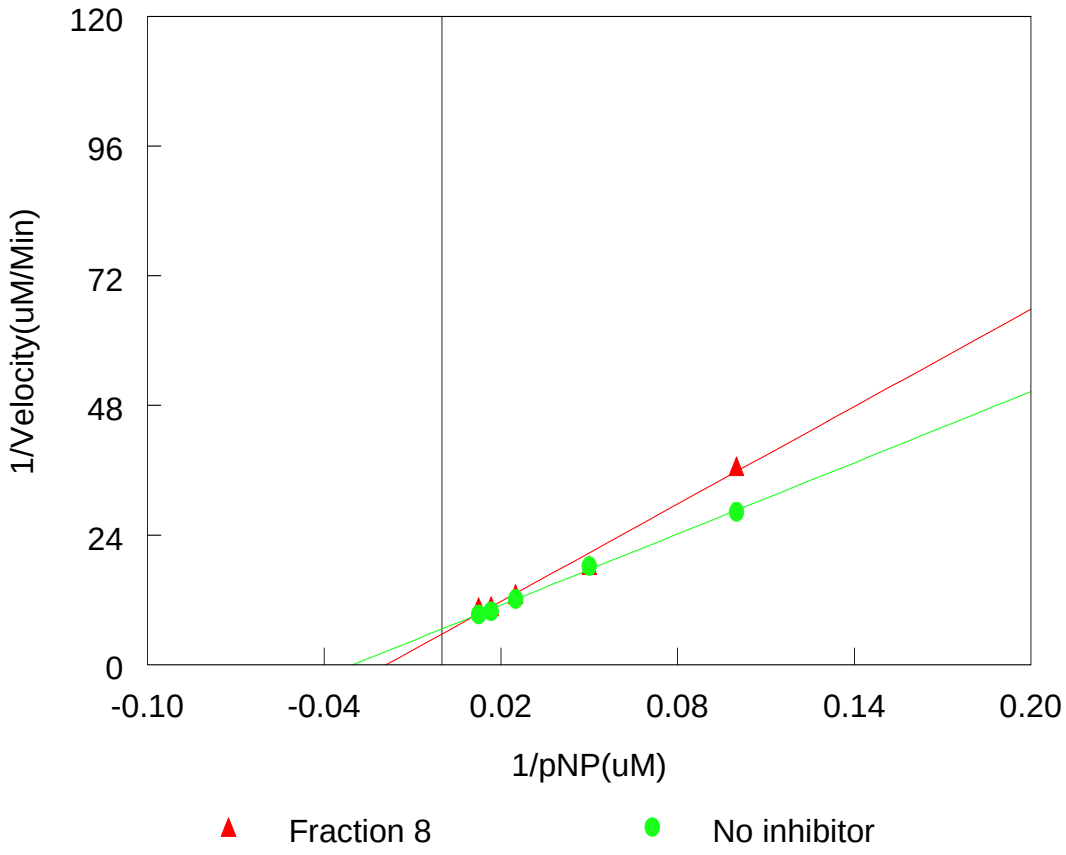

Figure 23. Lineweaver Burk plot of nitrophenol oxidation by 2E1 and inhibition by Spilanthes fraction 8

Figure 22, 23 indicates the results of inhibition effects of fraction 8 of Spilanthes by using pNP assay. The Michaelis-Menten plot and the Lineweaver Burk plot of fraction 5 of Spilanthes were showed in Figure 22 and Figure 23 respectively. From the above

two figures, it can be known that fraction 8 of Spilanthes showed a little inhibition on the activities of P450 2E1. We also cannot know what type of inhibition occurs since it has similar value of both K_m and V_{max}.

