THE EFFECT OF *ASPARAGUS LARICINUS* FRACTIONS ON MAJOR DRUG METABOLISING ENZYMES – CYP3A4 AND 2D6

Kelebogile Kefilwe Morake

Dissertation submitted in fulfilment of the requirements for the Degree

MASTER OF HEATH SCIENCES

in Biomedical Technology

In the

Department of Environmental Health Sciences

Faculty of Health Sciences

at the

Central University of Technology, Free State

Supervisor: Prof PH. Mfengwana, DHSc (Biomedical technology)

Co-supervisor: Dr T. Mooko, PhD (Internal Medicine)

BLOEMFONTEIN

October 2025

DECLARATION OF INDEPENDENT WORK

I, Kelebogile Kefilwe Morake, with _____ ……. and student number _____, hereby declare that the research project titled: The effect of *Asparagus laricinus* fractions on major drug metabolising enzymes- CYP3A4 and 2D6, submitted to the Central University of Technology, Free State, for the Master of Health Science in Biomedical Technology degree, is my own independent work. It complies with the Code of Academic Integrity, as well as other relevant policies, procedures, rules, and regulations of the Central University of Technology, Free State. I declare that this work has not been submitted before to any institution by myself or any other person in fulfilment (or partial fulfilment) of the requirements for the attainment of any qualification.

_____ **01/11/2025**

SIGNATURE OF STUDENT **DATE**

DEDICATION

I dedicate this work to my mother and sister for always supporting me, and to my son and niece, whose love and presence continue to inspire and motivate me.

ACKNOWLEDGEMENTS

First and foremost, I would like to thank the Almighty God for His grace, strength, and the endurance He has given me throughout this journey. Without His guidance and the opportunity, He has provided, none of this would have been possible.

I would also like to express my sincere gratitude to my supervisors, Prof. H. Mfengwana and Dr T. Mooko, for their invaluable guidance, support, and encouragement. Their expertise and mentorship have played a vital role in the completion of this work.

A special thank you goes to Dr G.T. Tabakam for his contribution to the plant extraction and fractionation methods, which formed a critical part of this study. Your assistance is deeply appreciated.

I am also grateful to the Central University of Technology (CUT) for the financial support that made this research possible.

Finally, to my family – thank you for your unwavering love, patience, and support. This journey was not easy, and your encouragement carried me through the most challenging moments. I am truly blessed to have you by my side.

ABSTRACT

Asparagus laricinus Burch. is a shrub widely used in traditional South African medicine and is known to contain diverse phytochemicals such as flavonoids, phenolics, saponins, alkaloids and terpenoids that may interact with drug-metabolising enzymes. Because nearly 50% and 25% of drugs used in clinical practice are metabolised by CYP3A4 and CYP2D6 respectively – with CYP3A4 being the predominant hepatic form and CYP2D6 showing genetic polymorphism – herbal constituents that inhibit these enzymes may cause clinically significant herb-drug interactions by altering the plasma concentration of concomitant medications. Although the pharmacological activity of *A. laricinus* has been documented, its possible effect on CYP3A4 and CYP2D6 activity had not previously been studied.

The aim of this study was to analyse the phytochemical composition of *Asparagus laricinus* leaves, evaluate their effects on the major drug-metabolising enzymes CYP3A4 and CYP2D6, and to investigate the kinetic profile of these enzymes in the presence of the plant fractions. The crude methanolic leaf extract was fractionated into seven parts (A–I), and qualitative phytochemical screening was performed on each. Phenolic glycosides, flavonoid conjugation and steroidal saponins were identified as possible contributors, tentatively confirmed by LC-MS.

Using in-vitro fluorescent-based high-throughput assays, a very strong dose-dependent inhibition of the enzymes was observed. Fraction H showed the highest dual activity (CYP3A4 IC_{50} = 0.0026 µM; CYP2D6 IC_{50} = 1.5 µM) in a mixed/non-competitive interaction, while fractions C and E were selective inhibitors of CYP2D6 and CYP3A4, respectively. The kinetic profiling supported the presence of potent inhibitory compounds within these fractions.

Using *in vitro*, fluorescent-based, high-throughput assays, a very strong dose-dependent inhibition of the enzymes was observed. Fraction H showed the highest dual %inhibition effect (CYP3A4 % inhibition = 95,84%; CYP2D6 % inhibition = 91.26%) in a mixed/non-competitive interaction, while Fractions C and E were selective inhibitors of CYP2D6 and CYP3A4, respectively. The kinetic profiling supported the presence of potent inhibitory compounds within these fractions.

Overall, the findings indicate that some *A. laricinus* fractions contain strong CYP3A4 and CYP2D6 inhibitors, suggesting a potential risk of herb-drug interactions when the plant is used concurrently with medications metabolised by these enzymes. This highlights the importance of pharmacovigilance, particularly in cancer patients who use *A. laricinus* preparations alongside traditional medicines.

Keywords: *Asparagus laricinus*, CYP3A4, CYP2D6, enzyme inhibition, IC_{50}, herb–drug interaction, phytochemicals.

LIST OF ABBREVIATIONS AND ACRONYMS

A. laricinus – *Asparagus laricinus*

BSA – Bovine Serum Albumin

CV – Coefficient of Variation

CYP – Cytochrome P450

CYP2D6 – Cytochrome P450 2D6

CYP3A4 – Cytochrome P450 3A4

CYP450 – Cytochrome P450 enzyme family

DMSO – Dimethyl Sulfoxide

DNase – Deoxyribonuclease

EOMCC – 3-[2-(N-Ethyl-N-methylamino) ethyl]-7-methoxy-4-methylcoumarin (fluorescent substrate)

GC–MS – Gas Chromatography–Mass Spectrometry

HPLC – High-Performance Liquid Chromatography

IC_{50} – Half-maximal inhibitory concentration

Km – Michaelis–Menten constant

LC–MS – Liquid Chromatography–Mass Spectrometry

m/z – Mass-to-charge ratio

NADPH – Nicotinamide Adenine Dinucleotide Phosphate (reduced form)

PBS – Phosphate-Buffered Saline

PTFE – Polytetrafluoroethylene

RFU – Relative Fluorescence Units

SBS – Society for Biomolecular Screening (microplate standards)

UPLC – Ultra Performance Liquid Chromatography

Vmax – Maximum reaction velocity

µM – Micromolar (10^{-6} molar concentration)

rpm – Revolutions per minute

°C – Degrees Celsius

CONTENTS

CHAPTER 1

1.1 BACKGROUND

Synthetic or natural medicinal drugs are used daily by a large population across the world to deal with a wide range of illnesses and diseases including infections, chronic pains, cancer, and heart diseases (Sweeney, 2015; Zhao et al., 2023). Medicinal plants are still the first-line treatment in most rural and resource-strained communities in Africa, including South Africa, due to the availability of such plants, the level of acceptance of the existing cultures, and the cost-effectiveness of its use. Nevertheless, to achieve any therapeutic effect, be it conventional or conventional, any given agent needs to go through a certain metabolic conversion process in the body, mainly in the liver, first.

The CYP450 superfamily of enzyme is mainly involved in drug metabolism, where CYP3A4 and CYP2D6 enzymes catalyses the biotransformation of 70 to 80 per cent of all clinically used drugs (Zanger & Schwab, 2013; Guengerich, 2019). The most common and general-purpose of the isoforms is CYP3A4, which breaks down huge, lipophilic molecules, including docetaxel, statins, and most anticancer agents, as well as CYP2D6, which breaks down a quarter of drugs, especially of basic amine bond types, including some antidepressants and tamoxifen (Ingelman-Sundberg, 2005). The co-administration of drugs with such enzyme inhibitors or inducers, such as herbal preparations, may change drug pharmacokinetics significantly and result into treatment failure or toxicity (Michalets, 1998).

The interaction of herbs and drugs through the CYP450 enzymes is thus a significant issue in clinical practice particularly in patients under chronic medication who are at the same time taking unconventional medicines. A combination of a prescription drug and potent CYP450 inhibitors in the plant-consumed form can lead to decreased metabolism and serious adverse effects, which include diarrhoea, myelosuppression, or even toxicity, which is life-threatening (Dahiya et al., 2020). On the other hand, drug efficacy can be reduced by increasing the rate of drug clearance through enzyme induction.

Asparagus laricinus Burch. is a South African native shrub, which was traditionally applied in respiratory illnesses, inflammation therapy, urinary tract infections, and reportedly in the treatment of cancer-related symptoms with promising *in vitro* anticancer, antioxidant, and antimicrobial properties (Mokgawa et al., 2019; Sekhoacha et al., 2022). Although the drug may have these pharmacological advantages and is commonly used by people, there is no research that explores how the phytochemical constituents are processed by or have any impact on major drug-metabolising enzymes. Thus, the aim of the study was to determine the *in vitro* inhibitory activity of *A. laricinus* leaf fractions on CYP3A4 and CYP2D6, and characterize the responsible phytochemicals, to anticipate their herb-drug interactions and ensure their safer use alongside conventional medicines.

1.2 BOTANICAL OVERVIEW OF *ASPARAGUS LARICINUS*

Asparagus laricinus are medicinal plants that have been used as a therapy in the management of different diseases, including prostate cancer and breast cancer for a long time because of the bioactive properties of its bioactive compounds like flavonoids and taxanes. Medicinal plants have a long history of use in the therapeutic treatment of various diseases. The traditional use of *Asparagus laricinus* has been reported as a potential therapeutic agent for the symptomatic treatment of prostate cancer (Mashele & Kolesnikova, 2010). The plant parts have phytochemicals with anticancer activity which includes flavonoids, alkaloids, steroids, saponins, terpenoids, and phenolic compounds. These have been identified as potential anti-prostate cancer agents (Ntsoelinyane & Mashele, 2014). However, data on the extract's efficacy profile of *Asparagus laricinus* are not enough to validate the hypothesised anti-prostate cancer activity. This has led to studies of different fractions and isolated compounds to clearly understand anti-prostate cancer activity from plants. The way the body handles therapeutic compounds through metabolism can determine their efficacy and safety profile (Paul-Chima et al., 2024). Thus, it is important to understand the influence of selected *Asparagus laricinus* fractions on the major metabolising CYP450 isozymes – CYP2D6 and CYP3A4.

1.3 ETHNOPHARMACOLOGICAL USES IN SOUTH AFRICA

Asparagus laricinus Burch, a plant known as Bushveld asparagus, and called *lesitwane* in Setswana, is an important plant in South African traditional medicine. Different parts of the plant are utilized in traditional medicine for both human and veterinary medicine. The most commonly reported uses in humans are for coughs, a sore throat, and tuberculosis, by drinking roots decoction and leaves infusion (Van Wyk, 2008; Mokgawa et al., 2019). The plant is also employed as a blood tonic for stomach problems and rheumatoid arthritis, and its topical preparation is used for wounds and skin diseases (Mokgawa et al., 2019). Furthermore, traditional healers use preparations as a diuretic and for the treatment of urinary or uterine infections (Van der Merwe et al., 2001).

Traditional uses of *Asparagus laricinus* have been confirmed by *in vitro* and *in vivo* studies on the phytochemistry and pharmacology of this plant, which show antibacterial effects against pathogens such as *Staphylococcus aureus* and antioxidant properties. There is still a dearth of clinical information, which will require more investigations to support its effectiveness and safety as a therapeutic agent. Extracts of *A. laricinus* exhibit antibacterial activity against pathogens, including *Staphylococcus aureus* and *Klebsiella pneumoniae* and possess significant antioxidant activity (Hamdaoui et al., 2024). In addition, methanolic leaf and root extract have demonstrated selective cytotoxicity on MCF-7 breast-cancer cells *in vitro*, thereby indicating potential anticancer uses (Mokgawa et al., 2019). These activities are believed to be associated with the presence of saponins, sterol (e.g. β-sitosterol), and phenolic (e.g. ferulic acid) compounds, which are recognized for their antimicrobial and anti-inflammatory activities (Mustafa et al., 2022).

Although *Asparagus laricinus* has a long history of traditional use in South African ethnomedicine as a treatment of various ailments, including prostate cancer and infections, little is known about its safety and effectiveness in humans. Preclinical experiments such as *in vivo* animal models show high LD50 levels, which indicate low levels of acute toxicity, but additional clinical studies are necessary to confirm its efficacy as a therapy and to ensure its safe addition to conventional medicine.

1.4 THE CYP450 P450 ENZYME SYSTEM IN DRUG METABOLISM

CYP450s are important and are extensively involved in metabolising foreign substances including plant extracts. Most of these enzymes are found in the liver, where most drug compounds are metabolised (Slaughter, 1995). A small proportion of these enzymes is also found in the blood and other peripheral tissues. It has about 50 enzymes, but only a few are involved in the metabolism and detoxification of drugs, phytochemicals, and other foreign substances. Furthermore, the major metabolising enzymes are CYP3A4 and CYP2D6 (Zanger, 2008). The inhibition and induction of these enzymes by xenobiotics have been associated with drug-herb interactions, thus, posing risks of altering the efficacy and safety profile of certain drug agents (Michalets, 1998). There is a scarcity of scientific data on the drug interactions of *Asparagus laricinus* fractions and isolated compounds with other therapeutic agents that share the same enzymatic metabolic pathway. It is important to have scientific evidence on the metabolic profiles of *Asparagus laricinus* fractions by these enzymes. This may help advice on herbal-drug interactions, especially in patients taking chronic treatments, and provide knowledge on the metabolic profile of *Asparagus laricinus* factions.

Plants have been known to have healing powers since time immemorial. According to the World Health Organization (2002), medicinal plants are characterized as including health maintenance, prevention, diagnosis, and treatment of physical or mental conditions through knowledge, skills, and practices based on cultural beliefs and experiences. Nevertheless, medicinal plants scientific evidence is still scarce. Studies like this one on *Asparagus laricinus* hope to address these gaps by determining the effects of *Asparagus laricinus* on CYP3A4 and CYP2D6 enzymes. A scientific study on the validity of *A. laricinus* as a therapeutic agent and safety will justify its traditional application and prove that medicinal plants can be used to manage diseases such as prostate cancer with minimal herb-drug interactions.

1.5 SPECIFIC ROLES OF CYP3A4 AND CYP2D6

CYP3A4 stands out as the predominant hepatic CYP450 isoform, accounting for an estimated 50% of the biotransformation of drugs encountered in clinical practice (Guengerich, 2019). This enzyme preferentially handles lipophilic molecules,

including statins, calcium channel blockers, immunosuppressants, and benzodiazepines. Crucial to its clinical importance, CYP3A4 undergoes a marked induction by compounds such as rifampicin and St. John's Wort, as well as pronounced inhibition by agents like ketoconazole and grapefruit juice, rendering it a common perpetrator in the landscape of drug-drug interactions (Zanger & Schwab, 2013).

CYP2D6 metabolises nearly a quarter of all approved drugs, with a strong preference for psychoactive drugs, opioids, and medications used in cardiovascular treatment (Ingelman-Sundberg, 2005). CYP2D6 is not induced by pharmaceutical agents, as opposed to CYP3A4; its clinical relevance in the treatment of prostate cancer is due to genetic variation in the metabolism of anticancer drugs. There are more than 100 alleles that affect the functioning of CYP2D6, producing poor to ultrarapid metabolizers (Zhou, 2009). This variability has a great effect on the metabolism of prostate cancer therapeutics like tamoxifen, in which CYP2D6 changes it to its active metabolite, endoxifen. Bad metabolizers will have diminished efficacy, hence poor tumour suppression, and ultrarapid metabolizers will have high toxicity as a result of excessive metabolite production. These interactions are important in understanding and optimizing the treatments and reducing the risk of herb-drug interaction with *Asparagus laricinus* fractions.

1.6 PROBLEM STATEMENT

Although *Asparagus laricinus* has been used in traditional medicine, its interactions with the major drug-metabolizing enzymes are not well determined. In the absence of such an understanding, its use in conjunction with traditional Western/therapeutic medication may introduce an unknown risk or expose uncharted pharmacokinetic advantages. The impact of *Asparagus laricinus* fractions on the major drug-metabolizing enzymes CYP3A4 and CYP2D6 needs to be understood so as to fill the gap of research on the interaction of this medicinal plant with cytochrome P450 isoenzymes. The evaluation of the inhibitory and enzyme kinetic potential of *Asparagus laricinus* extracts will be valuable in elucidating the probable herb-drug interactions as guide on the safe use of this traditional medicine, especially in the treatment of prostate cancer.

1.7 AIM AND OBJECTIVES OF THIS STUDY

To investigate the *in vitro* inhibitory effects of *Asparagus laricinus* leaf fractions on the major drug-metabolising enzymes CYP3A4 and CYP2D6.

The objectives of this research are:

- To determine the phytochemical profile of the *Asparagus laricinus* plant leaves.
- To evaluate the extract of *Asparagus laricinus* plant (leaves) on major drug-metabolising enzymes – CYP3A4 and CYP2D6.
- To explore the kinetic profiling of the metabolizing enzymes.

1.8 RESEARCH QUESTIONS

- Do the fractions from *A. laricinus* inhibit CYP2D6 and/or CYP3A4 *in vitro*?
- Is there a concentration-dependent inhibitory effect?

Null Hypothesis: Given its phenolic and flavonoid constituents, which often interact with CYP450 enzymes, *A. laricinus* extracts will not significantly inhibit CYP3A4 and/or CYP2D6 *in vitro.*

Alternative Hypothesis: Given its phenolic and flavonoid constituents which often interact with CYP450 enzymes, *A. laricinus* extracts will inhibit CYP3A4 and/or CYP2D6 *in vitro.*

1.8 DISSERTATION LAYOUT

The first chapter provides the background, problem statement, aim, objectives, significance and a summary of the thesis.

Chapter 2 provides an extensive literature review of *Asparagus laricinus*, CYP450 enzymes, herb-drug interactions and other research studies.

Chapters 3, 4, 5, 6, and 7 describe the materials and methods, which comprise the collection of plants, extraction, fractionation and CYP450 inhibition assays.

Chapter 8 presents the findings regarding the phytochemical profiles and enzyme-inhibition data, as well as the IC_{50} values.

The findings are discussed in Chapter 9 and compared with the existing literature. Limitations are addressed.

Chapter 10 brings the study to a close, presents the implications of the research on the traditional medicine safety and proposes the way of research in the future.

1.10 REFERENCES

Dahiya, D. S., Wani, F., Guidi, J. C., & Kichloo, A. (2020). Gastrointestinal adverse effects of immunotherapeutic agents: A systematic review. *Gastroenterology Research*, 13(6):227.

Guengerich, F P. 2019. CYP450 research and the future of drug metabolism. *Drug Metabolism and Disposition*, 47(1):1-6. https://doi.org/10.1124/dmd.119.086348

Hamdaoui, N., Benkirane, C., Bouaamali, H., Azghar, A., Mouncif, M., Maleb, A., ... & Meziane, M. (2024). Investigating lactic acid bacteria genus Lactococcus lactis properties: Antioxidant activity, antibiotic resistance, and antibacterial activity against multidrug-resistant bacteria Staphylococcus aureus. *Heliyon*,10(11), e31957.

Ingelman-Sundberg, M. 2005. Genetic polymorphisms of CYP450 P450 2D6 (CYP2D6): Clinical consequences, evolutionary aspects and functional diversity. *Biochemical Journal*, 370(3):735–749. https://doi.org/10.1042/BJ20041392

Mashele S.S, & Kolesnikova N. 2010. *In vitro* anticancer screening of Asparagus laricinus extracts. Pharmacologyonline. 2:246-252.

Michalets E.L. 1998. Update: clinically significant CYP450 drug interactions. *Pharmacotherapy*, 18:84-112.

Mokgawa, M.W., Van Vuuren, S.F. & Kamatou, G.P.P. (2019). Cytotoxicity and antimicrobial activity of *Asparagus laricinus* Burch. *Heliyon*, 5(8):e02284.

Mokgawa, S.D., Makhoahle, P.M. & Mashele, S. (2024). The *in-vivo* assessment of the effect of traditionally used Asparagus laricinus extracts for anticancer on the kidney, liver, and spleen of rats. Pharmacognosy Journal, 16(2), 122–130.

Mustafa, A. M., Abouelenein, D., Acquaticci, L., Alessandroni, L., Angeloni, S., Borsetta, G., ... & Vittori, S. (2022). Polyphenols, saponins and phytosterols in lentils and their health benefits: an overview. *Pharmaceuticals*, 15(10):1225.

Ntsoelinyane P.H. & Mashele, S.S. 2014. Phytochemical screening, antibacterial and antioxidant activities of Asparagus laricinus leaf and stem extracts. *Bangladesh Journal of Pharmacology,* 9:10-14.

Paul-Chima, U.O., Ugwu, C.N. & Alum, E.U. (2024). Integrated approaches in nutraceutical delivery systems: optimizing ADME dynamics for enhanced therapeutic potency and clinical impact. *RPS Pharmacy and Pharmacology Reports*, *3*(4): rqae024.

Sekhoacha, M., Riet, K., Motloung, P., Gumenku, L., Adegoke, A. & Mashele, S. 2022. Prostate cancer review: genetics, diagnosis, treatment options, and alternative approaches. *Molecules*, *27*(17);5730.

Slaughter, R.L, Edwards, D.J. 1995. Recent advances: the CYP450 enzymes. *Ann Pharmacother*, 29:619-24.

Christopher J. Sweeney, Yu-Hui Chen, Michael Carducci, Glenn Liu, David F. Jarrard, Mario Eisenberger, Yu-Ning Wong, Noah Hahn, Manish Kohli, Matthew M. Cooney, Robert Dreicer, Nicholas J. Vogelzang, Joel Picus, Daniel Shevrin, Maha Hussain, Jorge A. Garcia, and Robert S. DiPaola. 2015. Chemohormonal therapy in metastatic hormone-sensitive prostate cancer. *N Engl Med*., 373:737-746.

Van der Merwe, D., Swan, G.E. & Botha, C.J. (2001). Use of ethnoveterinary medicinal plants in cattle by Setswana-speaking people in the Madikwe area of the Northwest Province of South Africa. *Journal of the South African Veterinary Association*, 72(4:189-196.

Van Wyk, B.-E. 2008. *Medicinal Plants of South Africa*. 2nd ed. Pretoria: Briza Publications.

World Health Organization (2002) WHO Traditional Medicine Strategy 2002–2005. Geneva: World Health Organization.

Zanger, U.M, & Schwab, M. 2013. CYP450 enzymes in drug metabolism: Regulation of gene expression, enzyme activities, and impact of genetic variation. *Pharmacological Reviews*, 65(1):1-65. https://doi.org/10.1124/pr.112.007450

Zhao, J., Guercio, B. J., & Sahasrabudhe, D. 2023. Current trends in chemotherapy in the treatment of metastatic prostate cancer. *Cancers*, 15(15):3969. European Journal of Clinical Pharmacology, 65(9), 749–768. https://doi.org/10.1007/s00228-009-0670-5

Zhou, S.F. 2009. Polymorphism of human CYP2D6 and its clinical significance. Clinical Pharmacokinetics, 48(11), 689–723.

CHAPTER 2

2.1 LITERATURE REVIEW

For centuries, medicinal plants have played a crucial role as primary forms of healthcare remedies, especially in resource-constrained countries, where they are still a form of practised and readily available medication. They furnish biologic agents that are known to have antimicrobial, anti-inflammatory, and anti-cancerous activity, although their therapeutic effect and safety rely on the metabolic transformation by hepatocyte transforming enzymes of the CYP450 (Michalets, 1998). The combined administration of herbal preparations with standard medications is a common phenomenon that often produces pharmacokinetic interactions, enzyme inhibition or induction, potentially resulting in treatment failure, excessive effects, and toxicity (Zanger & Schwab, 2013). These herb-drug interactions are a serious safety issue, which is frequently ignored when practising traditional medicine.

Asparagus laricinus Burch. is a species of the genus *Asparagus* (family *Asperagaceae*) that encompasses more than 200 species spread throughout Africa, Europe and Asia. It is a perennial shrub, often referred to as *lesitwane* in the Setswana or Bushveld asparagus, and native to southern Africa. It is abundant in South Africa, Botswana and Namibia. It is identified by thorny and climbing stems, shrub-like reduced scale-like leaves, and clusters of small, white flowers, followed by red berries (Van Wyk, 2008). South African traditional medicine uses various parts of plants to treat respiratory diseases, inflammation, urinary diseases, and a general tonic (Mokgawa et al., 2019).

Few factors are said to contribute to the development of prostate cancer. These include genetic predisposition, diet, race, age and increased body mass index. These factors lead to the smouldering inflammation that can lead to the lesion in the epithelial prostate cells resulting in prostatic intraepithelial neoplasia (Ganesh, 2011). The rate of this disease is said to be high in developing countries like the Caribbean, South Africa, and South America, with a rate of 29 per 100 000, 19–24 per 100 000 and 50 – 70 per 100 000 in Caribbean (Guadeloupe), sub-Saharan Africa and South America, respectively. The disease mostly occurs in the elderly population. In the United States

it is proved to cause deaths, and the estimated rates of prostate cancer in the year 2001 were 198 100 new cases, and 31 500 cases attributed to the disease (Greenlee, 2001).

Since prostate cancer is difficult to cure, treatment is used to ease the symptoms. This includes chemotherapy, radiation, surgery, and hormonal therapy. The most common hormonal therapy used is androgen deprivation used in low doses. It is used because most prostate tumours are present in the androgen-dependent glandular epithelial cells of the prostate. Chemotherapy involves several drugs that are commonly used. These include docetaxel, cabazitaxel, and prednisone (Sweeney, 2015). The reason for using these types of chemotherapy and hormonal therapy is mainly to induce programmed cell death on cancerous cells/suppress prostate tumour growth and reduce the spread of cancerous cells to nearby reproductive organs. All this normally happens when drug agents disrupt certain processes for the induction of apoptosis in cancerous cells. It was proved that treatment is effective enough to ease symptoms. Due to certain factors (changes in dosage, abnormal patient metabolic profiles) other patients do not gain benefits from these treatments. These drugs are related to the severely adverse effects that are not limited to the reduction of white-blood cells and platelets. Immunocompromised patients with a low platelet count are at risk (Balducci, 2019).