3.4.5 Fraction 9

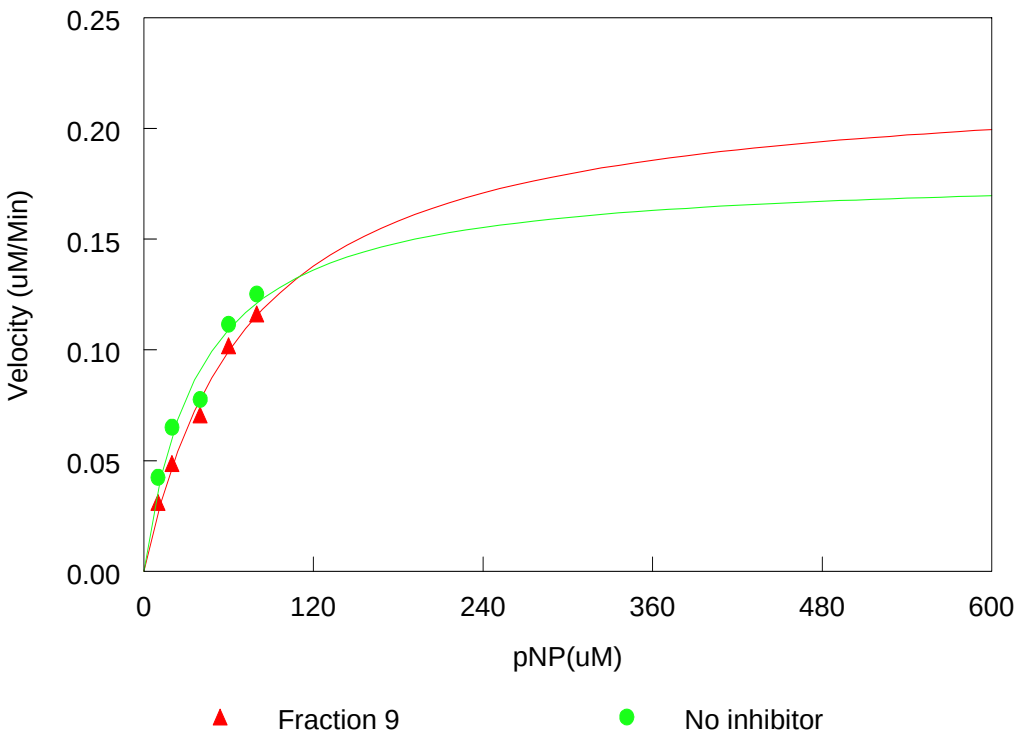

Figure 24. Michaelis Menten plot of nitrophenol oxidation by 2E1 and inhibition by Spilanthes fraction 9

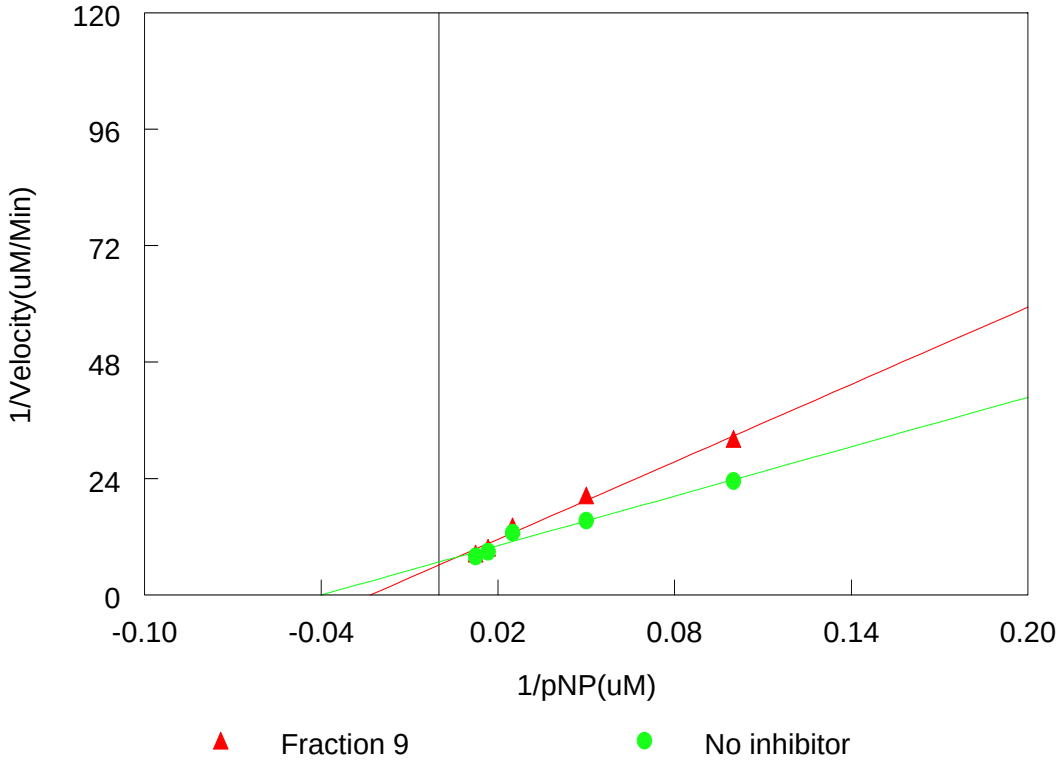

Figure 25. Lineweaver Burk plot of nitrophenol oxidation by 2E1 and inhibition by Spilanthes fraction 9

Figure 24 and Figure 25 showed the inhibition effects on 2E1 of fraction 9 of Spilanthes. Figure 24 is Michaelis-Menten plot and Figure 25 is Lineweaver-Burk plot of fraction 9 of Spilanthes. There is a little inhibition effect on 2E1 of fraction 9 which can

be seen from Figure 24. From Table 10, the competitive inhibitor can be determined because the V_{max} of fraction 9 and control are 0.225 μM/Min and 0.181μM/Min.

3.4.6 Fraction 10

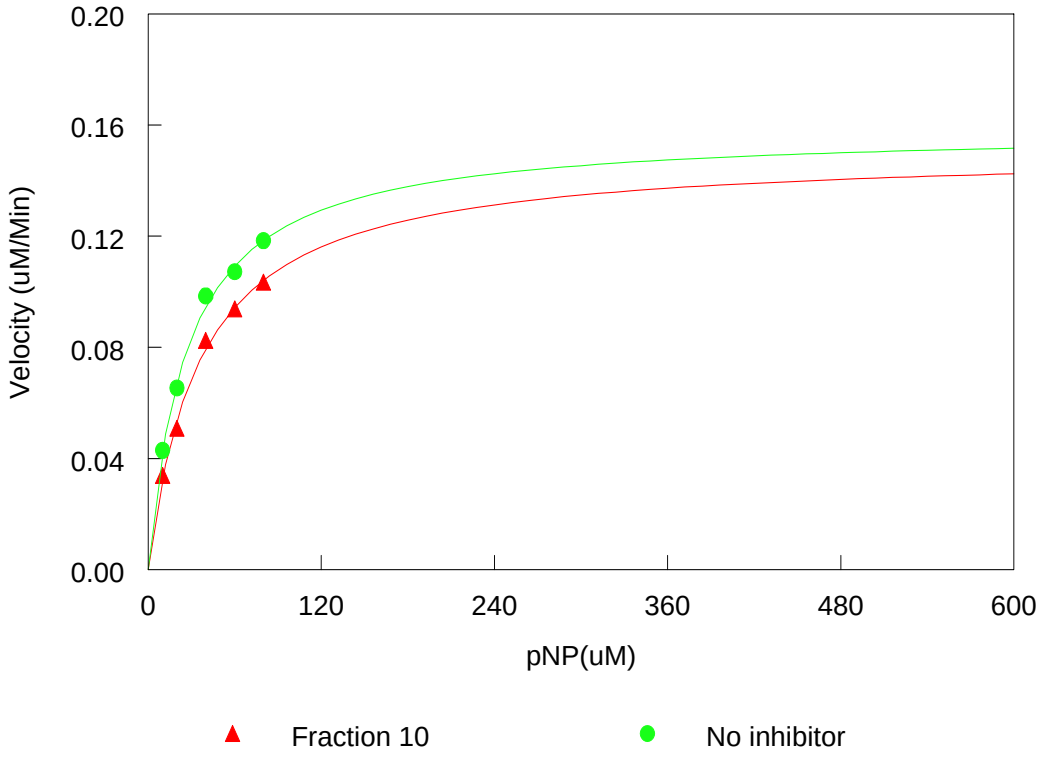

Figure 26. Michaelis Menten plot of nitrophenol oxidation by 2E1 and inhibition by Spilanthes fraction 10