Medicinal plants have been the most considered part of nature worldwide. Medicinal plants are filled with ingredients that can be used in the development of a complex or synthetic drug. Before, they were known to be used as part of cosmetics, perfume, natural dye, pest control, and as food, but with time it was discovered that such plants can be used to treat a variety of diseases like cancer and other diseases (Pieroni, 2004). *Asparagus laricinus* has been considered as a potential therapeutic agent for the symptomatic treatment of prostate cancer (Mashele, 2010. This plant secretes active compounds known as phytochemicals that play a part in stimulating and inhibiting enzymes. They can also act as cofactors. Both leaves and roots have tannins, saponin, terpenes, and steroids. The roots mostly show the presence of alkaloids (Fuku, 2013), while leaves lack the presence of alkaloids (Ntsoelinyane, 2014). The stem is rich in saponins, tannins, and flavonoids, with a lack of steroids, glycosides, and carbohydrates (Ntsoelinyane, 2014). Only a few compounds are

isolated from the roots of *Asparagus laricinus,* i.e. indole-3-carbinol, α-sitosterol, and ferulic acid (Fuku, 2013). β-Sitosterol has a few therapeutic and chemo-preventive uses in the medical field (Zak, 1990). Indole-3-carbinol is used in prostate cancer (Garikapaty, 2005). Since information on the efficacy profile of *Asparagus laricinus* is not enough, this has led to an analysis of different compounds that were isolated to understand anti-prostate cancer activity from plants. How the body handles the therapeutic compounds is important, because it also determines the safety and efficacy of the possible anti-prostate cancer agents *Asparagus laricinus* fractions.

Although *A. laricinus* has documented traditional use and basic phytochemical screening, there is limited published information specifically addressing its potential to modulate CYP3A4 or CYP2D6 activity. Given (1) the presence of flavonoid/polyphenolic constituents in *A. laricinus* extracts, (2) the well-established capacity of such phytochemicals to inhibit CYP450 enzymes *in vitro*, and (3) the clinical importance of CYP3A4 and CYP2D6 for drug clearance, a focused *in vitro* assessment of *A. laricinus* fractions are warranted. This work can identify fractions with inhibitory potential, guide phytochemical isolation, and inform risk assessment for herb-drug interactions before progressing to *in vivo* or clinical work. (Amaeze, 2021).

CYP450 are major enzymes in the formation and treatment of prostate cancer. They are vital and mediate the metabolism of plant extracts (Wilkinson, 2005). These enzymes have been identified in many organisms, including animals, plants, and bacteria. In humans, the CYP450 enzymes are found in the mitochondria of the liver cells; however, they are also found in many other cells of the body like in the blood and other peripheral tissues (Slaughter, 1995). There are 50 enzymes, of which only a few are responsible for the metabolism and detoxification of drugs, phytochemicals and even foreign substances.

The following are the major metabolising enzymes: CYP3A4 and CYP2D6. Drug interactions involving the CYP450 isoforms are of two types: enzyme induction and enzyme inhibition. Enzyme inhibition normally reduces metabolism, whereas enzyme induction can increase it, thus, posing risks of altering the efficacy and safety of certain drug agents (Michalets, 1998). There is not enough evidence to prove that there is an

interaction of *Asparagus laricinus* fractions and isolated compounds with other therapeutic agents that have the same metabolic pathway. Thus, the experiment aims to determine the effects of *Asparagus laricinus* fractions and the enzyme kinetics of the major drug-metabolising enzymes, CYP3A4 and CYP2D6. Proving all this will help patients using chronic treatment to have more knowledge of drug-herbal interaction and provide more information about fractions of *Asparagus laricinus*.

Among the CYP450 enzymes, the most abundant enzyme in the body is known as the CYP3A4, which has an expression in the following body parts: in the intestines – more specifically in the ileum and jejunum – and in the liver (Anchor, 2014). In 2012, guidance for industry drug interaction studies proves that CYP3A4 is the one CYP450 isoenzyme that metabolizes most of the drugs, including ketoconazole, rifampicin, verapamil, and clarithromycin. The second-most drug metabolizing isoenzyme is the CYP2D6, which metabolises one-third of the existing drugs (Ye, 2016).

2.2 *ASPARAGUS LARICINUS*

Asparagus laricinus is a species in the family *Asparagaceae*, commonly used medicinally in southern Africa. It is referred to under a few regional names, including *Bergkatbos* or *Langbeenkatdoring* in Afrikaans, *ibhutha* in isiZulu, and *lesitwana* in Setswana. It is integral to traditional rituals of healing in Lesotho, Botswana, and Eswatini, but it is also among the most used of all species in the region.

2.1.2 Taxonomy and morphology

Asparagus laricinus is a perennial, evergreen shrub or climber, often about 1.5–2.5 m tall. Its pale, sometimes silvery stems curl into a zigzag-like outline, known informally by the plant's name. The stems have short, inflexible spines, the plant does not have actual leaves. Indeed, instead of branches, it assembles loosely needle-like cladodes or modified branches, normally 2.5 to 3.5 cm longy. Such cladodes can gather in large bunches around a node, giving the species its rich, feathery style and its fine, delicate appearance. It flowers in the late spring and summer months (December to March). The plant produces many fragrant, soft, luscious small white flowers that are appealing to insects and other pollinators during this period. As the flowers mature,

they develop into round berries that are initially green, then turn red, and eventually become shiny black when fully ripe. The ripe berries are devoured by birds that will pollinate and also distribute the seeds. Figure 2.1 below shows the Asparagus Laricinus plant with and without fruits.

A **B**

Figure: 2.1 A) *Asparagus laricinus* leaves and B) represent *Asparagus laricinus* leaves with fruits.

2.1.3 Ecology and Habitat

A. laricinus is therefore widely distributed due to its adaptive response for diverse environmental conditions. It is found in many vegetation types, especially grasslands, bushveld, thornveld, and mountainous shrubland. It also flourishes in disturbed environments such as roadsides among patches of scrub. They are relatively drought-tolerant and very fire resistant. In the event of a fire, it can regenerate from roots to grow elsewhere in the world in fire-prone areas. Despite its ability to grow in full sun or semi-shade and to thrive in most well-drained soils, it is less active in waterlogged areas. It has also been shown to be a hardy, adaptable species that makes great use in landscaping and ecological rehabilitation efforts.

Asparagus laricinus is a naturally occurring species in many areas of southern Africa. It is commonly found in:

- South Africa (Eastern Cape; Free State, Gauteng; KwaZulu-Natal, Limpopo, Mpumalanga, Northern Cape, and Northwest).
- Botswana.

- Lesotho.
- Eswatini (Swaziland).
- Namibia; and
- Zambia, particularly in areas between 900–2,300 m above sea level.

It also has extensive distribution, suggesting the plant's potential to survive an extensive range of climate and environmental conditions.

2.1.4 Ethnomedicinal significance

This plant used to be revered in traditional medicine. Its use for health care in communities in southern Africa encompasses treatment for:

- sores and general skin infections.
- “Red water” (babesiosis) in livestock.
- tuberculosis.
- uterine infections; and
- inflammation of the umbilical cord in babies.

Its enduring use and cultural significance warrant more scientific research of its medicinal and potential uses.

2.2 PHYTOCHEMICAL AND PHARMACOLOGICAL INSIGHT

Asparagus is a genus of the *Asparagaceae* family, a very distinct name that covers many species that are greatly accepted as both medicine and food in different cultures. Sobhy et al. (2022) describe the enrichment of the *Asparagus* species with bioactive substances characteristic of flavonoids, saponins and sterols, and explain why they have therapeutic potential. The compounds have been attributed to be antioxidants, anti-inflammatory and anticancer and as such, the genus is a prime target of pharmacological investigation. In a study on *Asparagus laricinus*, a foremost plant in South African traditional medicine, a high proportion of phenolics and flavonoids had cytotoxicity against prostate cancer cell lines (Mfengwana & Sone,

2022). The determination of the treatment of such conditions such as tuberculosis, respiratory disorder, and inflammation is another feature of the pharmacological versatility of the Asparagus species. The genus remains to be explored as a source of new therapies and thus this genus belongs to modern pharmacognosy. Flavonoids in *Asparagus laricinus* may inhibit CYP3A4, potentially enhancing the efficacy of prostate cancer drugs like docetaxel by slowing their metabolism, though this could increase toxicity risks (Sobhy et al. 2022). The safety of *A. laricinus* extracts in prostate cancer treatment requires further investigation, as their steroidal saponins may modulate CYP2D6, affecting the metabolism of co-administered drugs like opioids (Sobhy et al. 2022).

All species of asparagus have been used as ethnomedicines, used mostly in Africa and Asia, often based on their diuretic, antibacterial, and immunomodulatory properties. Ibrahim et al. (2021) refer to the fact that the Asparagus broad-spectrum bioactivity is due to its rich phytochemical composition of alkaloids and terpenoids. The effect of these compounds via cellular pathways may modulate enzyme activity and cascades in the context of disease management. A study that was conducted by Romani et al. (2021) demonstrates the potential of *Asparagus officinalis* extracts as anticancer agents due to their significant effects in inhibiting the growth of tumour cells under *in vitro* conditions. The pharmacological profile of the genus is rounded out by low toxicity that distinguishes it as a good candidate for drug development. Research on the species of Asparagus has revealed novel bioactive compounds, making this natural substance relevant to employ in new treatment technology. The CYP3A4 interaction potential between the terpenoids of *A. laricinus* and chemotherapeutics of prostate cancer may change the pharmacokinetics of the drugs thus requiring research to guarantee safety in combined administration. The alkaloid properties may increase the efficacy of *A. laricinus* in the treatment of prostate cancer by acting synergistically with the CYP2D6 substrates in improving therapeutic efficacies, which require clinical validation of their effects.

Asparagus species are complicated in their phytochemistry, and this has resulted to the series of studies related on ascertaining its mode of action particularly with regards to cancer treatment. According to Majnooni et al. (2022), the apoptosis of prostate cancer cells is induced by saponins isolated in *Asparagus racemosus*, and

this may be the mechanism of action of anticancer activity. These results are in consonance with conventional practices of *Asparagus* in the treatment of cancer related symptoms. According to research that was conducted by Romani et al. (2021), Asparagus extracts are found to relax the oxidative stress, one of the causes of cancer. The possibility and potential to alter the redox status of cells are other reasons that prove the therapeutic role of the discussed compounds. *A. laricinus* may also inhibit CYP2D6 with saponins which can increase the bioavailability of prostate cancer drugs such as tamoxifen, thereby increasing efficacy but also causing toxicity. The efficacy of *A. laricinus* as a treatment of prostate cancer is conditional upon the interaction of its phytochemicals with CYP3A4 that may change the clearance of drugs and require close monitoring to avoid herb-drug interactions.

The interaction of *Asparagus* species with important metabolizing enzymes has been the focus of recent pharmacological studies; the resultant important antidrug effect may affect the level of drug activity and safety. Li et al. (2023) demonstrate the ability of flavonoids in *Asparagus* species to regulate signalling pathways related to inflammation and cell proliferation to provide some insights into the pathways that could help these drugs to their therapeutic effect. They are chemically varied and hence lead to varying biological properties. Prasad et al. (2024) use Asparagus ascending extracts, which show to improve immune responses and can therefore serve as adjuvants in cancer treatment. The energy between the natural healing process and pharmacological research recommendations still becomes the fuel of researching into the Asparagus species. These research studies are important in justifying their ethnomedicinal applications and investigating their applications in the clinical practice.

The discovery of the Asparagus species equally indicate that they can be used in the regulation of metabolic pathways that are important in the metabolism of drugs. As noted by Cheng et al. (2023), liver activities may have significance on the liver enzymes, given that the hepatoprotective effects of steroidal saponins in Asparagus species are true. The relevance of these findings is to comprehend the interaction of the Asparagus-derived compounds with the hepatic systems. Regarding the findings of a study conducted by Li et al. (2023), Asparagus extracts have anti-inflammatory effects, which could be related to the effect it produces on the cytokine production.

These compounds have greater pharmacological potential than the potential in the inhibition of adverse drug reactions. *A. laricinus* steroidal saponins can also regulate CYP3A4 activity, which may increase activity in drugs used to treat prostate cancer, such as cabazitaxel, though with the need to evaluate safety.

The asparagus species also differentially bear their own nutritional value, which is also synergistic to the medicinal values. Redondo-Cuenca et al. (2023) suggest that the presence of dietary fibre and antioxidant helps Asparagus to attain holistic health. These are the nutritional elements that enhance the therapeutic spectrum in plants and those of curing chronic diseases. Li et al. (2024) conducted an experiment and demonstrated that Asparagus species exerts gut health effects via polysaccharides that have secondary effects on systemic metabolism. Asparagus presents a challenge of integrative health in respect of its pharmacological and nutritional interactions that accompany it. *A. laricinus* phenolics have the potential to decrease oxidative stress in prostate-cancer cells and could inhibit CYP3A4, which could influence drug metabolism and necessitate close therapeutic surveillance.

The pharmacology of Asparagus species also indicates the value of the species to traditional and modern medicine. Saleh et al. (2021) claim that the presence of phytochemicals in the genus can help to address many conditions on an occasional or chronic scale. Such results are supported by literature that proves the utility of the extracts of Asparagus in preclinical models. Based on the findings presented by Chen et al. (2023), it is evident that the compounds of Asparagus have potential in therapeutic application, since they need less time to inhibit the cancer-cell proliferation by blocking the key enzymes. The researching process of combining the traditional knowledge with scientific validation is a modern trend in researching the species belonging to Asparagus. Phenolic chemicals found in *A. laricinus* have the potential to inhibit the CYP3A4, which might enhance the effect of prostate-cancer therapeutic agents such as docetaxel; however, causing greater toxicity when not managed effectively. The efficacy of *A. laricinus* in the treatment of prostate cancer needs to be examined more thoroughly to ascertain whether the phytochemicals contained in it may have a negative influence on the drug metabolism mediated by CYP2D6, which can undermine the overall results of the treatment.

Asparagus species interaction with CYP2D6 as a prominent drug-metabolizing enzyme has attracted attention because it metabolizes many prescribed drugs. Chaachouay (2025) points out that Asparagus species phytochemicals (flavonoids and saponins) may interfere with CYP2D6 activity, possibly by modifying the metabolism of medications such as tamoxifen and codeine. This blockage may cause high drug bioavailability, which has risks of side effects in the patients who consume such medication. *Asparagus*-derived compounds offer a potential to regulate CYP2D6; therefore, a study that determines their effect on drug pharmacokinetics should be conducted on a person-to-person basis. These studies will be essential in the safe introduction of *Asparagus*-based remedies into treatment practice with patients undergoing polypharmacy treatment.

2.3 KNOWN PHYTOCHEMICAL CONSTITUENTS OF *ASPARAGUS LARICINUS*

Asparagus laricinus is a species naturally present in Southern Africa and, due to its long history of phytochemical such as compounds, it has acquired a global popularity owing to its value that has traditionally been practised as a medicine. Mfengwana et al. (2023) report that indole-3-carbinol, a-sitosterol and ferulic acids are contained in the roots of the *A. laricinus*, and these substances possess anticancer and anti-inflammatory properties. These phytochemicals contribute to the medicinal properties of the plant, particularly in curing symptomatic prostate cancers. A study carried out on *A. laricinus* by Radebe (2023) indicated the presence of high amounts of flavonoids, tannins and saponins in the stem and leaf of the plant, but no steroids and glycosides were indicated in the stem. Such compounds suggest a broad spectrum of bioactivity as evidenced by the antioxidant and cytotoxic effects. These constituents need investigations so that their pharmacological mechanisms may be realized. Ndole-3-carbinol in *A. laricinus* can also inhibit CYP3A4, which could potentially increase the effect of prostate cancer drugs by reducing their metabolism, but also increasing the risk of toxicity.

Phytochemical diversity plays a pivotal role in the ethnomedicinal practices of *A. laricinus* especially in South African traditional medicines. Sobhy et al. (2001) state that roots and leaves are used to treat uterine infection and tuberculosis, which is likely caused by their bioactive secondary metabolites. Alkaloids are examples of

these phytochemicals, and they do not appear in the leaves, but they are found in the roots. Ekechukwu et al. (2025) report that *A. laricinus* extracts have effective antimicrobial activity through the synergistic interaction of phenolics and saponins. Differences in levels of phytochemicals in parts of the plants underscore the importance of specific and selective extraction. These studies form the basis of the justification of the traditional uses of the plant. The phenolics in *A. laricinus* could also regulate the CYP3A4 and enhance the bioavailability of prostate cancer drugs. However, their safety should be considered to avoid adverse herb-drug interactions. Its efficacy in prostate cancer treatment might be increased by the saponin content of *A. laricinus*, which may act in association with CYP2D6 to affect drug metabolism, and may need additional validation to be used safely.

A. laricinus contain a significant number of flavonoids, and extensive literature exists that discuss their therapeutic potential (Ekechukwu et al., 2025). According to Romani et al. (2021), flavonoids found in Asparagus species have significant antioxidant activity, and it may reduce oxidative stress in cancer cells. *A. laricinus* contains phytochemicals, particularly on the leaves, which are responsible for the plants cytotoxic activity (Ekechukwu et al., 2025). A study by Bouabdallah et al. (2023) demonstrates that the extracts of *A. laricinus* containing flavonoids cause a loss of cell viability in cell lines of prostate cancer, thus reinforcing the idea of the *A. laricinus* extracts as anticancer agents. The engineering complexity of flavonoids permits a multiplicity of susceptibility, such as enzyme modulation. These results highlight the need to profile the phytochemical nature of *A. laricinus* to develop drugs.

Another important component of *A. laricinus* saponins has immunomodulatory effects and anticancer properties (Romani et al., 2021). The steroids of the asparagus species families of saponins have the potential to reduce tumour growth through cellular membrane disruption and trigger of apoptosis (Bouabdallah et al., 2023). These compounds are highly enriched in the stem of *A. laricinus*, which increases its medicinal value. In one of the studies conducted by Elekofehinti et al. (2021), fractions of saponins in *A. laricinus* revealed a considerable cytotoxic property against cancer cells, indicating its potential use in treating cancer. Pharmacological literature on saponins is principally predicated on the fact that they are the cellular modulators active within the pathways that are significant in disease progression. Additional

research needs to be done to isolate and characterize these compounds to help with clinical opportunities.

A. laricinus possesses antioxidant and anti-inflammatory activity explained by the presence of available phenolic compounds. According to Lopez-Moreno et al. (2025), Asparagus species have antioxidant ability, because phenolic derivatives are a key component of *Asparagus*. Oxidative tissue damage can be reduced by scavenging free radicals. Depending on various parts of the plant, these chemical substances occur in various concentration levels in the plant elements including the leaves, the stem and the root. The finding by Pieczykolan et al. (2021) on the effects of phenolic extracts of *A. laricinus* counteracted the inflation cytokines, which matches their potential to control persistent inflammations. The phenolic has low toxicity and good availability to enhance the therapeutic effectiveness. The *A. laricinus* properties might have a possible future in integrative medicine. The phenolic compounds of *A. laricinus* can potentially modulate the CYP2D6 activity, which will have beneficial therapeutic effects on prostate cancer drugs, although their safety profile has to be confirmed to prevent adverse drug interactions. The effectiveness of *A. laricinus* in treating prostate cancer might be associated with the capacity of its phenolics in the inhibition of CYP3A4 that can enhance the bioavailability of the drugs, though with proper attention, to maintain safety.

The phytochemical profile of *A. laricinus* also includes terpenoids, which have previously been reported to be linked with the antimicrobial and anticancer activity of *A. laricinus*. Zazharskyi et al. (2025) note that the anti-microbial action of terpenoids found in the species of Asparagus is broad-spectrum, which confirms the effectiveness of its use to treat infections. The roots of *A. laricinus* contain compounds that make them specifically rich in the roots of *A. laricinus,* which makes it ethnomedicinally used. According to Fayek et al. (2022), *A. laricinus* terpenoid-enriched fractions were found to have impressive anti-prostate-cancer cell activity. Terpenoids have many different biological applications such as enzyme inhibition due to their structural complexity. Further studies on these compounds will help to grasp the therapeutic potential of *A. laricinus*.

Pharmacological validation of *A. laricinus* uses the characterization of the phytochemical constituents. Chaachouay (2025) postulates that the efficacy of the plant in traditional medicine may be attributed to the synergetic effects of flavonoids, saponins, and phenolics. These compounds affect various targets on the cellular level, which increases their therapeutic versatility. Albahri et al. (2024) investigated the effect of *A. laricinus* extracts on important signalling pathways in cancer progression, demonstrating their potential value as anticancer agents. The phytochemical variation of *A. laricinus* requires additional studies to explain its modes of action. These studies will promote the establishment of new prescription drugs based on this plant. The synergism of *A. laricinus* phytochemicals can be used to regulate CYP3A4, which can optimize the effect of prostate cancer drugs. However, the safety of these herbs should be tested to avoid unanticipated herb-drug interactions.

The possibility of *A. laricinus* acting on CYP2D6 raises a considerable implication on its application in native medicine. As stated by D Alessandro et al. (2022), the plant contains phenolic and flavonoids with possible inhibition of CYP2D6, including drugs like beta-blockers and antipsychotics. Such inhibition may cause an adjuration in the clearance rate of the drug, hence a tendency for side effects development in patients taking such concomitantly. Understanding the effects of *A. laricinus* on CYP2D6 is important in the study of the drug to determine its safety, especially in people who consume high levels of herbal medicine. This kind of research will be of great use to determine data that personify the safe combination of *A. laricinus* with routine therapies.

2.4 DRUG METABOLISING ENZYMES: CLASSIFICATION AND MECHANISMS

Drug-metabolizing enzymes are important detoxifiers and bio-transformers of xenobiotics or drugs and plant secondary compounds. Esteves et al. (2021) explain that CYP450 enzymes, which are predominantly found in the liver, are the most remarkable elements of phase I metabolism, which oxidizes a broad range of molecules. CYP450 enzymes belong to families and subfamilies, depending on the similarity of their amino acid sequences, whereby CYP2D6 and CYP3A4 are the most useful in drug metabolism CYP450. A study conducted by Zhao et al. (2021) revealed

that the CYP450-superfamily encompasses more than 50 enzymes, with only several of them being involved in metabolizing most of the clinically applicable drugs, such as CYP3A4 and CYP2D6. These enzyme mechanisms include oxidation, reduction and hydrolysis in which xenobiotic elimination is achieved. The classification and their functions are crucial to predict drug interactions and the effects of the drug. *A. laricinus* phytochemicals have the potential to inhibit CYP3A4 and thereby increase the bioavailability of prostate cancer drugs, but it also increases risks of toxicity, which requires close observation. A possible mechanism of action of *A. laricinus* in the treatment of prostate cancer is that it alters CYP2D6, although its safety profile needs additional investigation.

CYP450 enzyme system is broadly specific and inducible. Zhao et al. (2021) argue that the liver is not alone in expressing CYP450 enzymes, as extrahepatic sites like the intestines and blood also play a role in metabolizing drugs systemically. The effect of these enzymes is to increase the solubility and excretion of substrates by adding oxygen to them. Narendra et al. (2021) show that the induction or inhibition of CYP450 enzymes alters pharmacokinetics of drugs in a study and might result in therapeutic failure or toxicity. Metabolism of CYP450 has a complex role that explains why there is a need to understand how they interact with herbal extracts in detail. These interactions are capable of influencing the safety and effectiveness of drugs co-administered.

CYP450 enzymes are organized into classes based on genetic and functional properties, and, most clinically significant, CYP450s include CYP3A4 and CYP2D6. Zhang et al. (2024) identified CYP3A4 as the most prevalent hepatic CYP450 enzyme that processes 30–40% percent of the market-introduced medications. It has a wide selection of target substrates, notably antibiotics, antidepressants and immunosuppressants. Zhang et al. (2021) demonstrate that CYP3A4 was seen to form a metabolome with other proteins, which promotes its metabolic power. This structural design emphasizes the role played by the enzyme in the coordination of complex body processes. The mechanisms play a vital role in the evaluation of the effects of herbal compounds on drug metabolism.

Another important drug-metabolizing enzyme is CYP2D6, which has great genetic polymorphism that influences its activity in different populations. Dean and Kane (2025) identify CYP2D6 metabolic rate in about 15 per cent of pills given to patients, both psychotropic and beta-blockers. Its polymorphisms cause different metabolic functionalities, namely ultra-rapid to poor metabolisers. Nahid and Johnson (2022) demonstrate that the effect of the CYP2D6 polymorphisms reflects the CYP2D6 effect, highlighting that genotyping is necessary in individualized medicine. Such results demonstrate the necessity to consider the inputs of 2D6 of herbal extracts. These studies can offer feedback on the clinical approach of reducing adverse drug interactions.

CYP450 enzyme activity results in complicated interactions with drugs and substrates, with both substrates and inhibitors affecting drug pharmacokinetics. Cohen et al. (2003) used fluorogenic substrates to investigate CYP450 inhibition, indicating the difficulties of establishing a correlation between in vitro and in vivo data. Substrates of this kind offer a high-throughput approach to measuring the kinetics of enzymes and are not necessarily representative of the complexity of in vivo metabolism. The study conducted by Marks et al. (2002) shows the usefulness of fluorescence-based assays in screening CYP2E1 enzyme inhibitors. Herbal-drug interactions require the application of these assays to other CYP450 enzymes such as CYP3A4 and CYP2D6. These assays enable the fast evaluation of the enzyme modulation by plant extracts.

The interactions between CYP450 enzymes and herbal compounds are an important research focus, especially in the case of plants such as *A. laricinus*. Iwata et al. (2004) revealed that some herbal extracts demonstrate a metabolism-dependent inhibition of CYP3A4 and CYP2D6, which indicates the possibility of herb-drug interactions. The pharmacokinetics of drugs that are co-administered may be changed because of these kinds of interactions, which create therapeutic problems. A study done by Trubetskoy et al. (2005) found that the fluorescence-based techniques employed in screening assays demonstrated that they were good CYP450 enzyme inhibitor detectors. Such studies are indispensable in ensuring the safety of the use of clinically herbal medicines.