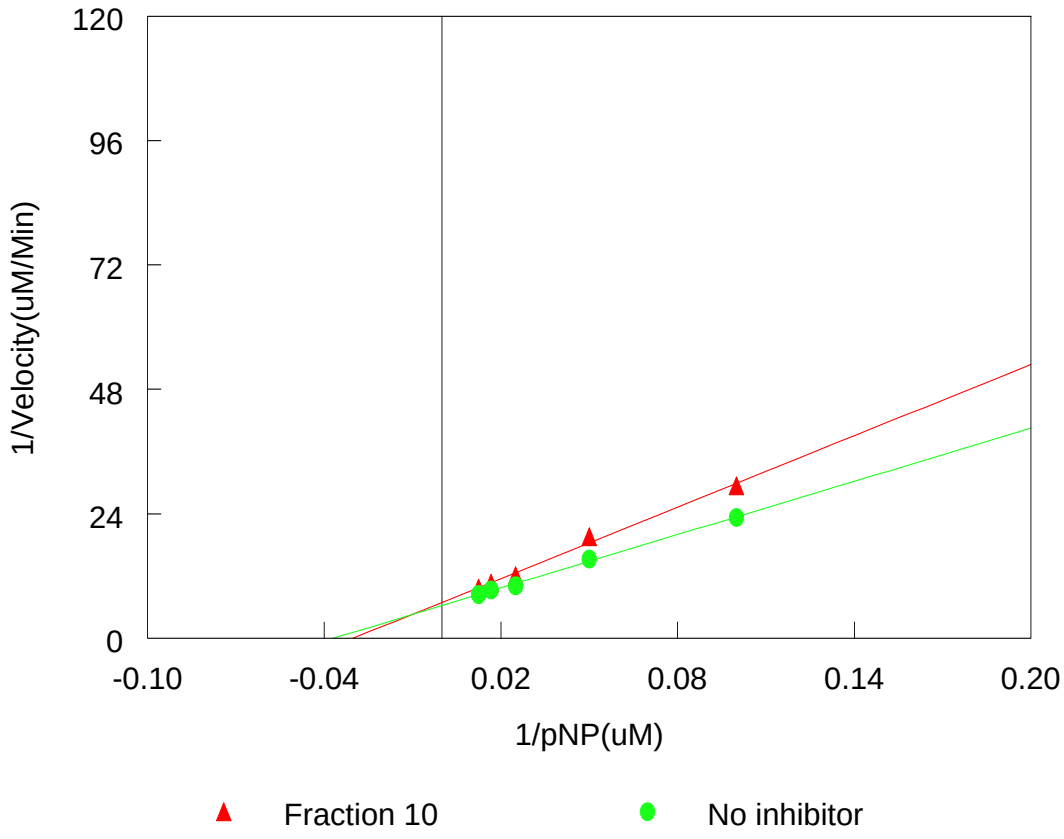

Figure 27. Lineweaver-Burk plot of nitrophenol oxidation by 2E1 and inhibition by Spilanthes fraction 10

Very similar to fraction 9 of Spilanthes, there is a little inhibition effect on 2E1 of fraction 10 which can be seen from Figure 26. From Table 10, the competitive inhibition

can be determined because the V_{max} of fraction 10 and control are 0.151uM/Min and 0.158uM/Min respectively.

3.4.7 Compared 2E1 with microsome of Fraction 4

Since fraction 4 has great inhibition effect on P450 2E1's activity, the comparison of fraction 4's inhibition on P450 2E1 and Human Microsome was examined.