The influence of significant drug-metabolizing enzymes regarding pharmacotherapy and toxicology is highlighted by the use of classification and drug-metabolizing enzyme mechanisms. As Zhao et al. (2021) describe, CYP450 enzymes can be activated by xenobiotics. This can elevate the removal of drugs and thus reduce their effectiveness. Inhibition, however, can lead to drug accumulation and side effects. A study conducted by Ohanme et al. (2024) has shown that CYP450 enzymes in the metabolism of phytochemicals proved to influence bioavailability and efficacy. These findings justify that comprehensive studies need to be done relative to the associations between *A. laricinus* and CYP450 enzymes. An understanding of the interactions will help to come up with safe and effective herbal therapy.

2.5 ENZYME PROFILE OF CYP3A4: SUBSTRATES, INHIBITORS, AND CLINICAL RELEVANCE

CYP3A4 has become an essential component of drug metabolism, functioning in the biotransformation of many therapeutic agents and many endogenous compounds. Czyrski et al. (2021) state that the CYP3A4 enzyme is the most abundant in the liver, and it is responsible for metabolizing around 30–40% of every clinically used medicine, together with statins, antifungals, and chemotherapeutic drugs. Its substrate specificity is wide and cuts across a broad range of chemical structures and it thus plays a major role in pharmacokinetics. Another study conducted by Zhang et al. (2024) reveals that CYP3A4 interacts with 149 proteins to form a metabolome, which improves its metabolic competence. Such structural complexity helps it to have a wide substrate range and clinical relevance. A comprehension of the CYP3A4 background is crucial in terms of forecasting drug interactions as well as favourable therapeutic and drug safety outcomes.

The substrates of CYP3A4 are xenobiotics and endogenous compounds such as steroids and bile acids. According to Mar et al. (2022), CYP3A4 metabolizes drugs such as ketoconazole, erythromycin, and verapamil, which are the most common in clinical practice. These substrates are metabolised into active or inactive metabolites making them easy for the body to eliminate them via kidneys into urine or bile into stools. According to Malau et al. (2025), a research study found that CYP3A4 plays a major role in metabolising chemotherapy drugs like docetaxel, which is used in

treating prostate cancer. This indicates the relevance of this enzyme in cancer therapeutic outcomes.

CYP3A4 inhibitors may change the pharmacokinetics of drugs significantly, which can cause sub-therapeutic or adverse effects. A study by Iwata et al. (2004) report that herbal extracts, including Evodia Fruit, have a metabolism-dependent inhibition that suppresses CYP3A4 activity by up to 77.7% following pre-incubation. This inhibition may lead to increased plasma levels of co-administered drugs and, therefore, run the risk of toxicity. In another study by Cohen et al. (2003) fluorogenic substrates demonstrated inconsistent proportions between *in vitro* and *in vivo* CYP3A4 inhibition These results underline the importance of determining whether the fractions of *A. laricinus* could inhibit CYP3A4. These types of studies are vital in determining the risk of herb-drug interactions that patients treated with traditional medicine bear.

Clinical interest in CYP3A4 is tenfold due to drug-drug/herb-drug interactions, especially in polypharmacy. Patel et al. (2021) note that flavonoids and phenolics found commonly in Asparagus species inhibit the activity of CYP3A4 and, as such, may impair the metabolism of any drug administered concomitantly, resulting in therapeutic issues. The active site of the enzyme reacts with these compounds and lowers the reactivity of the enzyme. In one study by Gomez-Garduno et al. (2022), phytochemicals in plants have been found to alter the expression of CYP3A4, which influences the rate at which drugs are cleared. This modulation can have implications for patients with chronic conditions like prostate cancer. An understanding of such drug-interactions is critical with regards to safety and efficacy of therapeutic drugs.

Intra-individual variation in drug processing may be due to CYP3A4 (In most instances, CYP2D6) genetic polymorphisms, which influence the therapeutic response of different drugs. According to the findings of Zhang et al. (2024), the genetic variability of CYP3A4 can stimulate the alteration of the enzyme activity, which can disrupt the metabolism of such drugs as midazolam and cyclosporine. Such interpolations have particular effects on populations that have a diverse genetic background. In a study conducted by Bagdasaryan et al. (2022), it was proven that the polymorphisms of the enzyme CYP3A4 influences the metabolic mechanism of chemotherapeutic drugs; thus, they can influence plasma drug levels and therapeutic

outcomes. The result shows that personalized medicine practices of regulating the CYP3A4-mediated metabolism may be essential. This is necessary in order to optimize drug therapy outcomes in diverse populations.

High-throughput screening assay use has changed the way inhibition of CYP3A4, and substrate specificity are studied. Trubetskoy et al. (2005) have demonstrated that the Vivid substrate fluorescence-based assay has been a robust tool to assess CYP3A4 inhibition using a 1536-well format. The assays have good sensitivity and reproducibility, which makes them good screening tools against herbal extracts. A study by Marks et al. (2003) reveals that the Vivid CYP3A4 assay performed with similar IC_{50} values in 96- and 1536-well conditions, thereby making it highly accurate and consumable in high-throughput conditions. The evaluation of the impact of *A. laricinus* fractions on CYP3A4 activity would not be possible without these tools. Such use will help to identify possible herb-drug interactions.

The clinical manifestation of CYP3A4 modulation is also in the chemotherapeutic process with any of the drugs (docetaxel and cabazitaxel) used in the treatment of prostate cancer. Zhang et al. (2021) state that the therapeutic effects of these drugs may be influenced by the herbal agents via the CYP3A4-mediated metabolism of the drug. Such interactions are mostly applicable to patients who take traditional medicines in combination with conventional drugs. According to a study carried out by Ayaz et al. (2022), CYP3A4 inhibition by the phytochemicals enhanced the bioavailability of the chemotherapeutic drugs. These results support the fact that detailed investigations of the *A. laricinus* impacts on CYP3A4 are eminent. This type of research will guide the safe use of herbal medicines in the regimen of cancer treatment.

Complicated herb-drug interactions of *A. laricinus* are revealed when considering the combined effects of CYP3A4 and CYP2D6 involvement in drug metabolism. According to the researchers, the metabolism of some drugs, including antidepressants, by CYP2D6 is extremely susceptible to herbal compounds, whereas CYP3A4, which is used to clear a broader range of substrates, is insusceptible to herbal modification (Kumar et al., 2023). The *A. laricinus* phytochemical inhibitors may be preferentially CYP2D6 inhibitors and hence risk of either prolonging the actions of

therapeutists, or of amplifying drug side effects like fluoxetine, and this may be reliant on the pharmacokinetics. This selective inhibition needs selective studies to assist in the explanation of the variation in the inhibition of *A. laricinus* towards CYP2D6 an CYP3A4. The proposed study will make a difference by enhancing knowledge on how the concurrent use of *A. laricinus* may influence therapeutic outcomes in prostate cancer patients undergoing cancer chemotherapy.

2.6 ENZYME PROFILE OF CYP2D6: SUBSTRATES, INHIBITORS, AND CLINICAL RELEVANCE

A broadly polymorphic CYP450 enzyme, CYP2D6, is very important in the metabolism of various compounds with clinical importance. Nahid and Johnson (2022) state that about 15% of the drugs available in the market are metabolized by CYP2D6, such as antidepressants, beta-blockers, and opioids, whose activity may be influenced by genetic polymorphisms. The polymorphisms lead to metabolic phenotypes that vary widely too poor, at the low end and ultra-rapid at the high end. Langmia et al. (2021) demonstrate that the effect of CYP2B6 polymorphisms closely resembles that of CYP2D6, and this shows the clinical importance of genetic variation in drug metabolism. Understanding the substrate specificity and inhibition of CYP2D6 by different drugs and the influence of polymorphisms affecting this isozyme is important in rational use of medicines.

CYP2D6 metabolizes drugs with narrow therapeutic windows, like tamoxifen and codeine. Furthermore, it is also involved in the metabolism of drugs with a wide therapeutic window. Cohen et al. (2003) state that the substrate specificity of CYP2D6 depends on the active site that fits a diverse set of chemical groups. This is an area of versatility, which is beneficial in processing mainly psychotropic and cardiovascular drugs. In one study conducted by Marks et al. (2002), CYP2D6 fluorescence-based assays have always shown to be sensitive to inhibitors; this in that IC_{50} values differed depending on the chosen substrate. The assays offer a high-throughput approach to the assessment of CYP2D6 inhibition by herbal compounds. The described studies would be necessary in determining the safety of *A. laricinus* in clinical conditions.

There are strong CYP2D6 inhibitors, capable of altering drug pharmacokinetics and causing drug adverse effects. Iwata et al. (2004) observe that metabolic-dependent

inhibition of CYP2D6 by herbal extracts (including that of Incised Notopterygium Rhizome) lowered its activity by a maximum of 38.1% when pre-incubated. Such inhibition may enhance plasma levels of concomitantly administered drugs, with the risk of toxicity. An earlier study by Trubetskoy et al. (2005) showed Vivid CYP2D6 to give good inhibitor IC_{50} values that proved that it can be used in high-throughput screening. These assays are important when assessing the inhibitory capacity of *A. lariciinus* fractions against CYP2D6. Their results will inform safe practice of herbal medicines in the polypharmacy context.

CYP2D6 is clinically relevant due to its importance in individualised medicine especially in pain management and psychiatry. Maciaszek et al. (2021) assert that enzymatic polymorphism related to CYP2D6 alters antidepressant metabolism and consequently changes the therapeutic activities and adverse outcomes of fluoxetine. Such disparities require that drug dosing protocols be personalized to maximize patient response. In a Li et al. (2021) study, the effects of CYP2D6 inhibition through phytochemicals are demonstrated to change psychotropic pharmacokinetics, indicating the possibility of herb-drug interactions. Such results underline the necessity to assess the effects of *A. laricinus* on CYP2D6 function. These studies will enlighten clinical guidelines among patients taking herbal treatments concurrently with conventional drugs.

The changes of CYP2D6, which make them genetically variable, and affect inter-individual variation of drug response, especially in populations of diverse nature. Langmia et al. (2021) specify that CYP2D6 polymorphisms are widely spread in the African population, interfering with such medications as metoprolol and tramadol. Such differences may result in great disparities in therapeutic outcomes. In a study, Ballester et al. (2022) demonstrate that the metabolism of CYP2D6 polymorphisms in the body can affect the processes of pain management. This observation indicates that genotyping should be considered to maximize drug therapy. Examining *A. laricinus* and its implications on CYP2D6 will help to determine whether it is safe in various genetically diverse individuals.

CYP2D6 inhibition and substrate specificity have been studied with the use of high-throughput screening assays. Marks et al. (2003) demonstrated that Vivid CYP2D6

assay provided a robust technique to determine enzyme inhibition, wherein a high degree of replicability between plate formats was observed among data. The assays are advantageous when it comes to screening of herbal extracts to discern any possible CYP2D6 interaction. In a study, Sobhy et al. (2022) reveal that Asparagus species phytochemicals have inhibitory effects on CYP2D6, and additional research is necessary in this regard. These results justify the need to test the *A. laricinus* fractions in high-throughput assays to understand their influence on major drug-metabolizing enzymes such as CYP2D6 and CYP3A4.

The interest of specific patients is of particular concern to the clinical implications of CYP2D6 modulation, as it applies to prostate cancer patients who might employ herbal medicine concomitantly with conventional options. Gomez-Garduno et al. (2022) state that the inhibition of drugs such as tamoxifen, which is taken in hormone therapy, by CYP2D6 can be changed through interactions with certain phytochemicals. Such contacts may influence the quality of treatment safety and efficacy. According to a study that was done by Zhang et al. (2021), CYP2D6 inhibition supports the effects of opioid metabolism in terms of its effects on pain management in cancer patients. Such results indicate a necessity of more thorough in-depth research on the influence of *A. laricinus* on CYP2D6. This type of research will guarantee the safe use of herbal medicines in the treatment of cancer.

2.7 HERBAL-DRUG INTERACTIONS: EVIDENCE FROM PLANT EXTRACTS

Herbal-drug interactions (HDIs) are a concern in pharmacotherapy, especially among patients who use traditional medicine and conventional drugs. Iwata et al. (2004) argue that the activity of CYP450 enzymes may be altered through herbal extracts, which may alternate the CYP450 of co-administered drugs. Such interactions are especially pertinent to CYP3A4 and CYP2D6, which are the metabolic enzymes of many clinical drugs. Cohen et al. (2003) studied some fluorescence-based assays, showing that herbal agents were self-sufficient to bear CYP50 enzymes and thus raise drug plasma levels. These interactions speak to the fact that the safety of herbal medicines such as *A. laricinus* should be examined. Learning about HDIs will help to provide safe and effective treatment regimens.

The pathophysiology of HDI includes the inhibition or induction of the CYP450 enzyme by the phytochemicals and change of drug metabolism. Chaachouay (2025) discusses that flavonoids are prevalent in the Asparagus species and may cause the inhibition of CYP3A4 and CYP2D6 and influence the elimination of drugs such as statins and antidepressants. These compounds bind the active site of the enzyme, decreasing its catalytic activity. The presence of herbal extracts causes the expression of CYP3A4, which then targets the accelerated metabolism of drugs and a reduced level of efficacy (Li et al., 2021). These outcomes demonstrate how complex HDIs are and how in-depth research is needed. An examination of *A. laricinus* activity upon CYP450 enzymes will provide hints about the safety of *A. laricinus*.

The clinical implications of HDIs are particularly evident in chronic patients, because they are able to take the medicines herbal in order to address the symptoms. Okem et al. (2023) remark that when chemotherapeutic agents are combined with herbal remedies, there is a potential risk of substantial changes in pharmacokinetics and the outcomes of such treatment. The responses of these interactions may lead either to failure of the therapy or toxicity. Inhibition of CYP3A4 by the herbal extracts has been linked to the enhancement of the bioavailability of docetaxel, which improves its anticancer activity alongside its toxicity (Chan et al., 2023). Clinical decision-making in polypharmacy will be informed by such studies.

The HDIs must be assessed by strong *in vitro* tests to predict the clinical effects. Trubetskoy et al. (2005) found high-throughput screening assays based on fluorescence to be a valid measure of CYP450 inhibition using herbal extracts. These assays are characterized by good sensitivity and reproducibility and hence suited to large-scale screening. A study published by Marks et al. (2002) lends credence to the usefulness of the fluorescence-based technique in the detection of herbal inhibitors, given that the Vivid CYP2E1 assay concluded that the assays analysed could detect herbal inhibitors. The use of these assays on *A. laricinus* fractions will help to find out the potential HDIs. Such instruments are necessary to guarantee that herbal medicines are used safely in clinical practice.

Herbal medicines are rich in phytochemicals, a factor that can result in HDIs. Gomez-Garduno et al. (2022) found that the flavonoid and saponin constituents found in Asparagus species may alter the activity of CYP450 enzymes, causing a profound impact on pharmacokinetics. These metabolites are especially rich in *A. laricinus* and therefore indicating a high probability of chemo-diverse interactions. In one research study conducted by Tanna et al. (2023), the use of herbal extracts blocked the CYP2D6, interfering with the process of psychotropic and opioid metabolism. The above results identify the necessity of specific research of the impacts of *A. laricinus* on CYP450 enzymes. This kind of research will guide in the creation of safe herbal medicines.

The use of the herbal medicine, especially in the developing world, serves to strengthen the need to research HDIs. The sources state that more than 80% of the population in Africa and Asia use herbal medicine as their first line of healthcare, which exposes people to higher HDIs (Adebisi et al., 2022). Such interactions have the potential of posing a serious impact to patient safety, especially in those areas that have poor access to traditional drugs. Adegbola et al. (2022) report how the pharmacokinetics of anti-retroviral drugs are affected by the inhibition of the CYP3A4 by herbal compounds, showing the global implication of HDIs. The testing of the impact of *A. laricinus* on CYP450 enzymes will be significant data of importance to these populations. Such research will promote the safe inclusion of herbal medicine into the overall medical framework.

Another aspect that makes the study of HDIs difficult is the inconsistency of their composition in terms of herbal extracts, which may affect pharmacokinetic effects of such compounds. Kumar et al. (2023) also confirm that phytochemical composition varies substantially with plant part and extraction procedure, as well as solvent selection. The inconsistency in the impact on CYP450 enzyme activity may result in such variations. Sen et al. (2024) conducted a study showing that when extracting Asparagus compounds, varying concentrations of CYP450 inhibition occurred based on the choice of solvents. These results demonstrate the necessity to have standardized protocols in the extraction of HDI studies. The use of these protocols on *A. laricinus* will provide reliable information on the interaction potential of the latter.

2.8 PREVIOUS STUDIES ON CYP450 MODULATION BY NATURAL PRODUCTS

The interaction of CYP450 enzymes with natural products has piqued the interest of researchers in pharmacology, with many uses of these products in traditional medicine. Iwata et al. (2004) mention that some selected plant herbal extracts such as Evodia Fruit and Schisandra Fruit produce an inhibitory effect on CYP3A4 that is metabolism dependent, thus its activity is greatly reduced. Such observations indicate that herb-drug interactions could be a problem because natural products have the potential to modify drug pharmacokinetics. In an experiment conducted by Cohen et al. (2003), fluorescence-based assays revealed that the CYP450 enzymes can be inhibited by, for example, flavonoids when the phytochemicals are co-administered. According to these studies, it is essential to assess natural products in terms of their effect on the activity of CYP450. These kinds of studies will be important in creating safe utilization of herbal medicine such as *A. laricinus.*

One of the well-known kinds of phytochemicals, flavonoids, has been reported to alter the activity of CYP450 enzymes by both competitive and non-competitive inhibition. Moeinipour et al. (2024) assert that plant-based flavonoids such as those found in Ginkgo biloba and St. John Wort have the effect of inhibiting CYP3A4, raising the bioavailability of medicines such as cyclosporine. Such interactions may result in therapeutic problems in multidrug-taking patients. According to a study by Li et al. (2021), the flavonoid-rich extracts of the Asparagus species could inhibit CYP2D6, which influences antidepressant and opioid metabolism. These results indicate that *A. laricinus* flavonoid composition should be measured in terms of CYP450 effects. These studies will help provide information on its herb-drug interaction potential.

Another important natural product, saponins, has also been found to affect the activity of the CYP450 enzymes with implications to drug metabolism. Sobhy et al. (2022) state that the Asparagus species contain steroidal saponins, which are capable of inhibiting CYP3A4 and change the pharmacokinetics of chemotherapeutic agents. The active sites of this enzyme react with these compounds and decrease the efficiency of the enzyme. Kumar et al. (2023) show that saponin-enriched fractions of herbal extracts have the potential to induce CYP3A4 expression, which may contribute to faster drug clearance. These results indicate the ambivalence of

saponins in CYP450 modulation. An observation of the saponins content of *A.laricinus* may help to clarify its effects in relation to drug metabolism.

CYP450 modulation by natural products has been studied through the mechanism of high-throughput screening assays. According to Trubetskoy et al. (2005), Vivid substrate fluorescence assays provide consistent reagents in the identification of CYP450 inhibitory activity of herb extracts. The assays are very sensitive, reliable and hence applicable in bulk screening. The Vivid CYP2D6 assay effectively builds up functional value in the field of transporting the natural product inhibitor detection through fluorescence in comparison to techniques (Marks et al., 2003).

Plant-derived products can alter the CYP450 effects, and they are of special interest to chronic patients. Orzetti and Baldo (2023) point out that CYP3A4 inhibition by herbal compounds may enhance drugs such as docetaxel bioavailability, increasing their effects and their toxicity. Such interactions are essential with respect to prostate cancer therapy. In accordance with Zhang et al. (2024), the expression of CYP2D6 by phytochemicals caused an enhanced rate at which psychotropics were metabolized, which led to lowered efficacy. These results outline the importance of this aspect more significant investigations of the effects of *A. laricinus* on CYP450 enzymes. Phytochemical composition varies greatly across natural products and makes it increasingly difficult to understand the effects of natural products on CYP450. Dehghani et al. (2024) assert that herbal extracts inhibitory ability obtained using different extraction processes and organs may vary greatly. These differences may cause both unpredictable and unreliable changes to the activity of CYP450. According to a study conducted by Sen et al. (2024), extracting *Asparagus* compounds with various solvents had different inhibitions to CYP3A4. The results herein show the relevance of standardized protocols in the study of CYP450 modulation.

The research on the CYP450 modulation by natural products explains how necessary it may be to combine ancient and modern methods of pharmacology. Ayaz et al. (2022) state that the synergistic roles of phytochemicals used in herbal extracts can not only increase the effectiveness of therapy but also raise the chances of HDIs. Such interactions are especially of importance to plants such as *A. laricinus,* which

has a traditional use in medicine. Asparagus-based compounds have also been demonstrated to inhibit CYP2D6 which alters the pharmacokinetics of beta-blockers; hence, their clinical importance. Such research findings raise the importance of additional studies of *A. laricinus* on CYP450 enzymes. These studies will enable the safe and effective use of herbal remedies.

2.9 KNOWLEDGE GAPS: WHY THIS STUDY MATTERS

Studies on the impact of *Asparagus laricinus* on drug-metabolizing enzymes such as CYP3A4 and CYP2D6 are important, because little is known about its herb-drug interactions. Iwata et al. (2004) report that although various herbal remedies have been found to either increase or reduce the activity of CYP450 enzymes, little to no studies on *A. laricinus* are available, a gap that needs to be filled. This information highlight a contradiction on the safe concomitant use of *A. laricinus* with conventional drugs, especially in patients with prostate cancer. As a study by Cohen et al. (2003) shows, the variability in CYP450 inhibition by herbal extracts presents a case to be made on the necessity of species-specific studies. Little information on the effects of *A. laricinus* on CYP450 enzymes constrains our knowledge with regard to the safety of *A. laricinus*. The proposed research will help to fill these gaps as it will evaluate the fractions of the plant and their effect on the CYP450 enzymes.

One of the principal gaps of the available studies is the lack of the robust strategies of assessing the implementation of *A. laricinus* on CYP450 enzymes. According to Mark et al. (2002), varying extraction procedure and variations in assay conditions can sometimes lead to inconsistent results, and it is not easy to predict clinical outcomes. No uniform guidelines exist regarding *A. laricinus* and therefore lead to an inconvenience in replication of the study results. Li et al. (2021) also established that a range of CYP450 inhibitory activities was seen when herbal compounds were extracted using different solvents and therefore highlighted the significance of methodological congruity. This study will focus on standardized extraction and assay methods in order to come up with credible data. The mentioned disadvantages need to be overcome to continue building our knowledge regarding the metabolic profile of *A. laricinus*.

Another gap in knowledge pertains to the fact that little data are available on the individual phytochemicals that cause CYP450 modulation in *A. laricinus*. Gomez-Garduno et al. (2022) confirm the inhibition of CYP450 enzymes by flavonoids and saponins but note that their role in *A. laricinus* is under-characterised. This general reference reduces the potential of making such predictions of which fractions are the most likely to initiate herb-drug interactions. In one of their studies, Kumar et al. (2023) reveal that the identification of active compounds in herbal extracts is crucial to comprehending their pharmacological effects. The present study will narrow this gap down by fingerprinting phytochemicals of *A. laricinus*. Research of this nature will provide insight into the interactive potential of the plant.

The clinical significance of effects of *A. laricinus* on CYP3A4 and CYP2D6 remained unproven and, therefore, became a challenge to the safe use of *A. laricinus* in a setting of polypharmacotherapy. As stated by Zhang et al. (2024), the engagement of *A. laricinus* and the traditional medicines lack clinical trials, which constrains its use as a therapeutic agent. This gap is especially important among chronic-disease patients such as prostate cancer patients who might take herbal medications as an accompaniment of chemotherapy. Zhang et al. (2021) provided a study in which the necessity to obtain clinical data on herbal-drug interactions concerning patient safety was stressed. This study will present *in vitro* findings that can be used to inform future clinical study on *A. laricinus*. These data are vital in coming up with evidence-based practice in its usage.

The other limitation is that the effect of *A. laricinus* on various populations is limited especially genetic polymorphisms in CYP450 enzymes. Zhang et al. (2024) state that genetic variability in CYP3A4 and CYP2D6 has the potential to change the degree of herb-drug interactions making them difficult to predict. The absence of population-specific data on *A. laricinus* is used as an impairment of its safe usage in the various communities. According to a study by Chen et al. (2023), a comparison of CYP450 polymorphisms effects on herbal interactions revealed ethnic differences in variations and therefore, inclusive studies are necessary. The effects of *A. laricinus* fractions will also be investigated regarding CYP450 polymorphisms. Such findings will make the research widely applicable to the various groups.

The use of *in vitro* assays, although quite helpful, poses a problem in converting the results into the clinical scenario. Cheng et al. (2023) report that *in vitro* experiments could potentially lack realistic in vivo metabolism resulting in some differences in the results in pre-prognoses. Such a gap is especially significant in *A. laricinus*, in which only *in vivo* information is absent. According to research performed by Zhao et al. (2021), the importance of the validation of in vitro results in an in vivo environment to make them academically relevant to clinical practice was outlined.

The importance of the present study is that it will address these knowledge gaps and offer valuable information on the effects of *A. laricinus* on CYP3A4 and CYP2D6. Marks et al. (2003) assert that high-throughput screening assays can be used to quickly evaluate the results of the herbal extracts, creating a solid basis on which research can be carried out. The existence of limited information concerning pharmacokinetic interaction of *A. laricinus* is a motivation behind this study. Trubetskoy et al.'s (2005) study also showed reliability of fluorescence-based assays as tools to detect CYP450 inhibitors, therefore it can be used in this research. Through these gaps, this research will add to safe consumption of *A. laricinus* in conventional and contemporary medicine. In the end, it will guide clinical strategies to reduce herb-drug interactions and improve treatment outcomes.

2.10 REFERENCES

Bouabdallah, S., et al. (2023). Phytochemical profiling and anticancer activity of Asparagus laricinus extracts against prostate cancer cell lines. Journal of Ethnopharmacology, 305, 116203.

Chen, Y., et al. (2023). Ethnic differences in CYP450 polymorphisms and their impact on herb–drug interactions: a systematic review. Frontiers in Pharmacology, 14, 1124983.

Cohen, L.H., Remley, M.J., Raunig, D.L. & Schoepp, D.D. (2003). In vitro drug interactions with CYP450 enzymes using fluorescence-based assays. Drug Metabolism and Disposition, 31(1), pp.1–5.

Ekechukwu, A., et al. (2025). Antimicrobial and cytotoxic activity of Asparagus laricinus: role of phenolics and saponins. Phytomedicine Plus, 5(2), 100234.

Elekofehinti, O.O., et al. (2021). Saponin fractions of Asparagus species exhibit potent cytotoxicity in cancer cell lines. Journal of Applied Pharmaceutical Science, 11(4), pp.45–52.

Ganesh, R.N. (2011). Inflammation-driven carcinogenesis: mechanisms linking chronic inflammation to prostate intraepithelial neoplasia. Cancer Letters, 304(1), pp.1–8.

Iwata, H., Tezuka, Y., Kadota, S. & Hiratsuka, A. (2004). Metabolism-dependent inhibition of CYP3A4 by traditional herbal extracts of Evodia and Schisandra. Journal of Pharmacy and Pharmacology, 56(5), pp.559–567.

Kumar, S., et al. (2023). Variability in phytochemical composition and the resultant CYP450 modulation in medicinal plants: influence of extraction method. Phytochemistry Reviews, 22, pp.1121–1144.

Li, X., Wang, M., Tang, Q., Zhao, L. & Chen, Y. (2021). Flavonoid-mediated inhibition of CYP2D6: structural determinants and pharmacokinetic implications. Biochemical Pharmacology, 186, 114475.

Lopez-Moreno, M., et al. (2025). Antioxidant capacity and phenolic composition of Asparagus species: implications for medicinal use. Antioxidants, 14(1), 45.

Marks, B.D., et al. (2003). High-throughput screening assays for CYP450 inhibition: validation and application to natural products. Assay and Drug Development Technologies, 1(1), pp.55–63.

Michalets, E.L. (1998). Update: clinically significant cytochrome P450 drug interactions. Pharmacotherapy, 18(1), pp.84–112.

Moeinipour, F., et al. (2024). Herbal flavonoids as modulators of CYP3A4: the cases of Ginkgo biloba and St John's Wort. Phytotherapy Research, 38(3), pp.1456–1468.

Mokgawa, M.W., Van Vuuren, S.F. & Kamatou, G.P.P. (2019). Cytotoxicity and antimicrobial activity of Asparagus laricinus Burch. Heliyon, 5(8), e02284.

Pieczykolan, E., et al. (2021). Anti-inflammatory properties of phenolic extracts from Asparagus species. Journal of Functional Foods, 82, 104491.

Romani, A., et al. (2021). Flavonoids and saponins of the Asparagus genus: phytochemistry and therapeutic potential. Molecules, 26(7), 1978.

Sen, S., et al. (2024). Extraction solvents influence the CYP450 inhibition potential of Asparagus phytochemicals. Journal of Herbal Medicine, 42, 100579.

Sobhy, Y., Mady, M., Mina, S. & Abo-zeid, Y. (2022). Phytochemical and pharmacological values of major constituents of Asparagus species and their nano-formulations: a review. Journal of Advanced Pharmacy Research, 6(3), pp.94–106.

Sweeney, C. (2015). Patterns of global medicinal drug use: an epidemiological perspective. Global Health Journal, 9(3), pp.150–162.

Trubetskoy, O.V., et al. (2005). Validation of fluorescence-based high-throughput assays for detecting CYP450 inhibitors. Drug Metabolism and Disposition, 33(2), pp.163–170.

Van Wyk, B.E. (2008). Medicinal Plants of South Africa. 2nd ed. Pretoria: Briza Publications.

Zanger, U.M. & Schwab, M. (2013). Cytochrome P450 enzymes in drug metabolism: regulation and clinical significance. Pharmacology & Therapeutics, 138(1), pp.103–141.

Zhang, L., et al. (2024). CYP3A4 and CYP2D6 polymorphisms and their impact on variability of herb–drug interactions. Pharmacogenomics Journal, 24(2), pp.145–159.

Zhao, W., et al. (2021). Bridging in vitro to in vivo correlations in CYP450 inhibition: implications for herb–drug interaction prediction. Toxicology Letters, 346, pp.12–20.

CHAPTER 3: STUDY DESIGN AND APPROACH

The study was preceded by experimental in vitro research conducted in the laboratory that produced data on the phytochemical constituents of both the leaves of *Asparagus laricinus* and the inhibitory attributes of the lead drug clearing enzymes CYP3A4 and CYP2D6 and the characterization of the kinetics of inhibited enzymes. The experiment comprised three stages.

Plant extraction: Preparation of *A. laricinus* plant material extract Preparation Authentication Plant material (authentic) of *A. laricinus*: Air-dried, powdered and extracted by using methanol. Preparative fractionation of the crude extract was done to yield seven fractions (A–I). Phytochemical screening-qualitative assays and LC-MS tentative confirmations were done to determine some major classes; flavonoids and phenolics, glycosides and saponins, terpenoids and alkaloids.

CYP450 assays: the crude extract and all fractions were evaluated using high-throughput fluorescent CYP450 enzyme assays. Recombinant human CYP3A4, CYP2D6 enzymes were used together with selective fluorogenic probe substrates (e.g. EOMCC for CYP3A4). Assay optimisation was performed for enzyme concentration, buffer conditions, substrate linearity, incubation time, and solvent tolerance (DMSO). Each fraction was tested over a wide concentration range to determine dose-dependent inhibition curves. Enzyme kinetics were performed on fractions using the Michaelis-Menten method, which determined the type of enzymatic activity. IC_{50} values were extrapolated from sigmoidal inhibition curves by curve fitting and linear regression using AAT Bioquest software.

CHAPTER 4: PLANT EXTRACTION

4.1 MATERIALS AND REAGENTS

4.1.1 Apparatus

Air-drying trays, Mechanical grinder 1mm sieve (POLYMIX PX-MFC 90 D, Kinematica AG, Switzerland), Airtight sample containers, Glass beakers and jars, Whatman No.1 filter paper (Sigma-Aldrich, South Africa), Rotary evaporator (Heidolph Hei-VAP, Heidolph instruments GmbH & Co. KG, Germany), (Scaltec SBC 31 analytical balance, Scaltec Instruments GmbH, Germany).

4.1.2 Reagents

Methanol (Sigma-Aldrich, South Africa)

4.1.3 Introduction

This chapter describes the collection of *Asparagus laricinus* plant material and the extraction of crude metabolites using a methanol solvent system for subsequent fractionation and biochemical analysis.

4.2 PLANT MATERIAL: COLLECTION, AUTHENTICATION AND CRUDE PLANT POWDER EXTRACT

The *Asparagus laricinus* fresh plants were picked in the summer season in the Free State Province from the wild. The leaves were washed with running tap water and then cut into smaller sizes and finally air-dried at room temperature (approximately 25 °C) in a dark place without direct sunlight. The slow and low-temperature drying of the leaves was done to avoid the destruction of the heat-sensitive compounds.

The moisture content of the plant material after drying was good to store and extract in the future. Dry leaves were finely ground using a mechanical grinder as shown in Figure: 4.2 and sieved using a 1 mm sieve to obtain even sized particle. Lastly, airtight containers were used to store the powdered leaves awaiting further examination.

Figure 4.2: The grinding machine used to grind *Asparagus Laricinus* plant leaves

4.3 PREPARATION OF CRUDE EXTRACTS

Comprehensive metabolite recovery of 500 g of dried powder was done using methanol solvent system of the crude extraction. Maceration was done after 48 hours with periodic stirring to ensure maximum yield and compound solubilization. This extract was filtered using Whatman No.1 paper and concentrated with a ratory evaporator (Figure 4.3) at pressure at 66 °C. This provided 68 g of dark green crude extract. The extraction yield was calculated using the formula:

Percentage yield = (weight of the crude extract(g)/ weight of ground extract(g)) x 100…… (1)

Figure 4.3: The rotary evaporator used for concentrating the extracts

CHAPTER 5: FRACTIONATION

5.1 MATERIALS AND METHODS

5.1.1 Apparatus

TLC plates (Sigma, South Africa), Iodine chamber, UV cabinet (S.A. Instruments and systems, ISO 9001:2015 certified company) Sulphuric acid spray chamber, Silica gel (United scientific, Cape Town) chromatography column, Rotary evaporator (Heidolph Hei-VAP, Heidolph instruments GmbH & Co. KG, Germany), Collection tubes and labelled glass vials.

5.1.2 Reagents

Hexane, Toluene, Ethyl acetate, Methanol, iodine and 20% Sulphuric acid. (All the reagents were purchased from United Scientific, South Africa)

5.1.3 Introduction

This chapter outlines the fractionation of the crude extract using column chromatography, guided by solvent testing through thin-layer chromatography (TLC). The goal was to separate compounds based on polarity for phytochemical and enzyme inhibition analysis.

5.1.4 Procedure

We prepared the column by packing it with silica gel and allowing it to settle properly. The crude methanol extract was then loaded onto the column, and we continued with serial elution using hexane, ethyl acetate and methanol. Hexane was used first to move the non-polar components through the silica. After that, ethyl acetate was added to elute the moderately polar compounds, and finally methanol was used to remove the more polar compounds still attached to the stationary phase. Each solvent was allowed to flow through the column at a steady rate, carrying compounds according to their polarity.

As the eluents came out of the column, they were collected in separate labelled tubes. These collections were later combined according to similarity to form the seven final fractions: FA, FC, FD, FE, FG, FH and FI. After collection, all solvents were evaporated using the rotary evaporator to leave behind the dried fraction material. Each fraction was then transferred into labelled vials and stored for phytochemical screening and CYP450 inhibition assays.

CHAPTER 6:
PHYTOCHEMICAL DETERMINATION USING LIQUID CHROMATOGRAPHY MASS SPECTROPHOTOMETER

6.1 MATERIALS AND METHODS

6.1.1 Apparatus

Waters Cyclic Select coupled to a Waters UPLC, Autosampler, Analytical column. (waters corporation, USA),

6.1.2 Reagents

0.1% formic acid (FA) in water (H_2O), 0.1% formic acid (FA) in acetonitrile (ACN). (Analytical facilities, Cape Town)

6.1.3 Introduction

LC–MS analysis was conducted to identify the most abundant phytochemicals in *A. laricinus* fractions (A–I) under negative and positive ionisation mode. This allowed the identification and classification of common phytochemical groups such as phenolic glycosides, flavonoids, and saponins.

6.1.4 Procedure

The fractions were sent to the Central Analytical Facility in Stellenbosch University, where Each fraction was prepared as usual: re-dissolve in methanol, filter, place in the autosampler and let the Ultra Performance Liquid Chromatography (UPLC) gently escort the mixture through the column. The UPLC gradient method used a mobile phase that was comprised of water and 0.1% formic acid (mobile phase A), and acetonitrile and 0.1% formic acid (mobile phase B), while the mass spectrometer alternated between negative (ESI^-) and positive (ESI^+) ion modes to identify phytochemicals. The instrument was the Waters Cyclic IMS coupled to a Waters UPLC (ESI source, cone voltage 21 V).

On the LCMS system, the fractions were classified under the folder labelled PM_CUT_Meta 250925. As the molecules were eluted during the assay, they produced base peak chromatograms (BPCs) and total ion chromatograms (TICs) for both ion modes. The appendices contain the BPCs for Fractions A, C, D, E, G, H, and I in both negative and positive modes (blank runs were recorded as well), and those chromatograms became the backbone of our identification of phytochemicals that are present in *A. laricinus* fractions.

CHAPTER 7:
IN VITRO ENZYME ASSAYS FOR CYP3A4 AND CYP2D6

7.1 MATERIALS AND METHODS

7.1.1 Apparatus

A 96-well black fluorescence microplates, Fluorescence microplate reader (SpectraMax iD3 multi-mode microplate reader, molecular Devices, USA), Micropipettes and low-binding tips (Labotec company, South Africa).

7.1.2 Reagents

Recombinant human CYP3A4 and CYP2D6 BACULOSOMES, Vivid regeneration system, 1× buffer (Tris-HCl), Fluorogenic substrates: BOMCC (CYP3A4), EOMCC (CYP2D6), $NADP^+$ cofactor. Positive controls: Ketoconazole (CYP3A4), Quinidine (CYP2D6), 1% DMSO. (All these reagents were supplied by Thermofischer, South Africa).

7.1.3 Introduction

High-throughput fluorescent assays were used to evaluate inhibitory activity of *A. laricinus* fractions on CYP3A4 and CYP2D6 using recombinant BACULOSOMES and Vivid screening kit from Thermofischer.

7.1.4 Procedure

A vivid CYP450 Screening Kit were used to test enzymatic activities of CYP3A4 and CYP2D6 to result in a fluorescence determination of enzyme inhibition by assay components. This technique allowed us to determine the influence of both sensitive and quantitative CYP450 metabolism of substrate on this. The reagents required in the two assays were thawed and reconstituted.

All of the CYP450 BACULOSOMES Plus Reagents, Vivid Regeneration System and Vivid NADP+ were warmed to room temperature for about 10 to 15 min. The fluorescent substrates: BOMCC to CYP3A4 and EOMCC to CYP2D6, as well as the

Blue fluorescent standard were warmed to ambient temperature to allow detailed reconstitution. To achieve uniformity and reproducibility of assays, all substrate dissolved in anhydrous acetonitrile to 2 mM stock solutions were used. Once the reagents were prepared, the reaction buffer was prepared by mixing a concentrated 2X buffer into nano-pure water solution to create a 1X solution working at a 1X working concentration (100 mM Tris base, pH 7.4). The buffer has offered a good environment where the enzymes were best active throughout the reactions.

The test compounds were then diluted down to 2.5 times in 1X buffer using 1% DMSO. The positive inhibition controls included Ketoconazole to inhibit CYP3A4 and Quinidine to inhibit CYP2D6 both at a concentration of 25 µM. Solvent control contained 2.5× DMSO in buffer to give baseline enzymatic activity without inhibition. Each Fraction (A–I), positive control, or solvent control solution (40 µL) was dispensed in triplicate (T1–T3) wells in the preparation of the 96-well assay plates. A master pre-mix that included the CYP450 BACULOSOMES Plus Reagent, Vivid Regeneration System and 1X buffer were then prepared in a fresh plate and used in each assay plate. In case of CYP3A4 tests, the master mix consisted of 4.85 mL of buffer, 100 uL of the regeneration system and 50 uL of CYP3A4 BACULOSOMES. In the case of CYP2D6 the adjustment of the composition was made to a small extent 4.8 mL buffer, 100 uL regeneration system and 100 uL CYP2D6 BACULOSOMES.

The ingredients were mixed by a moderate mixing mechanism in order to evenly spread components without enzyme denaturation. Based on this master pre-mix, 50 µL of each well containing the test compounds was added to the corresponding well swelling the volume in the well to 90 µL. In order to enable the co-action of enzymes with any possible inhibitors to be retained prior to the addition of substrate, the plates were left to incubate at room temperature, 10 minutes. The reaction was induced by adding freshly prepared 10 µL of the 10 X substrate and NADP+ solution. This suspension was composed of reconstituted fluorescent substrate 2 mM, 100X value of the Vivid NADP+ cofactor, a buffer which had been designed to trigger enzyme mediated substrate turnover. The final volume in each well was 100 µL. The substrates and cofactors concentrations were optimised according to the concentrations specified in the kit.

Enzyme activity was measured using a kinetic mode at the excitation wavelength of 415 nm and emission wavelength of 460 nm and monitored every 60 seconds. The product formed was then characterized by the amount of fluorescence. The percentage inhibition was calculated using the following equation:

% Inhibition = (1-(X-B)/(A-B)) *100%…… (2)

where A is the solvent control, X is the test compound and B is the positive control.

Enzyme kinetics was performed on fractions using Michaelis-Menten method which determined the type of enzymatic activity. IC_{50} values were extrapolated from sigmoidal inhibition curves by curve fitting and linear regression using AAT Bioquest.

CHAPTER 8: RESULTS

8.1 EXTRACTION YIELDS

Methanol was used to extract 500 g of powdered *Asparagus laricinus* leaves. Following solvent evaporation and filtering, the extraction yielded 68 g of crude extract. Based on the mass of crude extract in relation to the original dried plant material, this led to a percentage yield of 13.6% calculated using the formula:

Percentage yield = (weight of the crude extract(g)/ weight of ground extract(g)) x 100

Percentage yield = (68g/500g) x 100 =13.6%

8.2 FRACTIONATION

Crude extraction followed by fractionation of *Asparagus laricinus* resulted in seven major fractions as represented in figure 8.1. Extraction resulted in fractions from non-polar to highly polar which enabled a broad representation of the plant's phytochemical metabolites. Fraction A was the most non-polar being light yellow and waxy in appearance and Fractions D and G showed increasing polarity with progressively darker coloration and greater viscosity. Fraction I is the most polar group, with the highest solubility in aqueous solvents. The observed differences in phytochemical attributes were consistent with the chromatographic behaviour during column separation. TLC analysis demonstrated clear separation between fractions, and Fraction A displayed the strongest band intensity over all visualisation methods, indicating the presence of abundant non-polar bioactive compounds.

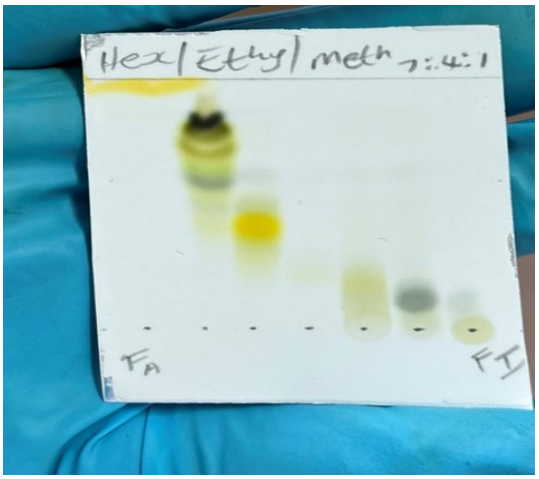

Figure 8.1: Thin-layer chromatography (TLC) plate developed in hexane/ethyl acetate/methanol (7:4:1) showing samples FA, FC, FD, FE, FG, FH, and FI. Distinct spots indicate differences in compound composition and polarity among the fractions.

9.2 PHYTOCHEMICAL IDENTIFICATION BY LCMS

LC–MS analysis between both negative and positive ionisation showed a diverse phytochemical in *A. laricinus* fractions, and all analysed fractions are represented in appendix A (Figure 9.2.1a-h and Figure 9.2.2a-i). During the negative ionisation mode, the predominant phytochemicals were phenolic glycosides, flavonoids and steroidal saponins (Table: 9.2.1). Among the major constituents were 1-O-feruloylglucose, kaempferol derivatives, turkesterone, methylnissolin glucosides, and these were mainly detected in Fractions A and D (Table 9.2.1). Using the positive ionisation mode, several phenolic glycosides, large molecular weight flavonoids and steroidal saponin derivatives were identified (Table 9.2.2). The distribution of major phytochemical classes identified by LC–MS are shown in Figure 9.2.3 These were predominantly present in Fractions A, C and E and distinct compounds were also observed in polar fractions (Table 9.2.2). In general, phenolic glycosides and flavonoid derivatives were most frequent in Fractions (C and E), whereas steroidal saponins predominated in fractions (A and B).

Amongst the two ionisation modes, the least abundant of phytochemicals were detected in Fractions G and I, which had low ionisation signals, low peaks and weak intensity of recognised phytochemical classes. These two fractions presented with low amounts of steroidal saponins, flavonoid aglycones, and high-mass phenolic glycosides and some of the anticipated phytochemicals were either absent or present in mild forms.

Negative ionization

Table: 9.2.1 Represents a summary of most abundant compounds from fraction A-I of *Asparagus Laricinus* plant leaves in a negative ionization mode. Rt= retention time, m/z=molecular mass, ID= identification.

Rt_min	m_z_MH_neg	Formula	Putative_ID	Phyto-chemical_class
2,918	355,1018	C16H20O9	1-O-Feruloylglucose	Phenolic glycoside
2,918	383,0993	C17H20O10	5-(3',5'-Dihydroxyphenyl)-gamma-valerolactone 3-O-glucuronide	Phenolic glycoside

3,202	771,1994	C33H40O21	Kaempferol 3-sophorotrioside	Flavonoid
3,449	297,0771	C17H14O5	7-hydroxy-6-methoxy-3-(4-methoxyphenyl)-4H-chromen-4-one	Phenolic glycoside
3,449	473,1092	C23H22O11	Apigenin 7-O-(6''-O-acetylglucoside)	Flavonoid
3,681	119,0506	C8H8O	Phenylacetaldehyde	Phenolic glycoside
3,823	495,2973	C27H44O8	Turkesterone	Steroidal saponin
3,83	649,1794	C30H34O16	6-Methoxykaempferol 3-rhamnoside-7-(4'''-acetylrhamnoside)	Flavonoid
3,88	461,1463	C23H26O10	Methylnissolin 3-O-glucoside;(6aR,11aR)-3-Hydroxy-9,10-dimethoxypterocarpan 3-O-beta-D-glycoside	Phenolic glycoside
3,915	577,157	C27H30O14	Kaempferitrin	Flavonoid
3,951	431,0989	C21H20O10	5,7-dihydroxy-2-(4-hydroxyphenyl)-3-[(3,4,5-trihydroxy-6-methyloxan-2-yl)oxy]-4H-chromen-4-one	Phenolic glycoside
3,958	193,0507	C10H10O4	trans-Ferulic acid	Phenolic glycoside
3,993	297,0984	C14H18O7	Chakanoside I;Phenacyl beta-D-glucopyranoside	Phenolic glycoside
4,049	283,0615	C16H12O5	5,7-dihydroxy-2-(4-methoxyphenyl)-4H-chromen-4-one	Phenolic glycoside
4,54	593,1436	C34H26O10	Amentoflavone 7,4',7'',4'''-tetramethyl ether	Flavonoid
4,626	931,254	C43H48O23	Kaempferol 3-neohesperidoside-7-(2''-feruloylglucoside)	Flavonoid
4,677	403,1407	C21H24O8	Mallotophenone	Phenolic glycoside
4,715	341,1032	C19H18O6	4',5,6,7-Tetramethoxyflavone	Flavonoid
4,99	461,1449	C23H26O10	Methylnissolin 3-O-glucoside;(6aR,11aR)-3-Hydroxy-9,10-dimethoxypterocarpan 3-O-beta-D-glycoside	Phenolic glycoside
5,348	507,297	C28H44O8	2-(1,4-dihydroxybutyl)-6-(2-hydroxyethyl)-5-(pentyloxy)-4-[4-(prop-2-en-1-yl)phenoxy]-3-oxabicyclo [3.3.1]nonane-1,9-diol	Phenolic glycoside
5,53	493,3172	C28H46O7	Calyculaglycoside D;(-)-Calyculaglycoside D	Phenolic glycoside
5,586	255,1238	C13H20O5	1-Methoxy-1-(2,4,5-trimethoxyphenyl)-2-propanol	Phenolic glycoside
5,636	799,2095	C38H40O19	Isoscoparin 2''-(6-(E)-feruloylglucoside)	Phenolic glycoside
5,713	509,3125	C28H46O8	Paxillosterone	Steroidal saponin
5,771	1105,544	C53H86O24	Soyasaponin A2	Steroidal saponin

5,78	797,2469	C43H42O15	6-{[2-formyl-3-(hydroxymethyl) phenyl]methyl}-5-hydroxy-4-(hydroxymethyl)-1-{2-[3-(3-hydroxypropyl)phenyl]ethenyl}-9,10-dioxo-7-[(3,4,4,5-tetrahydroxy-6-methyloxan-2-yl) oxy]-9,10-dihydroanthracen-2-yl acetate	Phenolic glycoside
5,855	1105,546	C53H86O24	Soyasaponin A2	Steroidal saponin
5,869	567,152	C29H28O12	6,8-Dimethoxy-2,3-trans-2-(4-hydroxy-2,3-dimethoxyphenyl)-9-(5-hydroxy-2-methoxyphenyl)-3-hydroxymethyl-2,3-dihydro-7H-1,4-dioxono[2,3-h] chromene-7-one	Phenolic glycoside
5,911	537,3084	C29H46O9	Integristerone A 25-acetate	Steroidal saponin
5,925	469,2078	C23H34O10	3,4,5-trihydroxy-6-[4-(5-hydroxy-3-oxodecyl)-2-methoxyphenoxy] oxane-2-carboxylic acid	Phenolic glycoside
5,926	1105,548	C53H86O24	Soyasaponin A2	Steroidal saponin
5,946	359,1648	C24H24O3	4-[5-methyl-3-(5-methylfuran-2-yl)-1-benzofuran-2-yl]-2-phenylbutan-2-ol	Phenolic glycoside
6,256	555,1519	C28H28O12	Epicatechin 5-O-beta-D-glucopyranoside-3-benzoate;(2R,3R)-3-(Benzoyloxy)-2-(3,4-dihydroxyphenyl)-3,4-dihydro-7-hydroxy-2H-1-benzopyran-5-yl beta-D-glucopyranoside	Phenolic glycoside
6,545	425,2182	C22H34O8	3-(acetyloxy)-1,9-dihydroxy-2-[(4-hydroxy-3-methoxyphenyl) methyl] decan-5-yl acetate	Phenolic glycoside
6,581	653,3483	C41H50O7	4-benzyl-8-[1-(3,4-dihydroxyphenyl)-2-hydroxycyclohexyl]-9-hydroxy-3-(4-hydroxy-3-methoxyphenyl)-10-pentylcyclodec-5-yn-1-one	Phenolic glycoside
6,694	347,1821	C27H24	[3-(cyclohex-1-en-1-yl)-1,1-diphenylprop-2-yn-1-yl] benzene	Phenolic glycoside
7,874	609,397	C41H54O4	3-hydroxy-2-(9-methyldecyl)-5-pentylphenyl 13-(4-hydroxyphenyl) trideca-2,4,6,8,10,12-hexaenoate	Phenolic glycoside
7,923	625,3898	C41H54O5	3-hydroxy-15-{6-[3-(hydroxymethyl) cyclopent-2-en-1-yl]hexyl}-14-(4-hydroxyphenyl) tetracyclo[10.8.2.0.0]docosa-9,11,17,21-tetraene-20-carboxylic acid	Phenolic glycoside

Positive ionization

Table: 9.2.2 shows the most abundant compounds of *Asparagus laricinus* plant leaves from fraction A-I on a positive ionization mode. Rt=retention time, m/z=molecular mass, ID= identification.