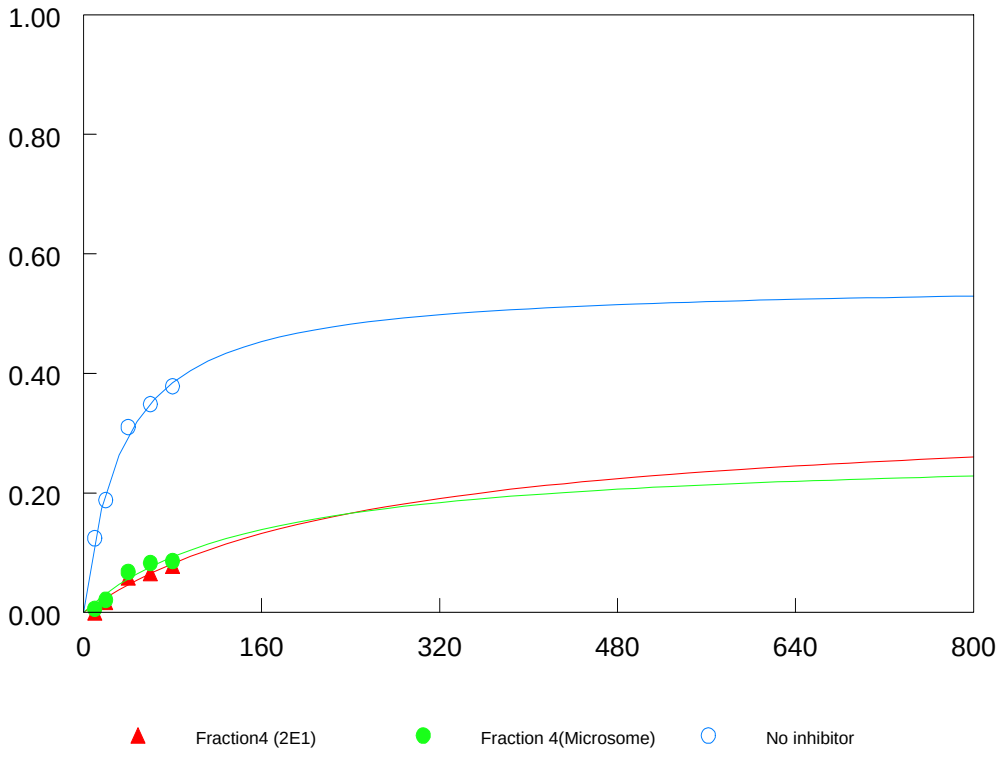

Figure 28. Michaelis Menten plot of nitrophenol oxidation by 2E1 and human microsome, and inhibition by Spilanthes fraction 4

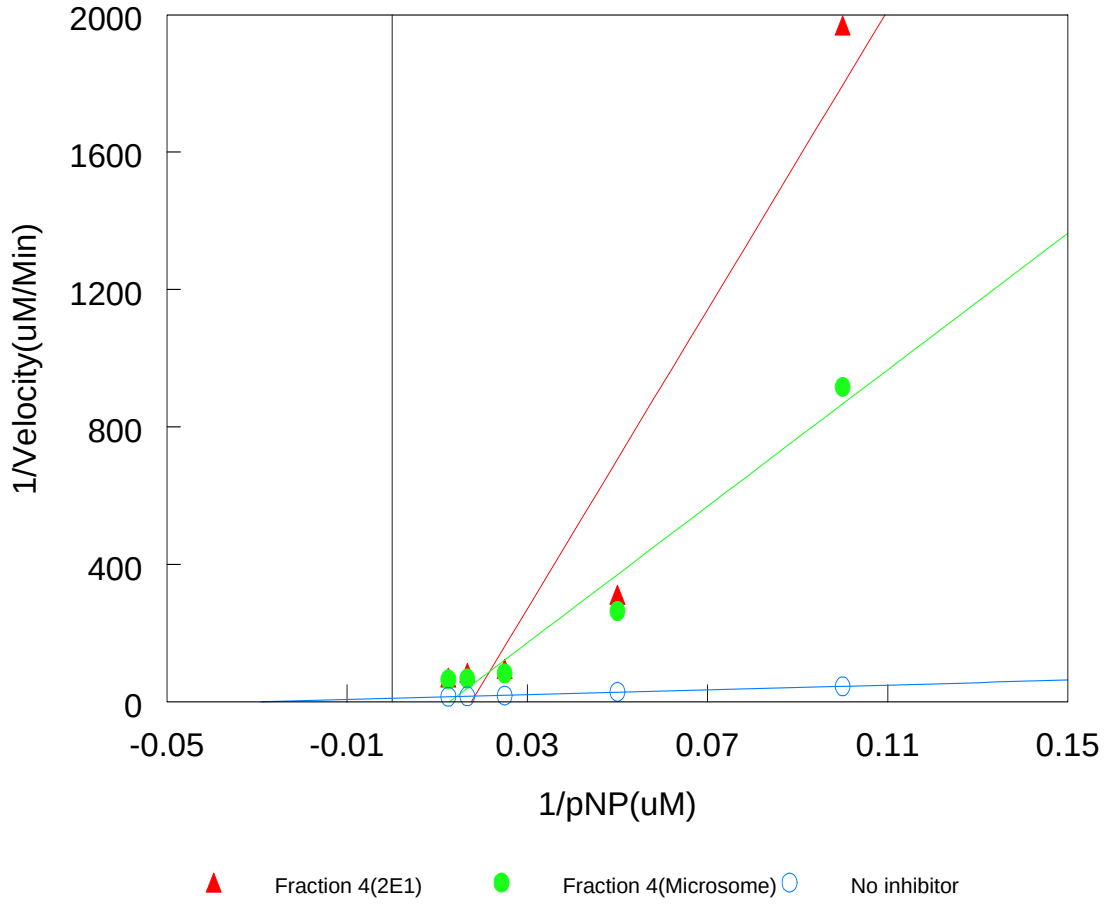

Figure 29. Lineweaver-Burk plot of nitrophenol oxidation by 2E1 and human microsome, and inhibition by Spilanthes fraction 4

Figure 28, 29 indicates the results of the comparison of inhibition effects of fraction 4 of Spilanthes on P450 2E1 and human microsome. From this Michaelis-

Menten plot, fraction 4 of Spilanthes has the similar inhibition on the activities of P450 2E1, while fraction 4 has a little more inhibition on P450 2E1 activity than that of human microsomes. From Figure 29, the type of inhibition cannot be determined from this plot because it has different K_m and V_{max} values.

Table 10. The values of V_{max} and K_m of different fractions of Spilanthes

			Control	
Fraction #	V_{max} (uM/Min)	K_m (uM)	V_{max} (uM/Min)	K_m (uM)
4	0.114±0.020	70.65±13.9	0.159±0.023	26.98±5.37
5	0.072±0.018	350.7±152	0.098±0.019	35.10±3.69
8	0.137±0.028	32.89±5.95	0.158±0.027	36.35±3.64
9	0.225±0.039	75.54±14.3	0.181±0.032	39.35±4.06
10	0.151±0.024	35.96±6.17	0.158±0.026	26.98±5.31

CHAPTER IV

DISCUSSION

4.1 Summary of Objectives

P450 2E1 activities were to be examined with respect to inhibition by four potential different inhibitors, including GTE, Goldenseal, Echinacea and Spilanthes. The concentration of each isobutylamide would be estimated using HPLC to generate a standard curve for a commercially available isobutyl amide. This standard curve could then be used to quantify all of the different isobutylamides in their purified forms. Inhibition experiments were performed in which different inhibitors were included in the reaction mixtures. The activities were compared to reactions that were with no inhibitor present. The inhibition constants then were calculated from Michaelis-Menten plots. The kinetic parameters obtained for all of these experiments, including V_{max} and K_m, were calculated for each experiment. The activity of individual fractions of GTE, Goldenseal, Echinacea and Spilanthes were determined to see which fraction could play an important role in this inhibition.

4.2 Green Tea Extract (GTE)

Four constituents of GTE, including EGCG, EGC, EC and GTE, were examined for their ability to block 2E1 activity by using pNP assay. The concentrations of all four

inhibitor were 100ug/mL and the concentration of substrate was increased from 10uM to 100uM. From the data of green tea extract, all of them clearly didn't inhibit the activities of P450 2E1 very much. Only whole extract showed more inhibition on the activities of enzyme P450 2E1, but it was still not significant. We may conclude that there are no significant inhibitions inhibitors present in GTE, since the concentration used was very high (100ug/mL). The present data of GTE have been shown that the GTE and its components don't inhibit the activities of P450 2E1. For the future, other P450 enzymes, such as 3A4, can be used to measure the inhibition effects of GTE and its components.