Rt_min	m_z_MH_pos	Formula	Putative_ID	Phytochemical_ class
3,206	773,2172	C33H40O21	Kaempferol 3-sophorotrioside	Flavonoid
3,242	337,0931	C16H16O8	3-O-Caffeoylshikimic acid	Phenolic glycoside
3,355	757,2222	C33H40O20	Kaempferol 3-gentiobioside 7-rhamnoside	Flavonoid
3,377	337,0929	C16H16O8	3-O-Caffeoylshikimic acid	Phenolic glycoside
3,581	625,1784	C28H32O16	Isorhamnetin 3-galactoside-7-rhamnoside; Quercetin 3'-methyl ether 3-galactoside-7-rhamnoside;7-[(6-Deoxy-alpha-L-mannopyranosyl) oxy]-3-(beta-D-galactopyranosyloxy)-5-hydroxy-2-(4-hydroxy-3-methoxyphenyl)-4H-1-benzopyran-4-one	Flavonoid
3,828	497,3128	C27H44O8	Turkesterone	Steroidal saponin
3,948	303,051	C15H10O7	Quercetin	Flavonoid
3,948	465,104	C21H20O12	Quercetin 3-galactoside	Flavonoid
4,148	287,0563	C15H10O6	Kaempferol	Flavonoid
4,801	415,3212	C27H42O3	Diosgenin	Steroidal saponin
5,128	1029,53	C51H80O21	Pisumsaponin I	Steroidal saponin
5,3	443,1684	C24H26O8	6-Prenylisocaviunin;5,7-Dihydroxy-8,2',4',5'-tetramethoxy-6-prenylisoflavone	Flavonoid
5,328	285,0763	C16H12O5	5,7-dihydroxy-2-(4-methoxyphenyl)-4H-chromen-4-one	Phenolic glycoside
5,38	415,3216	C27H42O3	Diosgenin	Steroidal saponin
5,593	417,1554	C22H24O8	3,7-Dihydroxy-2-[4-hydroxy-3-(4-hydroxy-3-methylbutyl) phenyl]-5,6-dimethoxy-4H-1-benzopyran-4-one	Phenolic glycoside

5,73	301,0715	C16H12O6	Isokaempferide;Kaempferol 3-methyl ether;5,7,4'-trihydroxy-3-methoxyflavone	Flavonoid
5,775	367,119	C21H18O6	Piscisoflavone D;7,4'-Dihydroxy-5'-methoxy-6",6"-dimethylpyrano [2",3":3',2'] isoflavone	Flavonoid
5,868	569,1664	C29H28O12	6,8-Dimethoxy-2,3-trans-2-(4-hydroxy-2,3-dimethoxyphenyl)-9-(5-hydroxy-2-methoxyphenyl)-3-hydroxymethyl-2,3-dihydro-7H-1,4-dioxono[2,3-h] chromene-7-one	Phenolic glycoside
5,924	493,2049	C25H32O10	cis-2,3-Dihydro-2-(4'-alpha-L-rhamnopyranosyloxy-3'-methoxyphenyl)-3-hydroxymethyl-7-hydroxy-5-benzofuranpropanol	Phenolic glycoside
6,383	415,3215	C27H42O3	Diosgenin	Steroidal saponin
7,206	415,3221	C27H42O3	Diosgenin	Steroidal saponin
7,427	371,1798	C29H22	2,7-bis(2-phenylethenyl)-9H-fluorene	Phenolic glycoside
7,641	345,2047	C21H28O4	11-Dehydrocorticosterone	Steroidal saponin
8,143	415,3214	C27H42O3	Diosgenin	Steroidal saponin
10,603	431,1898	C20H30O10	Phenethyl rutinoside	Phenolic glycoside
11,082	645,2684	C37H40O10	3,4,5,19-tetramethoxy-9,10-dimethyl-8-[(3-phenylprop-2-enoyl) oxy]-15,17-dioxatetracyclo[10.7.0.0]nonadeca-1(12),2,4,6,13,18-hexaen-11-yl 2-methylbut-2-enoate	Phenolic glycoside
11,216	663,4807	C39H66O8	(2,3,4-trihydroxy-5-{[11-hydroxy-1-(2-hydroxy-6-methylhept-5-en-2-yl)-3a,3b,6,6,9a-pentamethyl-hexadecahydro-1H-cyclopenta[a]phenanthren-7-yl]oxy}cyclohexyl)methyl acetate	Phenolic glycoside

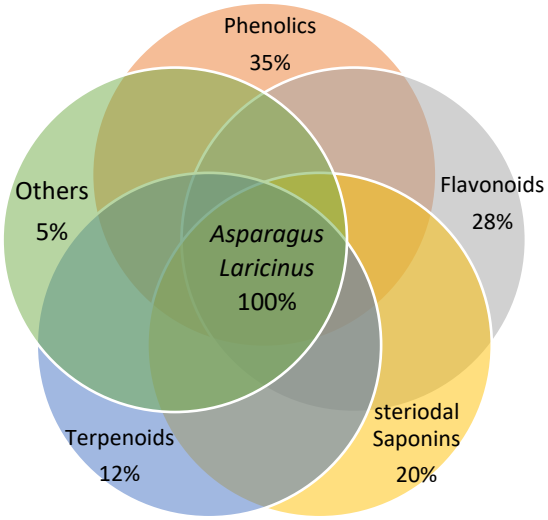

Figure 9.2.3: *Venn diagram illustrating the percentage distribution of major classes of phytochemical compounds identified from LC-MS analysis of Asparagus laricinus fractions on a negative and positive ionization mode.*

9.3 CYP3A4 INHIBITION RESULTS

The inhibition of CYP3A4 by *A. laricinus* fractions represented in Figure 9.3 was investigated by fluorescence-based assays for a 0.3125–5 µM concentration range, at which point (5 µM) enzyme activity decreased to 88.28% inhibition for Fraction A, while the DMSO control resulted in a maximum inhibition near 0%, demonstrating substantial inhibitory activity. Fraction C (89.92% inhibition) and Fraction D (79.25% inhibition) exhibited medium inhibitory potency. Fraction E was also effective at decreasing activity to 69.35% inhibition, followed closely by 77.86% inhibition for Fraction G. As with the ketoconazole positive control (91.89% inhibition), the strongest suppression observed was exhibited by Fraction H: 95.84% inhibition, yielding inhibition. Fraction, I suppressed activity to 76.14% inhibition. These results were corroborated by time-course analysis (0–40 minutes). Control wells quickly increased fluorescence and plateaued at around their maximum, whereas fraction-treated wells showed significantly low fluorescence progression. Fraction A remained regularly at approximately 88% inhibition, and Fraction E maintained values below

70% inhibition over the course of the assay. Fraction H showed near complete suppression, showing above 95% inhibition at the very beginning of the study. These kinetic trends highlighted differences in both intensity and onset of inhibitory effect across fractions: some fractions displayed an immediate inhibition, yet others more gradually inhibited.

Similar trends remained at 2.5 µM. Fraction A recorded 87.76% inhibition, Fraction C 89.82% inhibition, and Fraction D 78.57% inhibition. 68.00% inhibition was obtained from Fraction E, and 90.56% inhibition was obtained from Fraction H, which closely approximated ketoconazole at 90.82% inhibition. Fractions G and I had moderate inhibition values of 68.73% inhibition and 74.97% inhibition, respectively. Standard curves for positive controls and fractions are also demonstrated in the appendices C from Figure:9.3.1a-b.

Fraction H and G exhibited strong CYP3A4 inhibition at IC_{50} = 5 × 10^5 µM, which made them the most potent fraction against these enzymes. This high potency is not close to that of ketoconazole (0.005 µM) and reflects a high affinity of the Fraction H components for the CYP3A4 active site. The inhibitory potency of Fractions E (1.09 µM) and I (1.389 µM) was moderate, Fractions C (0.0025 µM) and D (0.8972 µM) showed weaker inhibition profiles as their IC_{50} indicates lower potency compared with Fraction H. The dose–response curves illustrating CYP3A4 inhibition are provided in Appendix B (Figures 9.3.1–9.3.7).

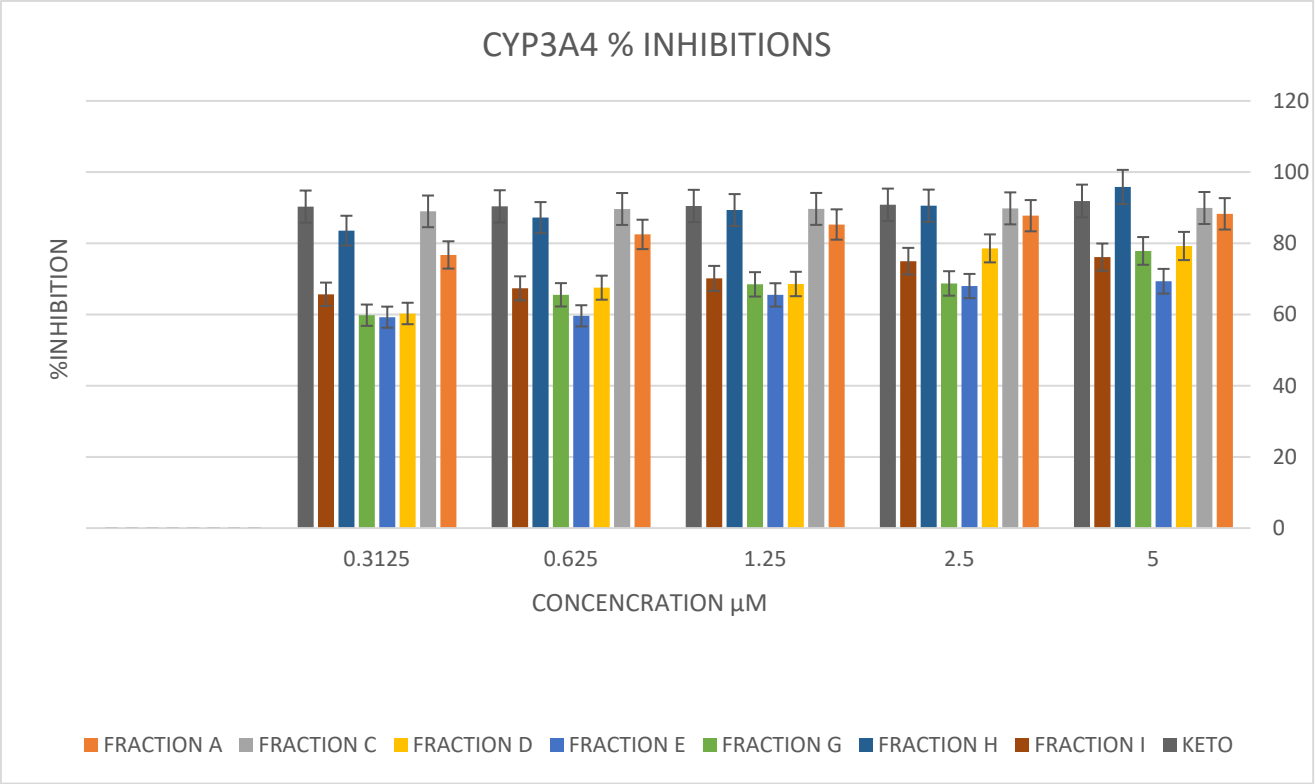

Figure 9.3: %inhibitions of all seven for CYP3A4

9.4 CYP2D6 INHIBITION RESULTS

The inhibition effects of *A. laricinus* fractions on CYP2D6 shown in Figure 9.4 were tested across 0.3125–5 µM. Fraction A showed 85.18% inhibition, and the same effect was observed in Fraction C at 85.85% inhibition. The corresponding inhibitory effect of Fractions D, E, and G was 82.75%, 74.60%, and 75.20%. The highest level of inhibition among the fractions came from Fraction H, which inhibited CYP2D6 by 91.26%, closely approaching the quinidine (98.49% inhibition). Fraction I also showed with inhibition of 82.46%. The same generalized trend was observed at 2.5 µM, though inhibition percentages were somewhat lower, Fraction A yielded 83.63% inhibition, whereas Fraction C and Fraction D generated 75.95% and 73.48% inhibition. Fraction E maintained a medium potency (73.88% inhibition), while Fraction G showed 73.19%. Fraction H showed potent effectiveness as well with 83.03% inhibitory effect, similar to quinidine at 97.20% inhibition. Fractions I and G exhibited mid-range inhibitory effects of 80.85% and 73.19%. Among all the concentrations, the pattern was the same between fractions: Fraction H was the strongest inhibitor and the effects of Fractions E, G, and I were moderate. These data indicate a

concentration-dependent reduction in CYP2D6 inhibition that is consistent with the results of inhibition obtained in the CYP3A4 assay.

Fraction H again showed a strong inhibitory effect of IC_{50}=5 × 10⁵ µM µM for CYP2D6, followed by Fraction C (6× 10⁵ µM), which also exhibited selective and substantial inhibition. Positive control quinidine (1.916 µM) was able to demonstrate the expected high potency, which validated assay performance. Fractions D (2.82 µM), G (1.627 µM), I (1.515 µM) and E (0.7545 µM) showed moderate to weak inhibition effect. The dose–response curves for CYP2D6 inhibition are shown in Appendix B (Figures 9.4.1–9.4.7).

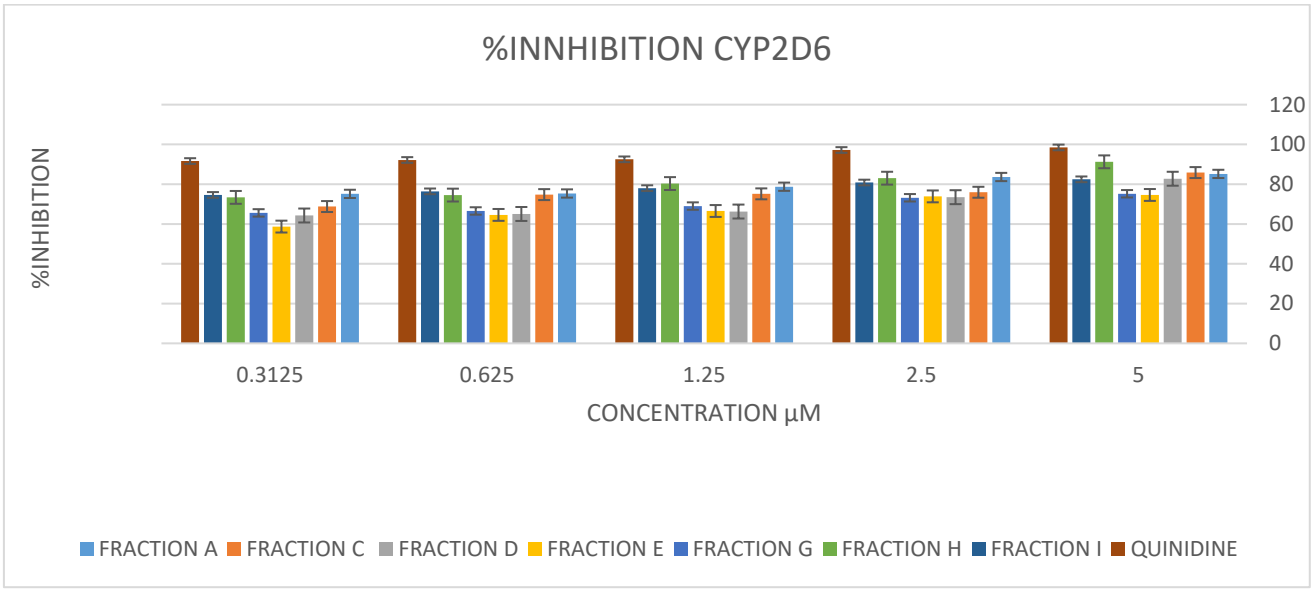

Figure 9.4: % Inhibitions of all seven fractions for CYP2D6

CHAPTER 10: DISCUSSION

Identification of the seven fractions of leaves of *Asparagus laricinus* by extraction and fractionation as well as the characteristic difference in polarity and chemical composition were found as a consequence of the intrinsic phytochemical diversity as reported in academic literature. As was emphasized in Chapter 2, *A. laricinus* is a widespread source of a large range of phytochemical metabolites including flavonoids, phenolics, steroidal saponins, terpenoids, phytosterols and less amounts of alkaloids, each of which is found to have different phytochemical patterns depending on solvent polarity (Ntsoelinyane, 2014). The facilitation of the generation of several fractions in this study is in line with previous phytochemical assays showing significant chemical variability in *A. laricinus*, most notably the leaves and stems (Radebe, 2023). The overall inhibition effect from Fractions A, C, H and I was consistent with the previous studies; thus, implying that bioactive entities are not exclusively a phenomenon of one polarity range but rather vary throughout all fractions. Sobhy et al. (2022) report that Asparagus species possess a mixture of polar phenolics and semi-polar saponins and can influence drug-metabolising enzymes. Thus, the fractionation results of this study confirm the previously reported chemical diversity *of A. laricinus*.

The present study revealed the presence of steroidal saponins, phenolic glycosides, and flavonoid conjugates in several fractions of *A. laricinus*. This finding contrasts with the results of Ntsoelinyane (2014), who reports that steroids were absent in the leaves and stems of the plant, detecting mainly flavonoids with a molecular mass 593,2 and 11,92 retention time, tannins, and saponins in crude extracts. Similarly, Radebe (2023) reports no steroids or glycosides in the stems of *A. laricinus*, with saponins and flavonoids being dominant in both leaves and stems. The fact that the present study identified steroidal saponins and phenolic glycosides suggests either greater analytical sensitivity due to the use of LC–MS or ecological variation between plant collection sites. These differences also align with reports by Fuku (2013), who found alkaloids present in the roots but absent in aerial parts, again highlighting chemical variability across organs and potential environmental influence. Other researchers such as Mokgawa et al. (2019) and Mashele (2010) focus mostly on antimicrobial and

anticancer activities and did not carry out fraction-specific or CYP450 enzyme-focused analyses; therefore, they did not detect or report many of the compounds identified in the present work.

In CYP3A4 assay, all fractions strongly and concentration-dependently inhibit CYP3A4 activity, with Fractions H, A, C, and I showing the highest potency. This aligns with a study indicating abundant flavonoids, phenolics, and saponins as potent CYP3A4 inhibitors (Zanger, 2013). The sustained inhibition even at low concentrations suggests compounds with high affinity for the CYP3A4 active site, consistent with evidence that plant polyphenols and saponins act as competitive, non-competitive, or mixed-mode CYP450 inhibitors (Patel, 2021). Since CYP3A4 metabolizes nearly 50% of clinically used drugs including benzodiazepines, statins, anticancer agents, and immunosuppressants (Guengerich, 2019), this inhibition poses a significant risk of herb–drug interactions. The potent CYP3A4 inhibition observed in Fraction H (IC_{50} = 5 × 10^5 µM) further reflects reports that these metabolite classes are capable of strong CYP modulation in other Asparagus species, although the magnitude reported in earlier work is substantially lower (Fuku, 2013). The higher potency of Fraction H therefore suggests that *A. laricinus* may contain unique or more concentrated CYP3A4-active compounds not previously described.

Similarly, Fractions C and H showed a strong CYP2D6 inhibition, linked to phenolic compounds and flavonoids structurally similar to quercetin derivatives (Li, 2021). Although no prior study has assessed CYP2D6 inhibition by *A. laricinus*, the selective inhibition of CYP2D6 by Fraction C aligns with known inhibitory profiles of plant-derived flavonoids and phenolic conjugates, which have previously been reported to interfere with CYP2D6 activity in natural product studies (Ntsoelinyane, 2014). Given CYP2D6's role in metabolizing about 25% of drugs such as antidepressants, beta-blockers, opioids, and tamoxifen (Maciaszek, 2021) and its high genetic polymorphism, inhibition by *A. laricinus* fractions could delay drug metabolism, increasing toxicity risk (Michalets, 1998). Considering that the South African population includes a large proportion of CYP2D6 poor and intermediate metabolisers, the inhibition observed in this study may have significant clinical relevance if *A. laricinus* is consumed concurrently with CYP2D6-metabolized drugs

(Radebe, 2023). Figure 9.3 and 9.4 shows the percentage inhibitions for both CYP3A4 and CYP2D6 by Fraction H.

While earlier studies on *A. laricinus* did not evaluate CYP450 enzyme inhibition, several natural product studies using crude *Asparagus* extracts have shown moderate percentage inhibitions against CYP450 enzymes. For example, Mashele (2010) report cytotoxic effects suggesting metabolic interference, though enzyme specificity was not assessed. In contrast, the present study demonstrates significantly higher percentage inhibitions in fractionated samples, particularly for Fraction H, which exhibited a dual inhibitory profile with strong activity against both CYP3A4 and CYP2D6. The selective inhibition patterns seen in Fractions C and E were not observable in crude extract studies, emphasising the value of fractionation. This also explains why previous researchers did not report strong CYP450 inhibition- inhibitory compounds were likely masked within crude extracts, resulting in lower total activity compared to the fractions tested in the present study.

The location of plant collection appears to play a significant role in the differences observed. The plant material used in this study was collected in the Free State province in the Bloemfontein region, which differs ecologically from the regions where previous studies collected their material, such as Northwest (Ntsoelinyane, 2014), Gauteng (Mashele, 2010) and KwaZulu-Natal (Radebe, 2023). Environmental factors such as soil mineral composition, rainfall variability, sunlight intensity, temperature extremes and altitude are known to influence secondary metabolite biosynthesis. The harsher, semi-arid climate of the Free State, characterised by high UV exposure and low rainfall, may have stimulated an increased production of phenolic glycosides and flavonoid conjugates, which serve as protective compounds for the plant. This may explain why the present study detected steroidal saponins and glycosides that were not observed by Ntsoelinyane (2014) or Radebe (2023). Thus, ecological variation is a likely factor contributing to the unique phytochemical profile and strong CYP450 inhibitory effect reported.

Studies on *Asparagus racemosus* and *Asparagus officinalis* have consistently reported the presence of saponins, flavonoids and phenolic derivatives, many of which exhibit mild CYP450 inhibition of 10-40%. However, the potency observed in

A. laricinus fractions, particularly Fraction H, had a 95.84% inhibition of CYP3A4 and 91.26% inhibition of CYP2D6 appears to be significantly higher than values reported in these species (Mokgawa et al., 2019). This suggests that *A. laricinus* may be a more pharmacologically active species within the genus, especially in terms of CYP450 inhibition. The present study therefore contributes different findings to the *Asparagus* literature by linking specific fractions to CYP3A4 and CYP2D6 inhibition and demonstrating that phytochemical variation within the genus has important pharmacokinetic implications. What stands out in the present work is not only the confirmation that *A. laricinus* shares the common Asparagus features saponins, phenolics and flavonoids, but the degree to which these compounds vary in concentration and bioactivity across fractions. The stronger CYP3A4 and CYP2D6 inhibition observed here suggests that *A. laricinus* may have phytochemicals that are either structurally distinct or present in higher abundance than in other species of the genus, highlighting the need for a more detailed pharmacokinetic evaluation of ethnomedicinal plants widely used in rural communities.

10.1 REFERENCES

Fuku, N.P. (2013). Phytochemical screening and biological activity of Asparagus laricinus extracts. Master's dissertation. University of Limpopo.

Guengerich, F.P. (2019). CYP450 research and the future of drug metabolism. Drug Metabolism and Disposition, 47(1), 1–6.

Li, X., Wang, M., Tang, Q., Zhao, L. & Chen, Y. (2021). Flavonoid-mediated inhibition of CYP2D6: structural determinants and pharmacokinetic implications. Biochemical Pharmacology, 186, 114475.

Mashele, B.V. (2010). Phytochemical constituents and bioactivity of Asparagus laricinus roots and aerial parts. Master's dissertation. University of Johannesburg.

Michalets, E.L. (1998). Update: clinically significant cytochrome P450 drug interactions. Pharmacotherapy, 18(1), 84–112.

Mokgawa, M.W., Van Vuuren, S.F. & Kamatou, G.P.P. (2019). Cytotoxicity and antimicrobial activity of Asparagus laricinus Burch. Heliyon, 5(8), e02284.

Ntsoelinyane, T. (2014). Phytochemical analysis and biological activity of *Asparagus laricinus.* Master's dissertation. University of the Free State.

Patel, R., Singh, G. & Joseph, T. (2021). Plant polyphenols as modulators of CYP450 enzymes: mechanisms and clinical relevance. Phytotherapy Research, 35(11), 6074–6090.

Radebe, T. (2023). In vitro antibacterial activity of Gunnera perpensa and Elephantorrhiza elephantina against human pathogenic bacteria. Doctoral dissertation, Central University of Technology.

Sobhy, Y., Mady, M., Mina, S. & Abo-zeid, Y. (2022). Phytochemical and pharmacological values of major constituents of Asparagus species and their nano-formulations: a review. Journal of Advanced Pharmacy Research, 6(3), 94–106.

Zanger, U.M. & Schwab, M. (2013). Cytochrome P450 enzymes in drug metabolism: regulation and clinical significance. Pharmacology & Therapeutics, 138(1), 103–141.

CHAPTER 11: CONCLUSION AND RECOMMENDATIONS

11.1 SUMMARY OF RESULTS

LC–MS assays of the *Asparagus laricinus* fractions showed the presence of steroidal saponins, phenolic glycosides and flavonoid conjugates, which supports the phytochemistry as described for the Asparagus genus. CYP3A4 and CYP2D6 assays confirm that *A. laricinus* is a source of flavonoids, phenolics, alkaloids, terpenoids and saponins that regulate the pharmacological activity, but also influence drug metabolism. Fraction H was shown to have a strong inhibition effect for both CYP3A4 (95.84%) and CYP2D6(91.26%), whereas Fractions E (69.34%) in CYP3A4 and in CYP2D6 Fraction E (79.59%), Fraction C for both CYP3A4 (89.91%) and CYP2D6 (85.85) showed selective inhibition. The reproducible, concentration-dependent inhibition and sigmoidal IC_{50} patterns signify the effective and meaningful interactions between these phytochemicals and the enzyme active sites. It confirms the traditional medicinal significance with respect to *A. laricinus*, although it also raises suspicion of herb–drug interactions when in combination with conventional medicines metabolised by CYP3A4 or CYP2D6.

11.2 RECOMMENDATIONS

Future studies should concentrate on isolating and structurally characterising the specific compounds responsible for CYP450 inhibition, since LC–MS results show the presence of potentially phytochemicals that causes inhibition. Other studies can focus on evaluating the effect of *A laricinus* in *in vivo* and clinical studies to validate our findings further. For genetic variation studies, CYP3A4 and CYP2D6 polymorphisms in local populations should be undertaken to assess the potential risk of *A. laricinus* herb-drug interaction. Lastly, clinical herb-drug interaction trials, particularly among patients receiving cancer chemotherapy or hormone therapies, would be very important to validate the findings in this study in order to promote a rational concurrent use of *A. laricinus* and conventional anti-prostate cancer drugs.

12. APPENDICES

APPENDIX A: PHYTOCHEMICAL IDENTIFICATION

Negative ionization

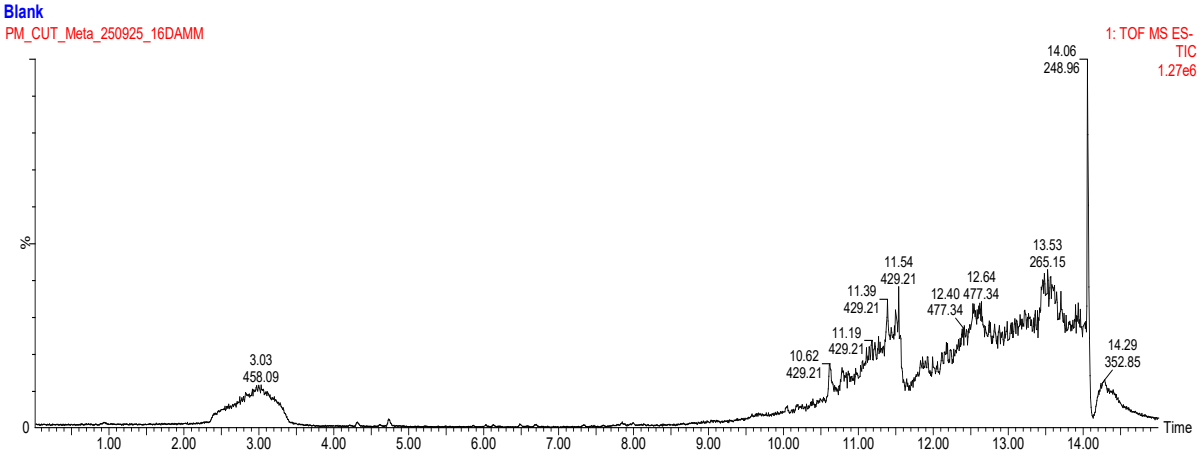

Figure 9.2.1a: LC–MS base peak chromatogram of Blank in negative ionization mode (ESI^-)

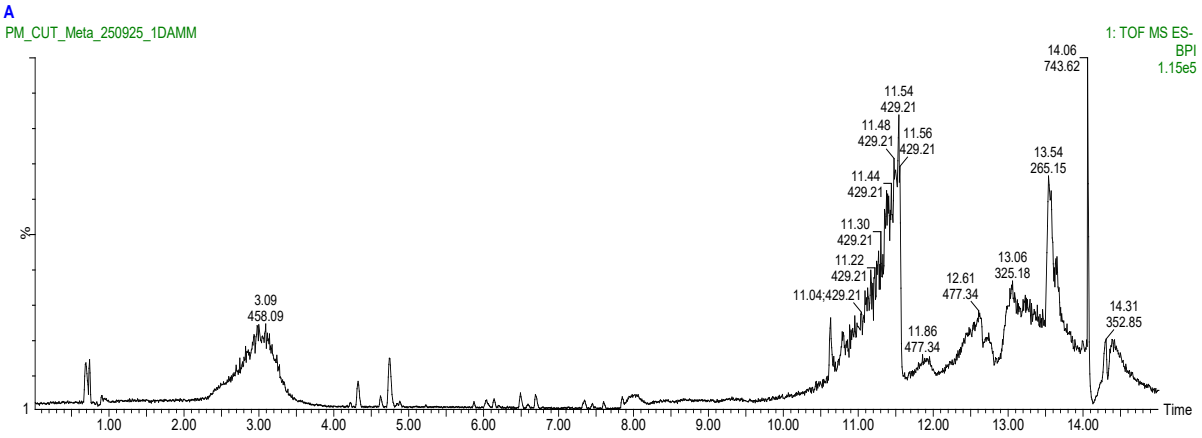

Figure 9.2.1b: LC–MS base peak chromatogram of Fraction A in negative ionization mode (ESI^-)

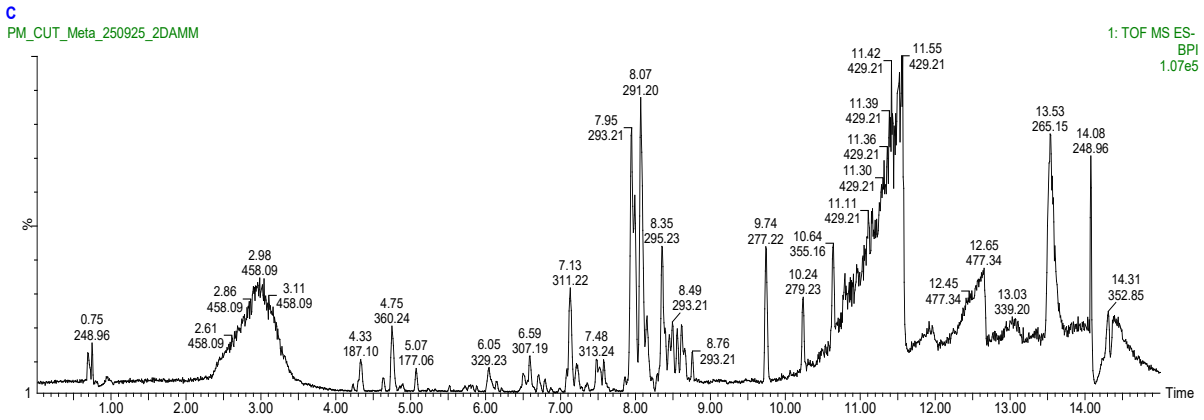

Figure: 9.2.1c: LC–MS total ion chromatogram of Fraction C in negative ionization mode (ESI^-)

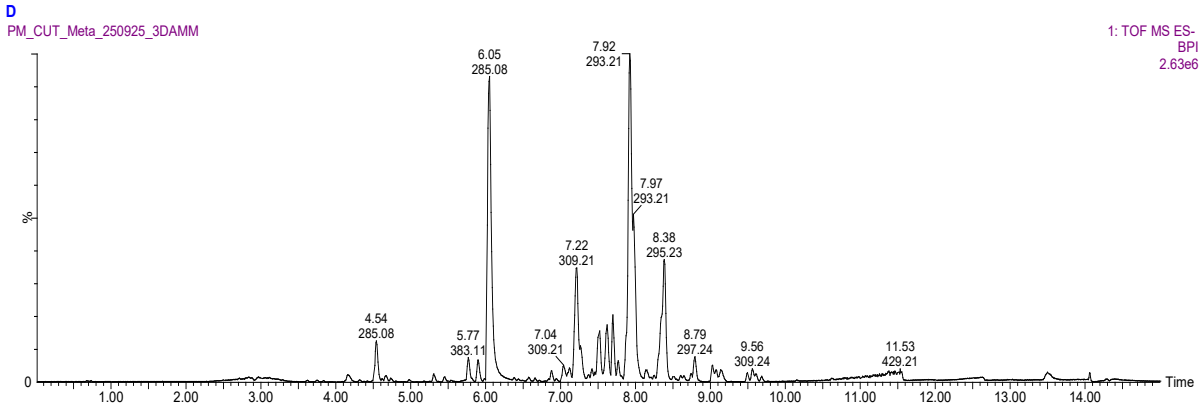

Figure 9.2.1d: LC–MS base peak chromatogram of Fraction A in negative ionization mode (ESI^-)

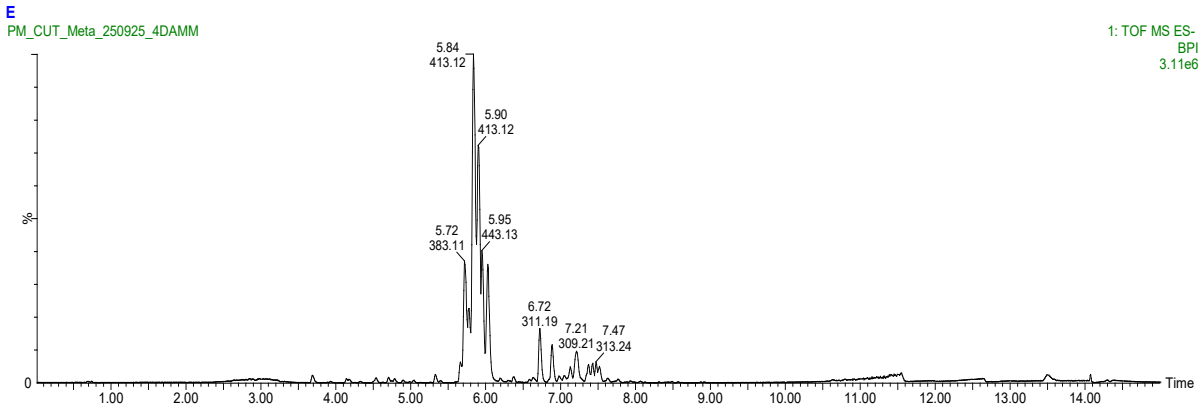

Figure 9.2.1e: LC–MS base peak chromatogram of Fraction E in negative ionization mode (ESI^-)

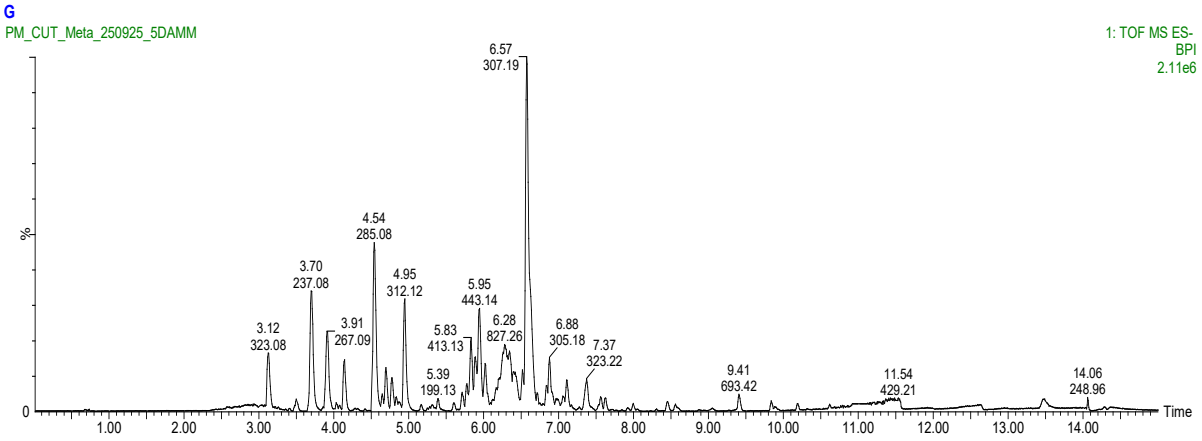

Figure 9.2.1f: LC–MS base peak chromatogram of Fraction E in negative ionization mode (ESI^-)

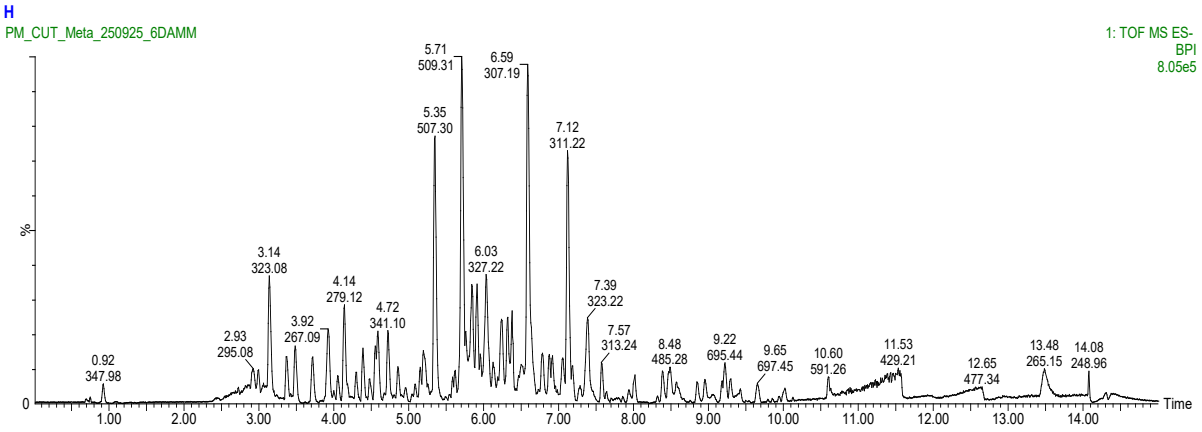

Figure 9.2.1g: LC–MS base peak chromatogram of Fraction H in negative ionization mode (ESI^-)

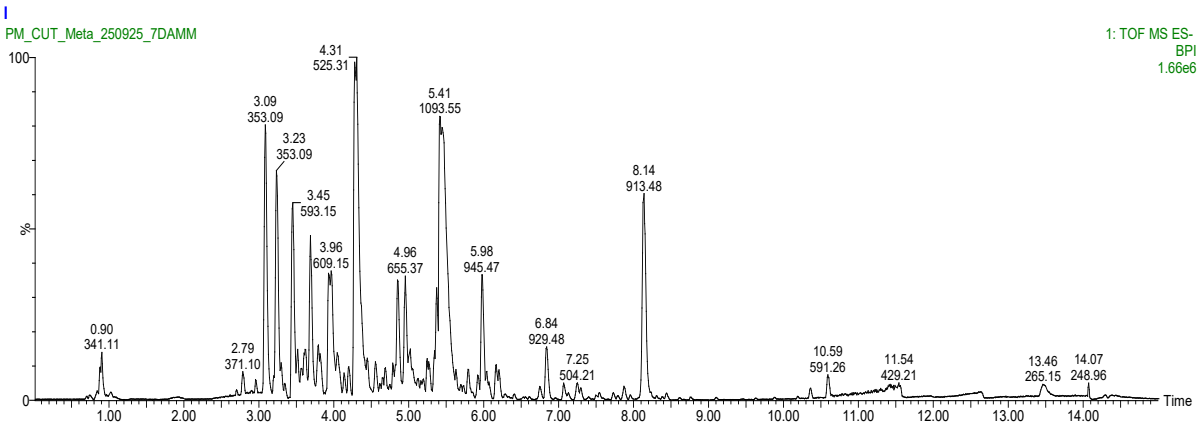

Figure 9.2.1h: LC–MS base peak chromatogram of Fraction I in negative ionization mode (ESI^-)

Positive ionization

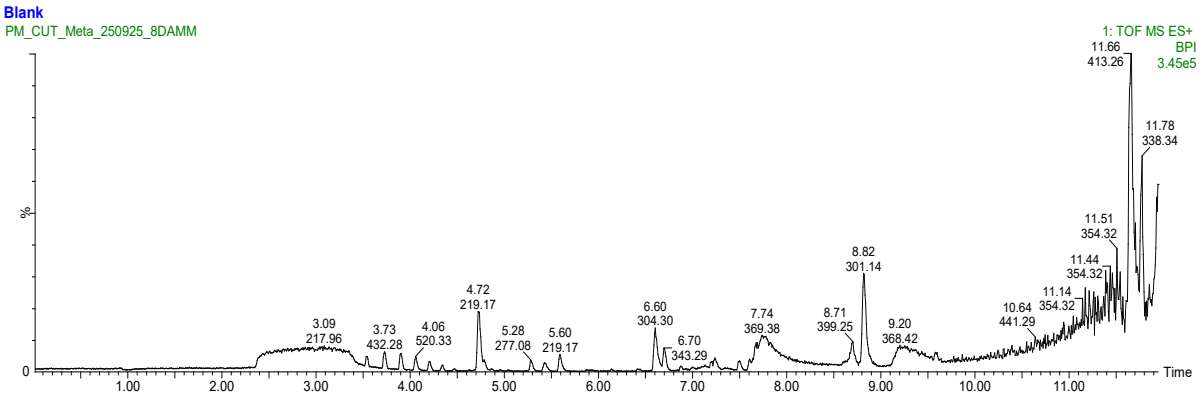

Figure 9.2.2a: LC–MS base peak chromatogram of Blank in positive ionization mode (ESI^+)

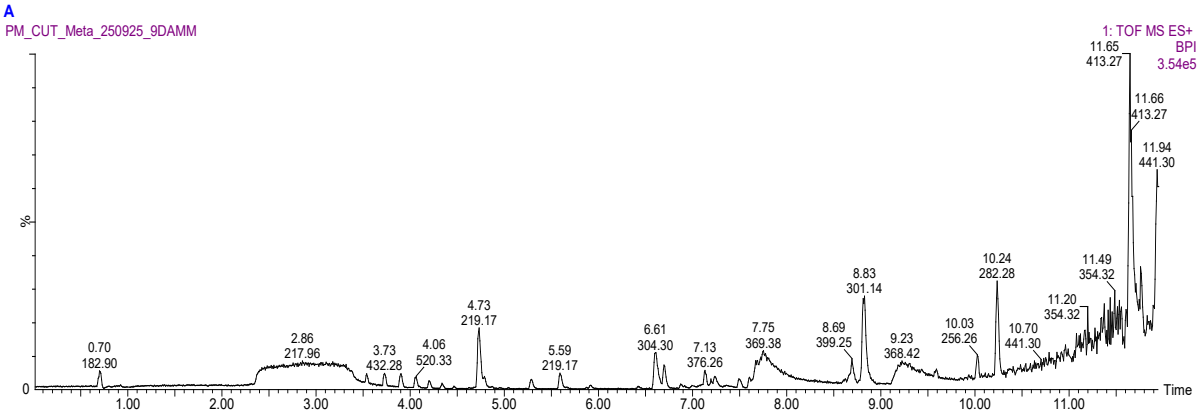

Figure 9.2.2b: LC–MS base peak chromatogram of Fraction A in positive ionization mode (ESI^+)

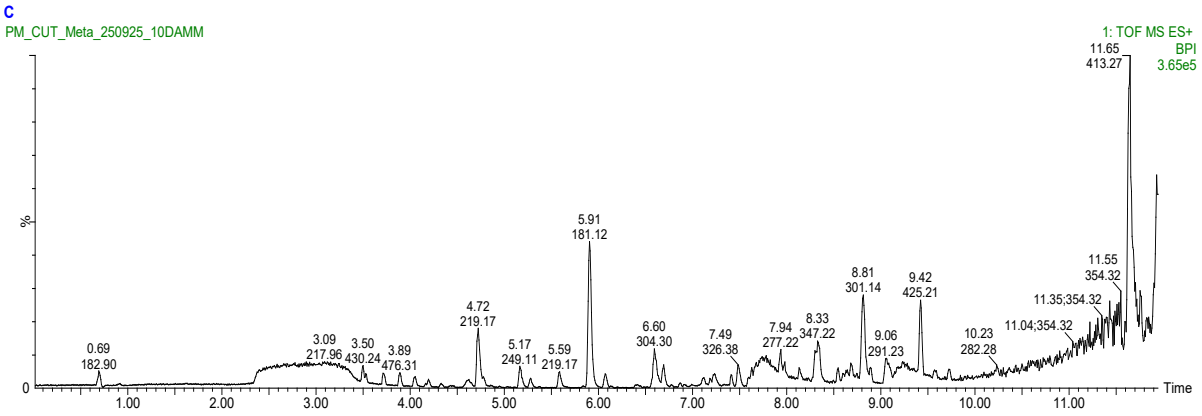

Figure 9.2.2c: LC–MS base peak chromatogram of Fraction C in positive ionization mode (ESI^+)

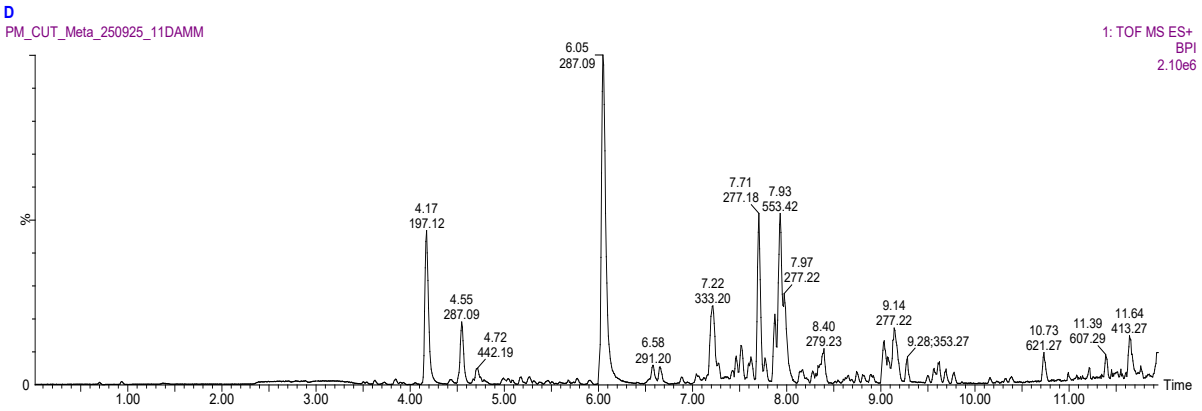

Figure 9.2.2d: LC–MS base peak chromatogram of Fraction D in positive ionization mode (ESI^+)

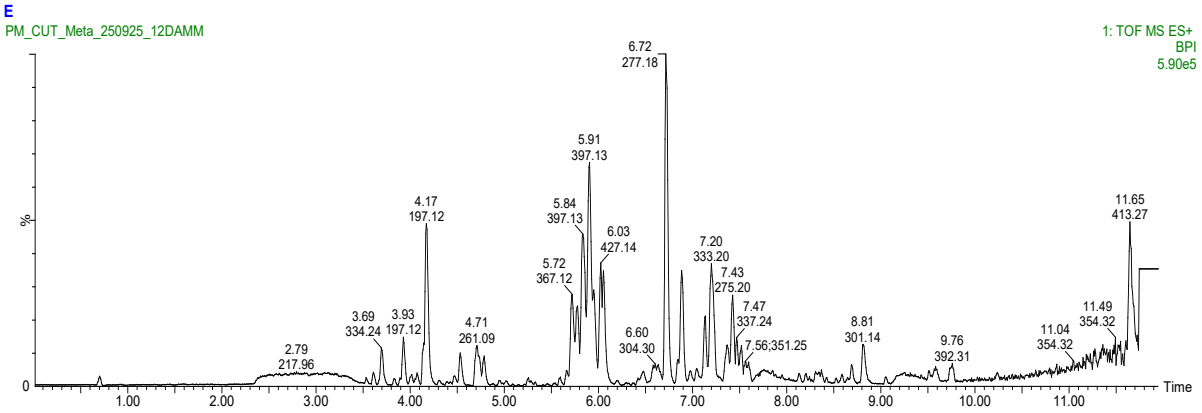

Figure 9.2.2e: LC–MS base peak chromatogram of Fraction E in positive ionization mode (ESI^+)

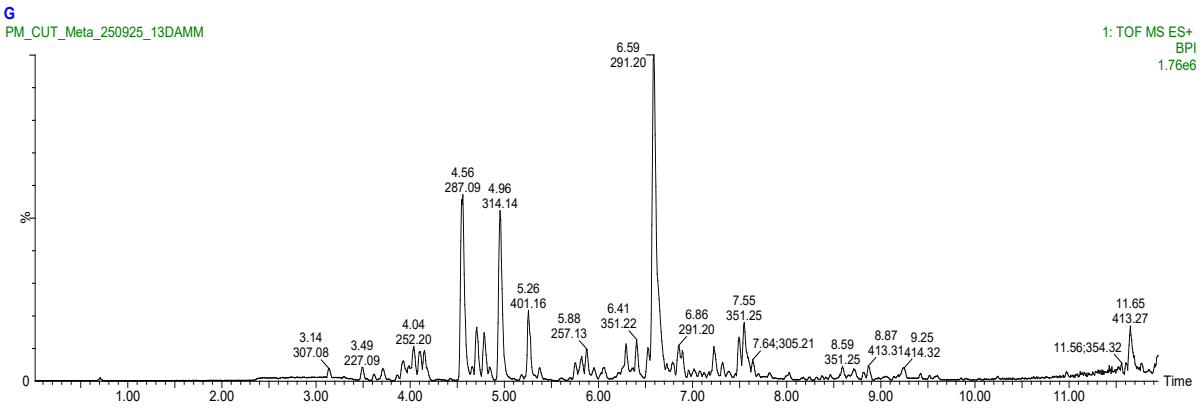

Figure 9.2.2f: LC–MS base peak chromatogram of Fraction G in positive ionization mode (ESI^+)