4.3 Goldenseal

Compared with GTE, the components of Goldenseal appear to show much more inhibition on the activities of P450 2E1. As indicated by the Michaelis-Menten curves, each component was capable of inhibiting P4502E1 activity significantly, even at low micromolar concentration. From the table 14, it indicates that the inhibitions of berberine and canadine are noncompetitive when the inhibitor's concentration is 4uM. Among these inhibitors, hydrastine appears to be the most potent inhibitor. This can be known from the inhibition constant of hydrastine, K_i = 3.2uM, while the K_i values of Berberine and Canadine are 18.4uM and 16.9uM respectively. Although Berberine and Canadine had been shown to inhibit P450 2E1 activity, their inhibitory effects were about one order of magnitude weaker than that of hydrastine. When the inhibitor's concentration is 100uM, berberine appears to be noncompetitive inhibitor while canadine and hydrastine

appear to be noncompetitive inhibitors. The Ki value of 86uM of hydrastine indicates that hydrastine is the most potent inhibitor on P450 2E1's activities. These kinetic data (K_i) strongly suggest that hydrastine is the most potent inhibitor among the components of Goldenseal investigated. We propose that hydrastine may be a strong enough inhibitor to exert effects *in vivo*. Although it is unknown how much of this compound may reach the liver, the low K_i value indicates further study is wanted.

Overall, it is evident that all three components of Goldenseal are capable of inhibiting the activity of P450 2E1 and hydrastine is the most potent inhibitor among all components. These observations support the concept that ingestion of goldenseal extract could potentially inhibit the metabolism of many drugs currently in therapeutic use.

4.4 Echinacea

The inhibition effects of Echinacea were examined by using pNP assay, which is described in the previous chapter. The comparisons of 6 different fractions of Echinacea were listed in chapter 3.3. The data of Echinacea indicates that all fractions are capable of inhibiting the activities of P450 2E1 at relatively high concentrations and fractions 2, 3, 4 are showing the most potent inhibition on P450 2E1's activities. From table 9, it can be seen that both V_{max} and K_m of the control are different from that of fraction 3, 6 of Echinacea. It indicates that the inhibitions are not strictly competitive or noncompetitive; rather it is possible that the inhibition is a mixture of competitive and noncompetitive inhibition. The competitive inhibition of fractions 2, 4 and 5 on P450 2E1 can be seen

from table 9. It appears that fraction 1 of Echinacea has the most significant inhibition on P450 2E1. It killed all activities of P450 2E1, and this may be caused by the high concentration of isobutylamide of Echinacea in fraction 1. From the comparison of fraction 2, 3, 4 of Echinacea, it is clear that these three fractions have the similar inhibition on the activities of P450 2E1. This also can be seen from kinetic parameter data in table 9. The V_{max} and K_m values were calculated from the Michaelis-MentenPlot by using Slidewrite Plus software.

In summary, the inhibition of P450 2E1 was observed in all fractions of Echinacea in the present study. Fraction 1 is the most potent compound inhibiting the activities of P450 2E1 at the concentration present in the extract, and fraction 2, 3, 4 have the similar inhibition effects on P450 2E1. The competitive inhibition of fraction 2, 4 and 5 of Echinacea could be determined on P450 2E1 activity, while fraction 3 and 6 appear to show the mixed inhibition on P450 2E1 activity. The results of these experiments give useful information to the scientific communities on the potential for inhibition of metabolic enzymes by herbal products, such as Echinacea.