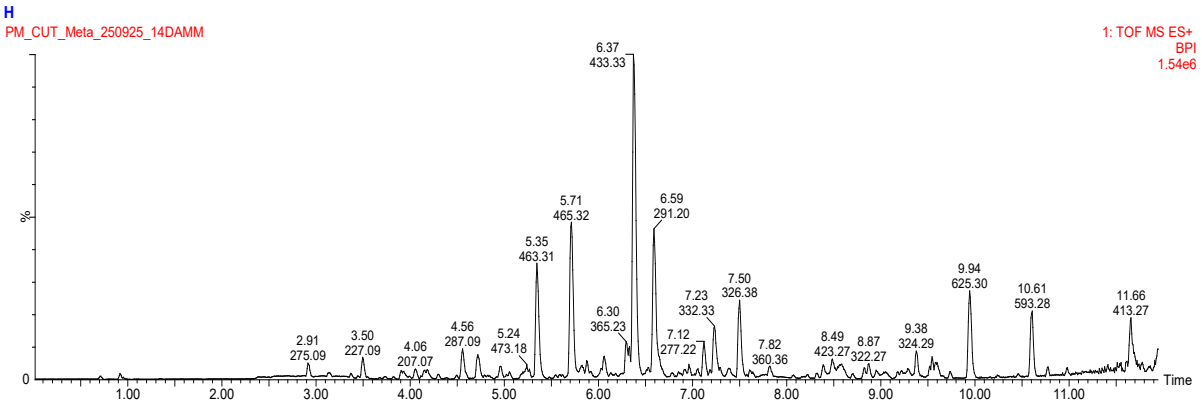

Figure 9.2.2h: LC–MS base peak chromatogram of Fraction H in positive ionization mode (ESI^+)

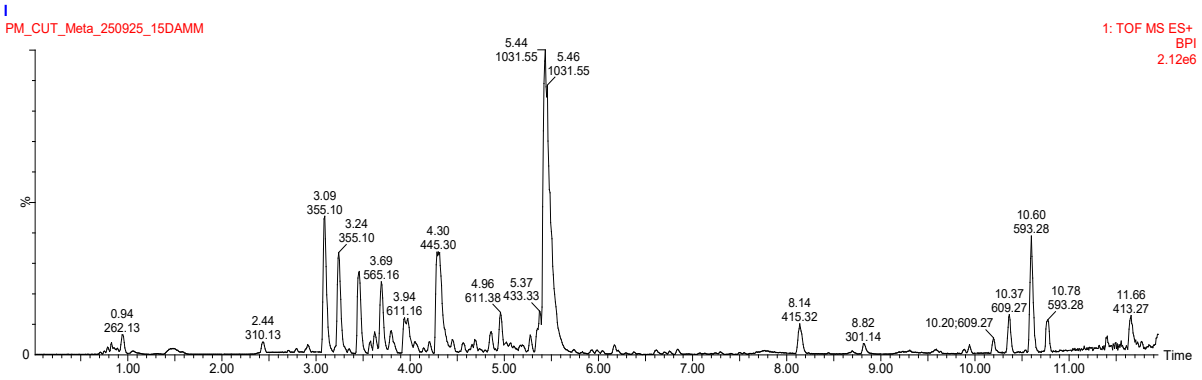

FIGURE 9.2.2I: LC–MS BASE PEAK CHROMATOGRAM OF FRACTION I IN POSITIVE IONIZATION MODE (ESI^+)APPENDIX B: IN VITRO ENZYME ASSAYS FOR CYP3A4 AND CYP2D6

CYP3A4 Results

CONCENTRATIONS (µM)	FRACTION A	FRACTION C	FRACTION D	FRACTION E	FRACTION G	FRACTION H	FRACTION I	KETO			DMSO		BUFFER
	RFU												
5	18607,5	17021,5	27347,5	36929,5	28688,5	11293,5	30358,5	15115			104037		7265,5
2,5	19112,5	17120,5	27999,5	38228,5	37526,5	16401,5	31485,5	16145					
1,25	21511,5	17268,5	37675,5	40643,5	37775,5	17565,5	36151,5	16459					
0,625	24199,5	17287,5	38680,5	46336,5	40616,5	19632,5	38857,5	16568					
0,3125	29782,5	17936,5	45694,5	46695,5	46158,5	23171,5	40476,5	16647					
CONCENTTRATION (µM)	%INHIBITION	%INHIBITION	%INHIBITION	%INHIBITION	%INHIBITION	%INHIBITION	%INHIBITION	%INHIBITION					
	FRACTION A	FRACTION C	FRACTION D	FRACTION E	FRACTION G	FRACTION H	FRACTION I	KETO					
5	88,27960712	89,9185194	79,2480224	69,34634681	77,86228383	95,83761748	76,13656913	91,88862423					
2,5	87,75775926	89,81621655	78,57427032	68,00400944	68,72942964	90,5592039	74,97197005	90,82426128					
1,25	85,27872359	89,66327896	68,57545868	65,50843998	68,47212247	89,35637042	70,15030252	90,49978558					
0,625	82,50104628	89,64364508	67,53692978	59,62550958	65,53634076	87,22041097	67,35402469	90,38714911					
0,3125	76,7317857	88,97299308	60,28892804	59,25453258	59,80944803	83,56334251	65,68101145	90,3055135					
0	0	0	0	0	0	0	0	0					

Table 9.3: CYP3A4 residual fluorescence inhibition at different concentration across all fractions (A–I)

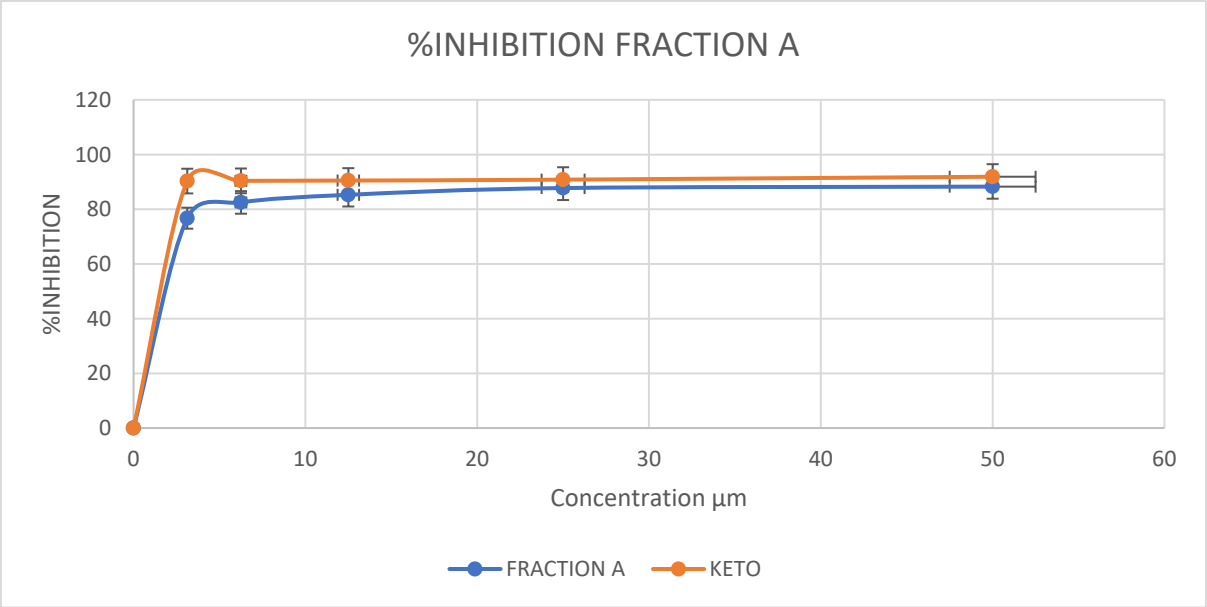

Figure 9.3.1: % inhibition of CYP3A4 by Fraction A

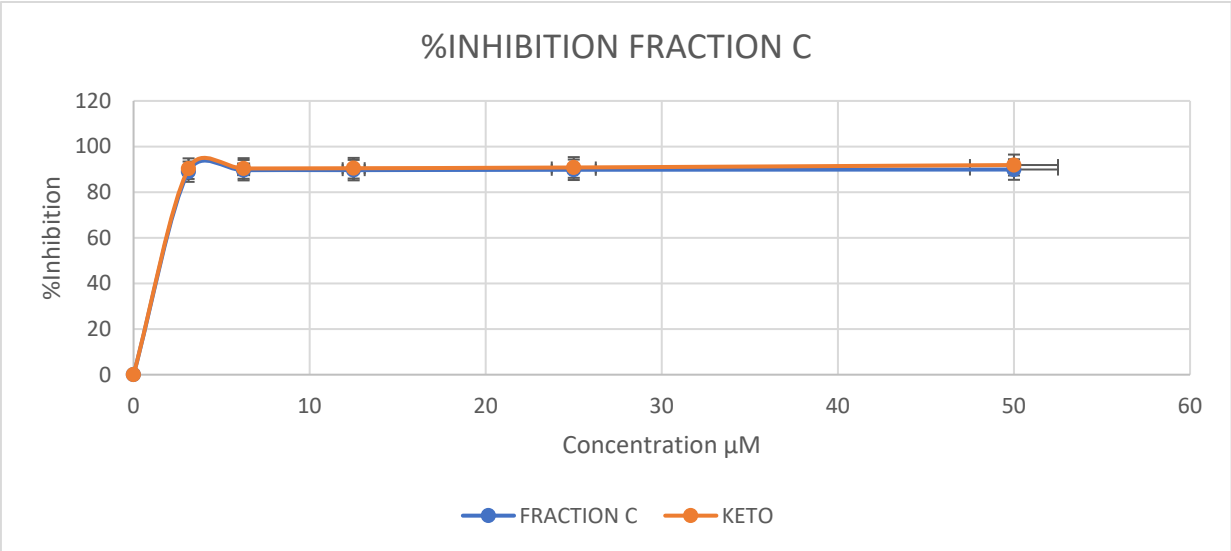

Figure 9.3.2: %inhibition of CYP3A4 by Fraction C

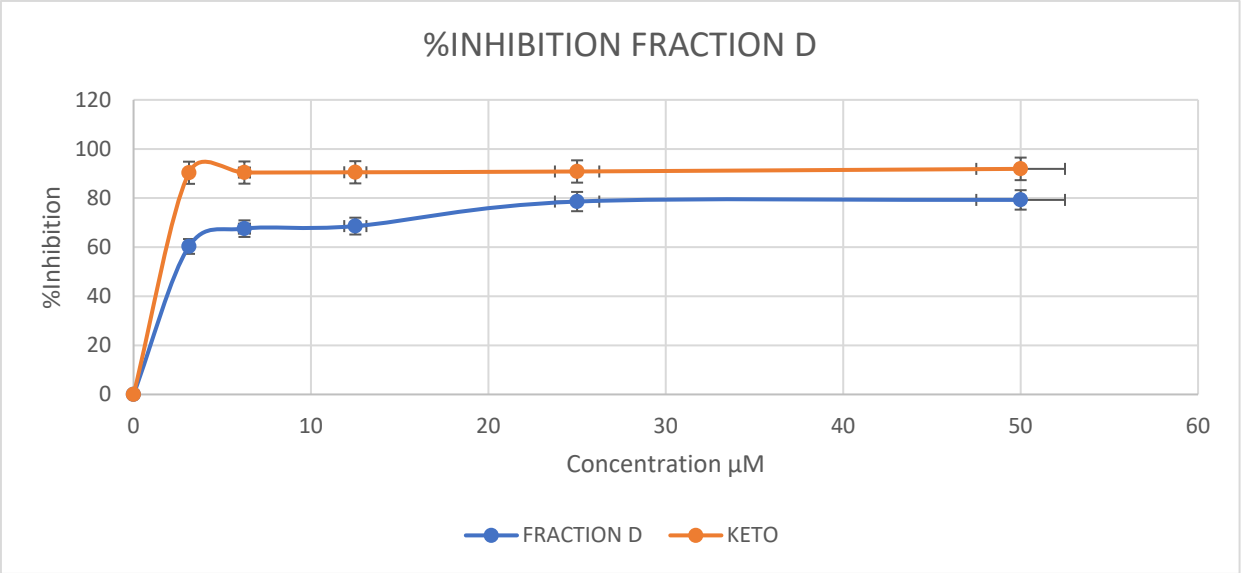

Figure 9.3.3: %inhibition of CYP3A4 by Fraction D

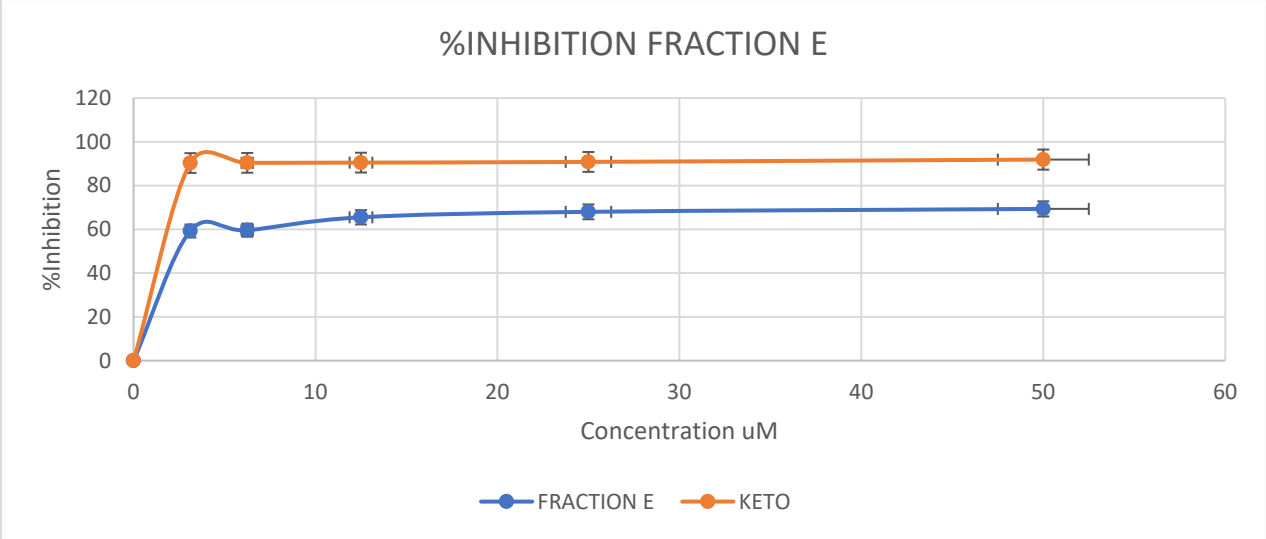

Figure 9.3.4: % inhibition of CYP3A4 by Fraction E

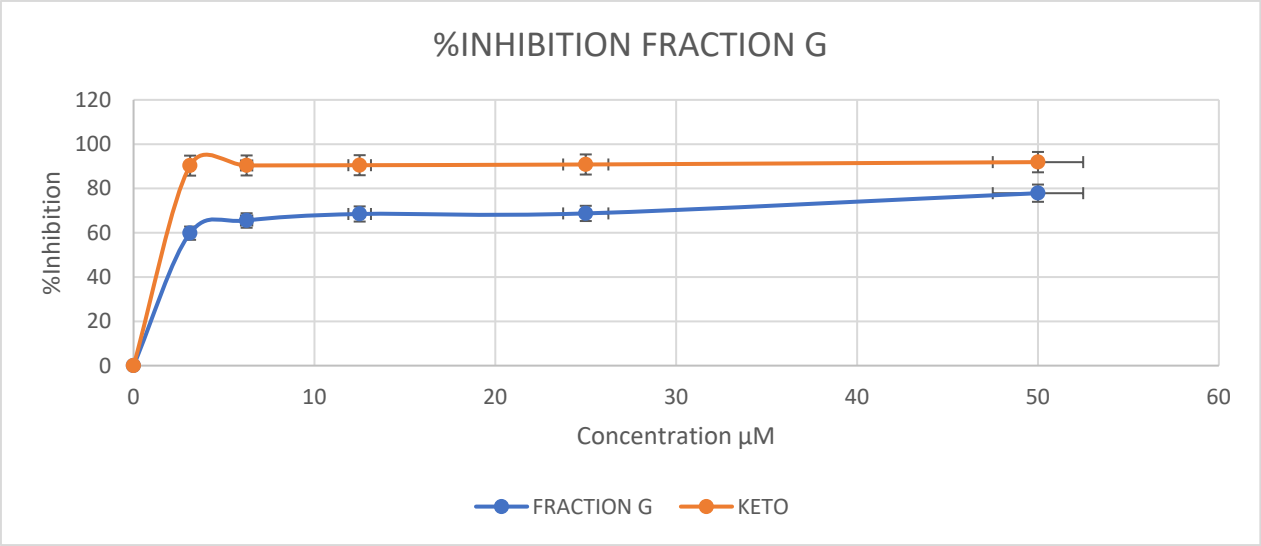

Figure 9.3.5: %inhibition of CYP3A4 by Fraction G

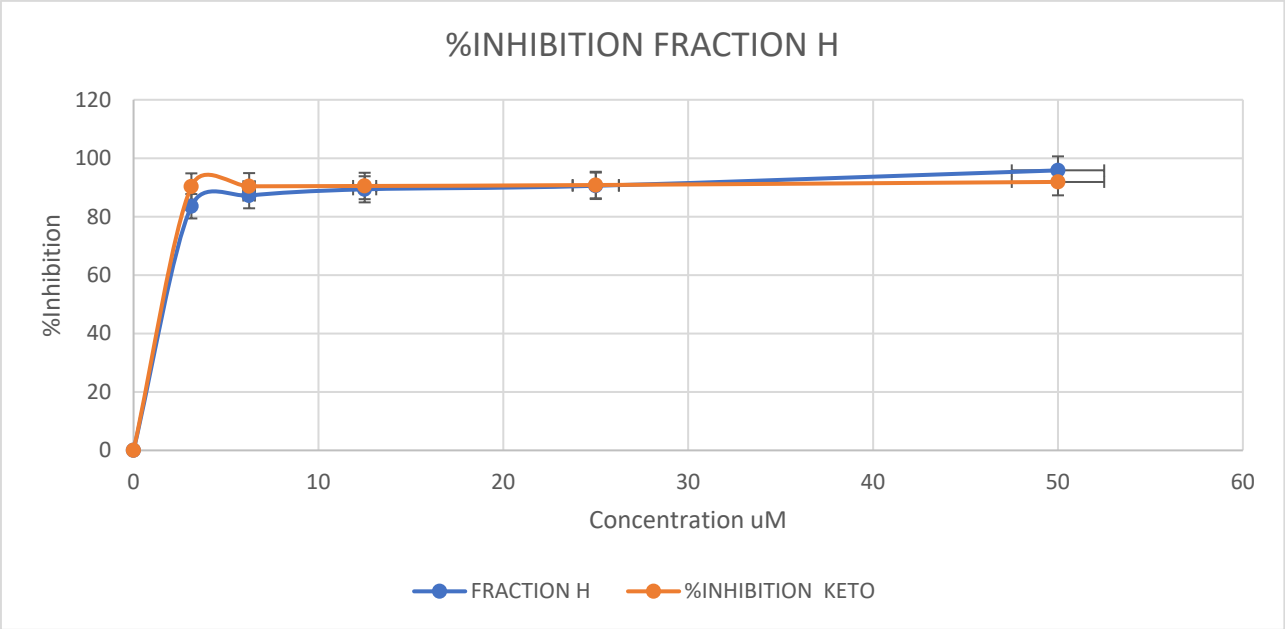

Figure 9.3.6: % inhibition of CYP3A4 by Fraction H

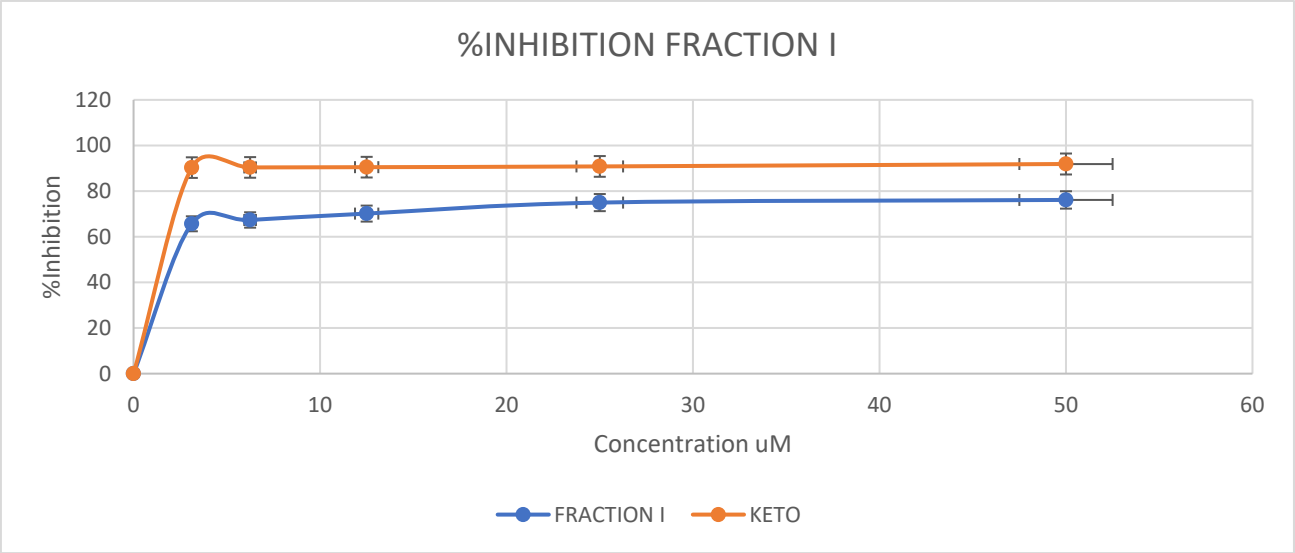

Figure 9.3.7: %inhibition of CYP3A4 by Fraction I

CYP2D6 Results

Table: 9.4**:** CYP2D6 residual fluorescence inhibition at concentration across all fractions (A–I)

CONCENTRATIONS (µM)	FRACTION A	FRACTION C	FRACTION D	FRACTION E	FRACTION G	FRACTION H	FRACTION I	QUINIDINE			DMSO	BUFFER
	RFU											
5	27412,5	10272	30197,5	39547,5	38857,5	20446,5	30531	12155			125080,5	10423
2,5	29194,5	9749	40826,5	40372,5	41165,5	29879,5	32383	13634				
1,25	34799,5	9716	49109,5	48786,5	45951,5	32976,5	35617	19005				
0,625	38698,5	9646	50506,5	51047	48777,5	39616,5	37471	19389				
0,3125	38919,5	8783	51389,5	57776	49885,5	40945,5	39531	19998				
CONCENTRATION (µM)	%INHIBITION	% INHIBITION	%INHIBITION	%INHIBITION	%INHIBITION	%INHIBITION	%INHIBITION	%INHIBITION				
	FRACTION A	FRACTION C	FRACTION D	FRACTION E	FRACTION G	FRACTION H	FRACTION I	QUINIDINE				
5	85,18239103	85,85024653	82,75341779	74,59869612	75,20048841	91,2578767	82,46255151	98,48941412				
2,5	83,62819702	75,95459236	73,48319997	73,87916185	73,18753679	83,0307655	80,84730611	97,19948542				
1,25	78,73972483	75,15193212	66,25907594	66,54078451	69,01336589	80,3296775	78,02673179	92,51509932				
0,625	75,33916229	74,77353515	65,04066459	64,56926062	66,54863398	74,5385169	76,40974206	92,18018882				
0,3125	75,14641432	68,77651645	64,27054488	58,70047751	65,58227765	73,3794126	74,6130868	91,64904171				
0	0	0	0	0	0	0	0	0	0			