4.5 Spilanthes

The pNP assay was used to determine the inhibition effects of fractions of Spilanthes on P450 2E1. Several fractions resulting from the separation of isobutylamides from Spilanthes were examined with respect to their inhibition of P450 2E1 as detailed in Chapter 3.4. From the plots and data of Spilanthes, it is clear that the fraction 4 has the

most potent inhibition on the activities of P450 2E1, and fraction 4 contains the isobutylamides spilanthol. It can be also seen that fraction 8, 9, 10 showed little inhibition on the activities of P450 2E1. For fraction 1, 2, 3, the data were not shown in this thesis because the product peak overlapped with other peaks and they cannot be separated using different mobile phase conditions. Since the fraction 4 had the most potent inhibition on P450 2E1, the kinetic parameters of inhibition of P450 2E1 in microsome were determined using the Michaelis-Menten model. From table 10, similar inhibition can be seen from the data, while fraction 4 has a little more inhibition on the activities of P450 2E1. The inhibition types of fraction 4, 5, 9 and 10 of Spilanthes on P450 2E1 appear to be the competitive inhibition, while fraction 8 of Spilanthes could not be determined.

This study provides evidence that fraction 4 from the Spilanthes ethanol extract had the most potent inhibition on the activities of P450 2E1. The K_m and V_{max} values of Spilanthes determined in this study should be useful in elucidating the extent to which this herbal product may influence the metabolism of drugs by cytochrome P450 2E1.

4.6 Improvements and Future Work

Since the product peaks of fraction 1, 2, 3 of Spilanthes were overlapped with other peaks, more techniques should be used to separate these peaks. For HPLC instrument, some samples are in autosampler for up to 20 hours and this could change the activity of samples. In order to get better results, all samples should be analyzed at the same time.

The inhibition effects of GTE and its components EGCG, EGC, EC were examined in this study, however they didn't inhibit significant on P450 2E1 activity. We will examine GTE and its major components in their ability to inhibit p-nitrophenol oxidation by other isoforms of P450, such as 2D6, 3A4 etc.

For Echinacea and Spilanthes, future studies involving isolated alkylamides will be carried out using samples prepared from both Echinacea and Spilanthes. The concentration of each alkylamides would be estimated by using HPLC. The objective is to purify enough each component of Echinacea and Spilanthes to calculate K_i values and to compare these K_i values to actual concentrations in different extracts. Further studies will be designed to examine potential interaction of these compounds with other P450 isoforms such as 3A4, 2D6, 2A6, 2C8, 1A1 and others.

4.7 Conclusion

GTE only show some inhibition effects on the activities of P450 2E1 even though the inhibition concentration is high enough (100ug/mL), but catechins of GTE did not show too much inhibition on P 450 2E1 activity. The inhibition of activities on other families of P450 can be examined in the future.

All three components of Goldenseal are capable of inhibiting of the activities of P4502E1 and the hydrastine has the most inhibition effects on the activities of P450 2E1, which can be seen from the lowest K_i of hydrastine. From kinetic data of goldenseal, it appears that components canadine and berberine are noncompetitive inhibitors of P450

2E1 in lower inhibitor concentration (4uM). Berberine appears to be noncompetitive inhibitor and canadine and hydrastine appear to be competitive inhibition in higher inhibitor concentration (100uM).

All fractions of Echinacea have inhibition effects on the activities of P450 2E1 and fraction 1 is the most inhibiting compound on the activities of P450 2E1 among all these six fractions. Fractions 2, 4, 5 of Echinacea appears to be competitive inhibitors for P450 2E1, while fractions 3 and 6 of Echinacea could have mixed inhibition on P450 2E1's activity.

From kinetic data of Spilanthes, it indicated that fraction 4, 5, 9 and 10 of Spilanthes showed competitive inhibition on P450 2E1's activity. Fraction 4 of Spilanthes had the most inhibition effects on the activities of P450 2E1. By comparing the fraction 4's inhibition on 2E1 and human microsomes, similar inhibition can be seen from the data and plots of fraction 4. In this study, the herbal products showed different extents of inhibition on P450 2E1, and it was successfully determined GTE did not show too much inhibition on P450 2E1's activities and Goldenseal, especially hydrastine, had good inhibition effect on P450 2E1's activities, and also Echinacea and Spilanthes showed the interesting inhibition effects varied from different fractions.

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