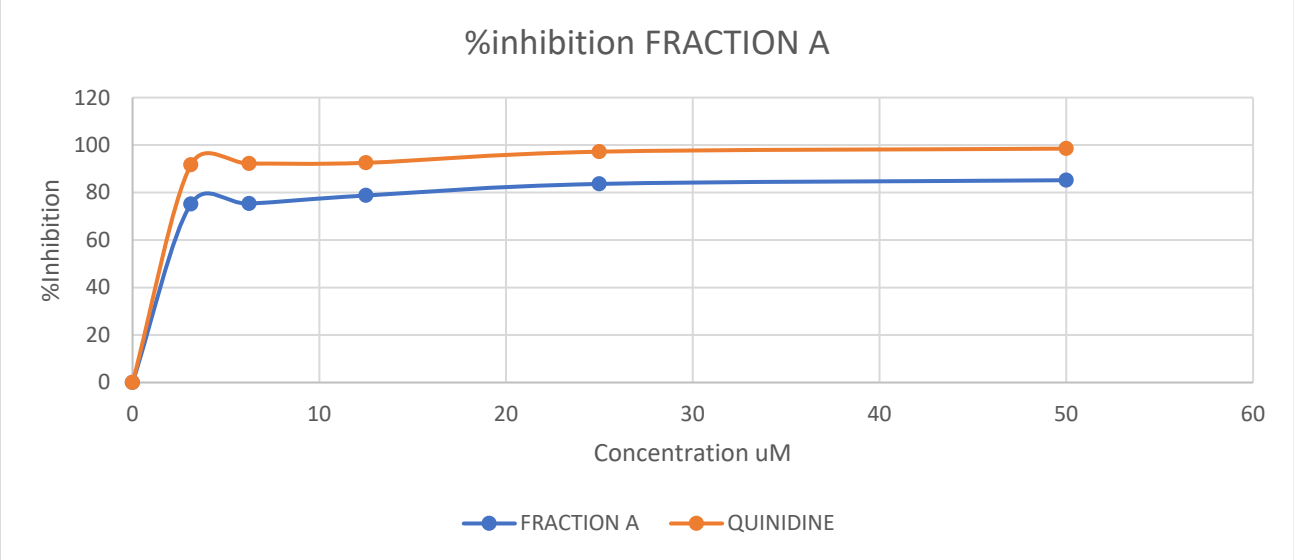

Figure 9.4.1: % inhibition of CYP2D6 by Fraction A

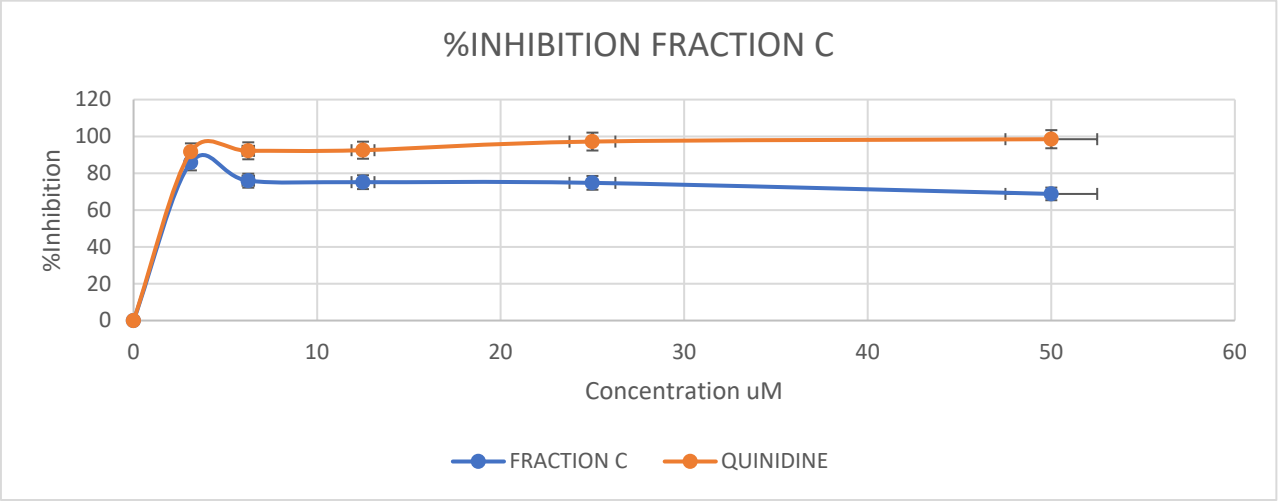

Figure 9.4.2: % inhibition of CYP2D6 by Fraction C

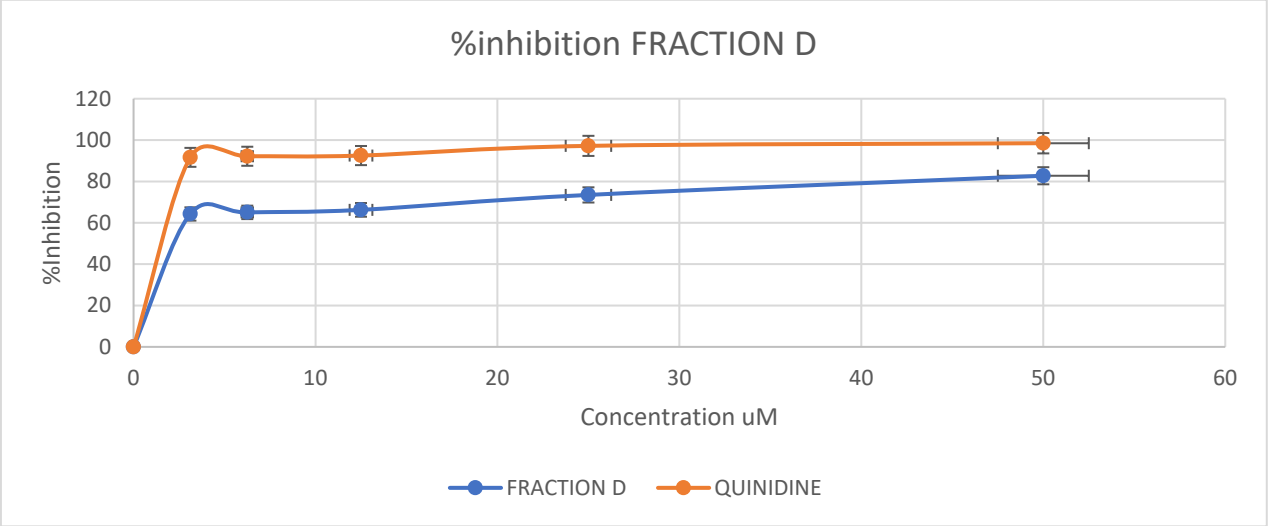

Figure 9.4.3: % inhibition of CYP2D6 by Fraction D

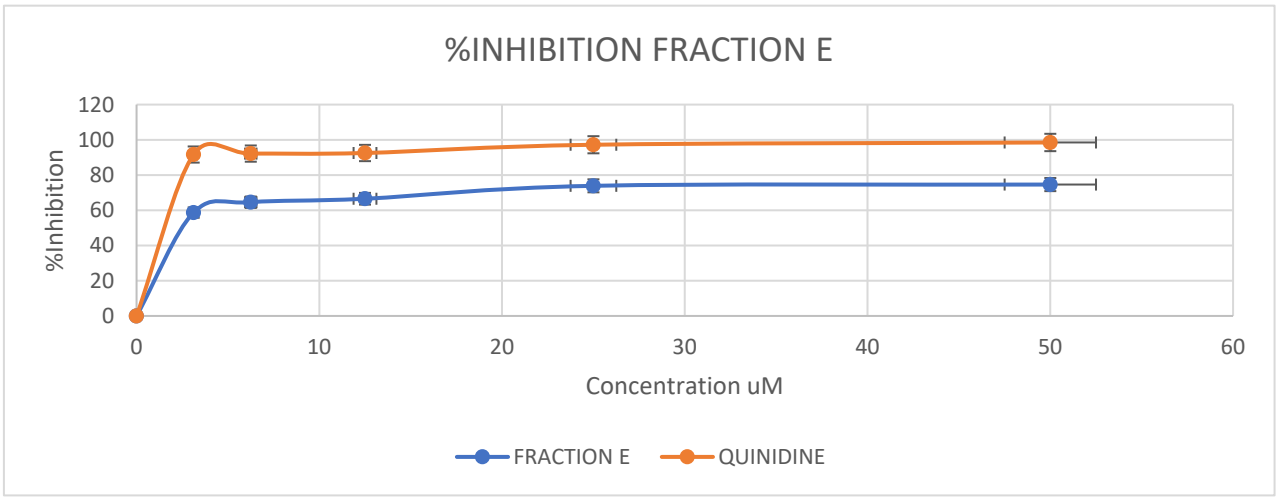

Figure 9.4.4: % inhibition of CYP2D6 by Fraction E

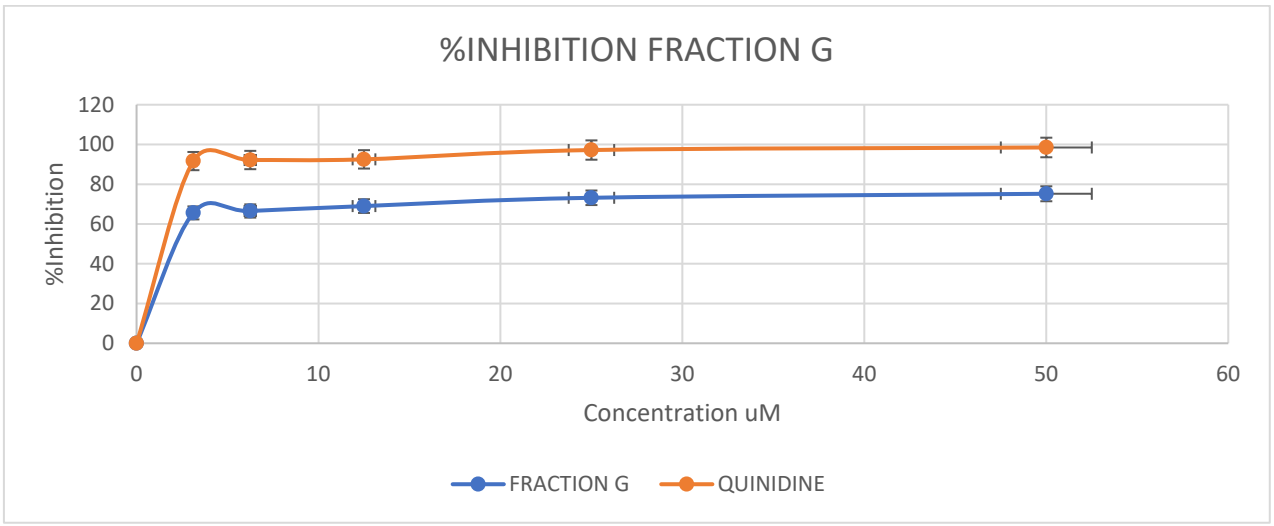

Figure 9.4.5: % inhibition of CYP2D6 by Fraction G

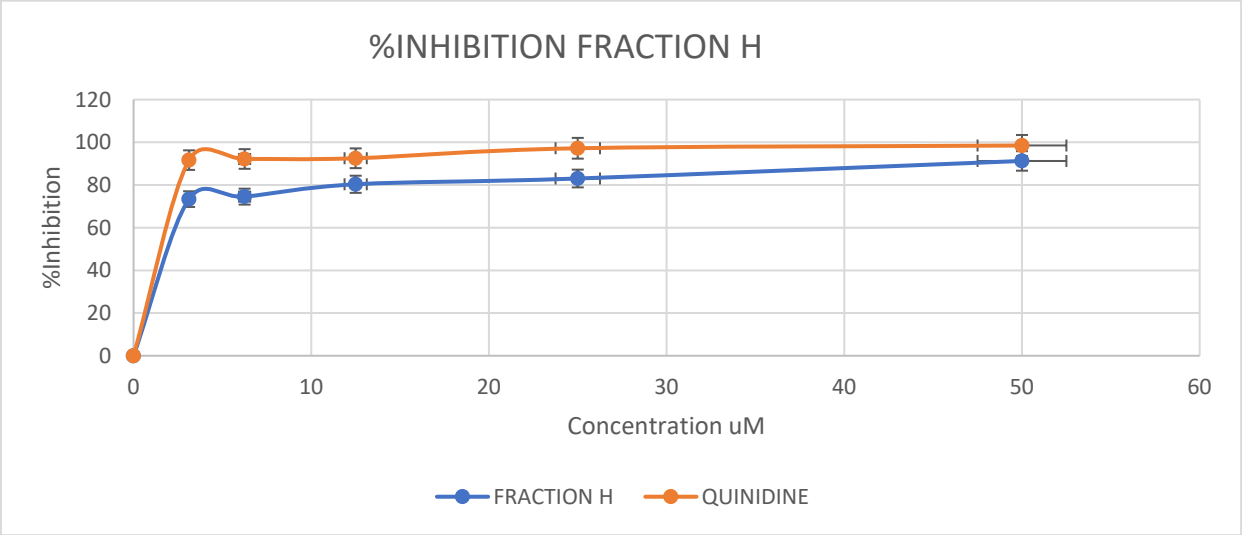

Figure 9.4.6: % inhibition of CYP2D6 by Fraction H

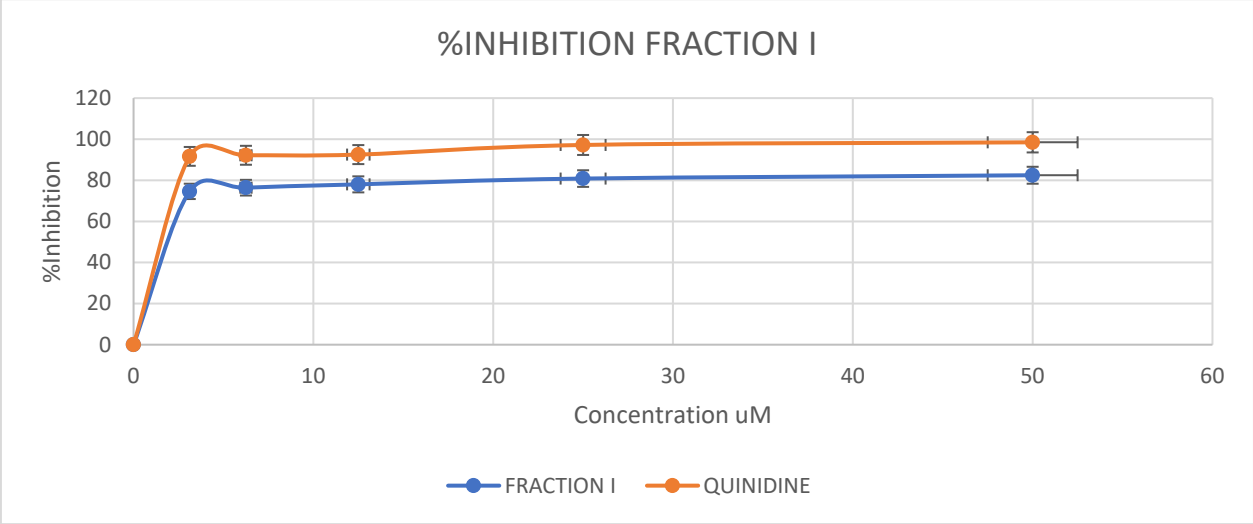

FIGURE 9.4.7: % INHIBITION OF CYP2D6 BY FRACTION IAPPENDIX C: IC50 CURVE-FITTING OUTPUTS

CYP3A4 IC_{50}

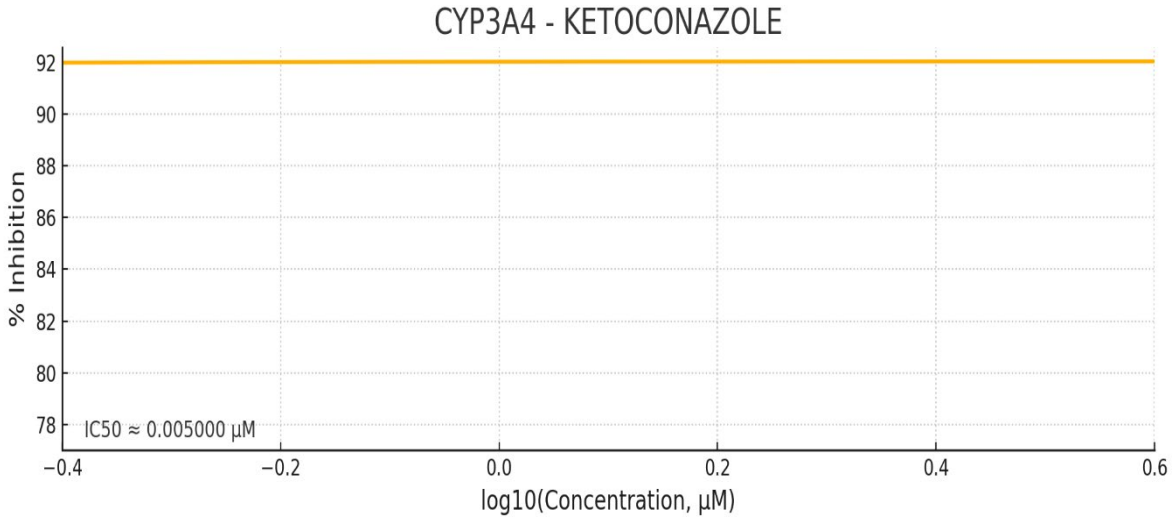

Figure 9.3a: Dose-response curve for CYP3A4 inhibition by the positive control ketoconazole

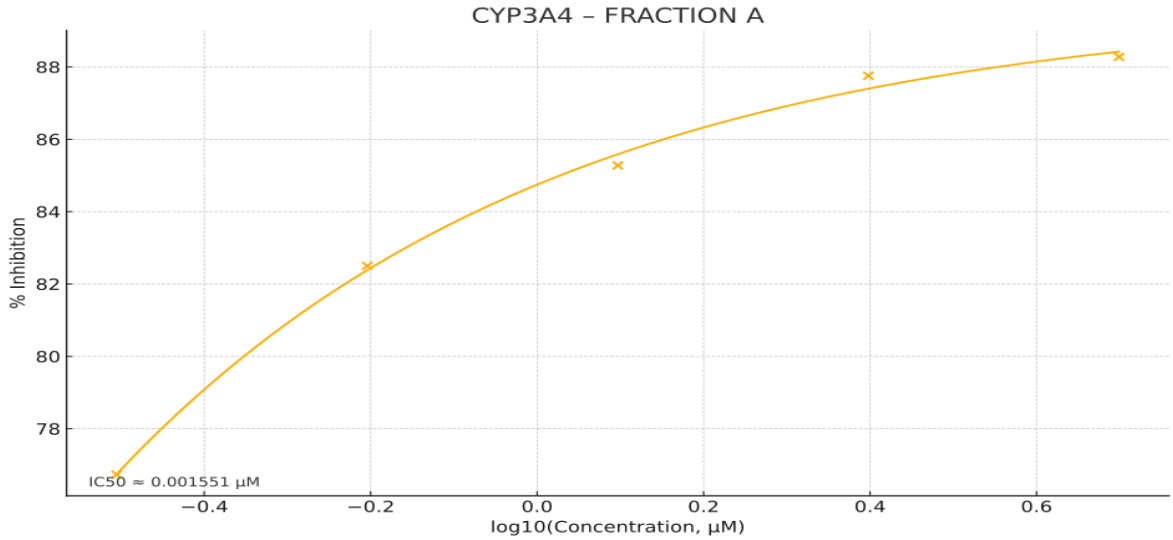

Figure 9.3b: Dose-response curve for CYP3A4 inhibition by Fraction A

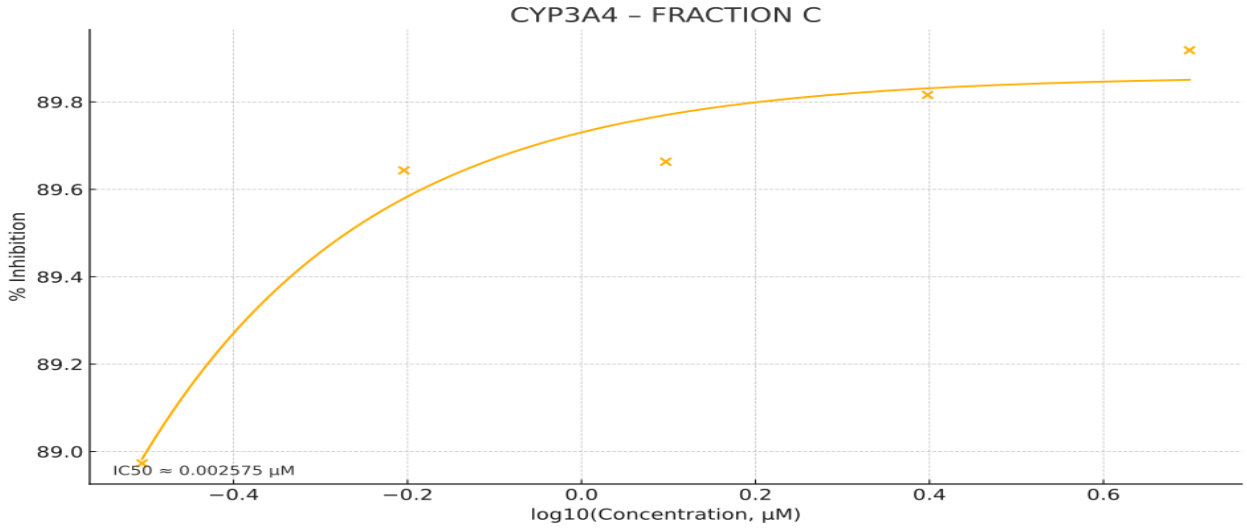

Figure 9.3c: Dose-response curve for CYP3A4 inhibition by Fraction C

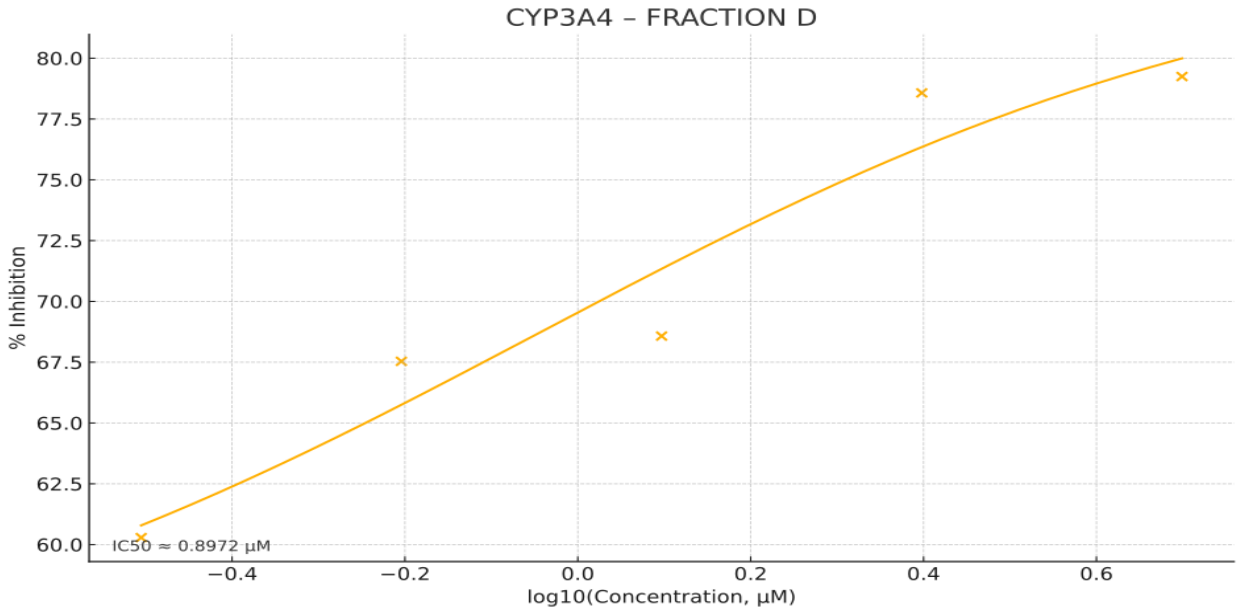

Figure 9.3d: Dose-response curve for CYP3A4 inhibition by Fraction D

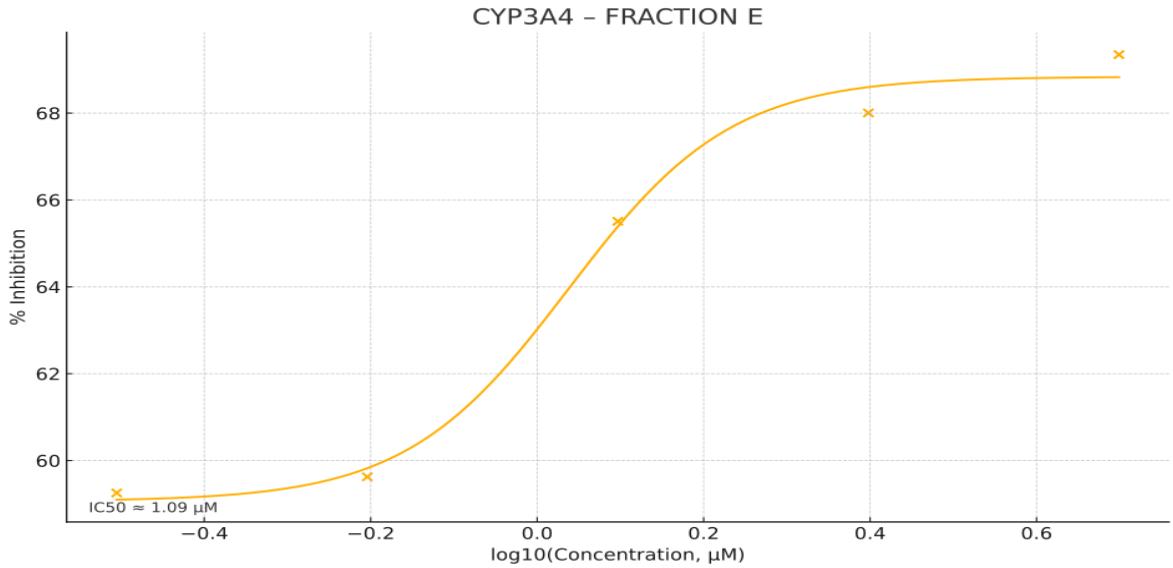

Figure 9.3e: Dose-response curve for CYP3A4 inhibition by Fraction E

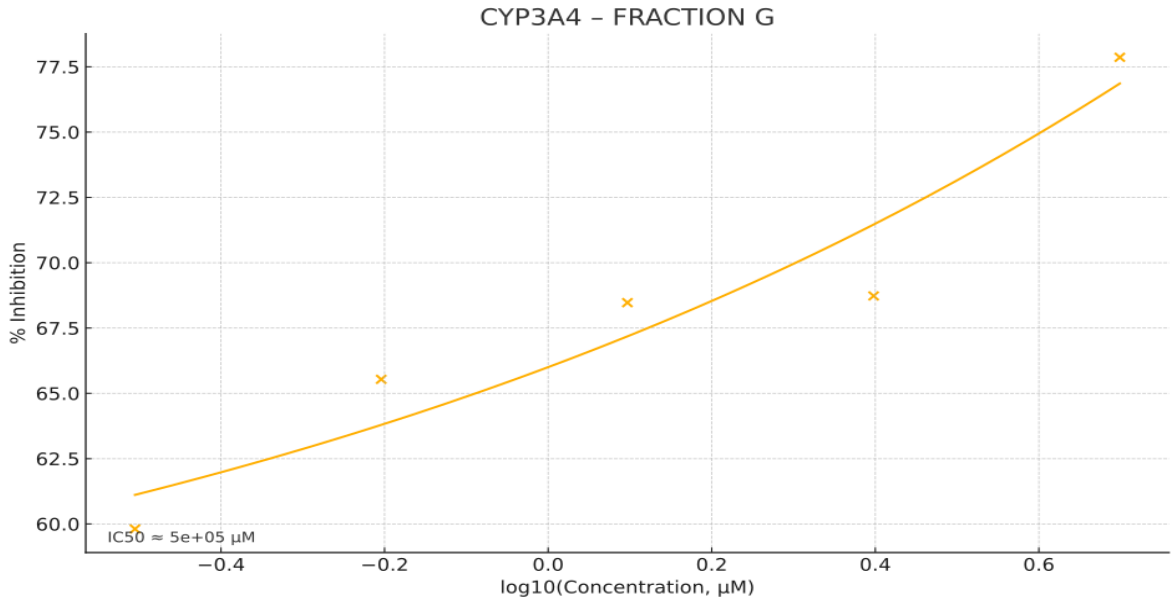

Figure 9.3f: Dose-response curve for CYP3A4 inhibition by Fraction G. where: 5e+05 µM = 5×10^{5} µM

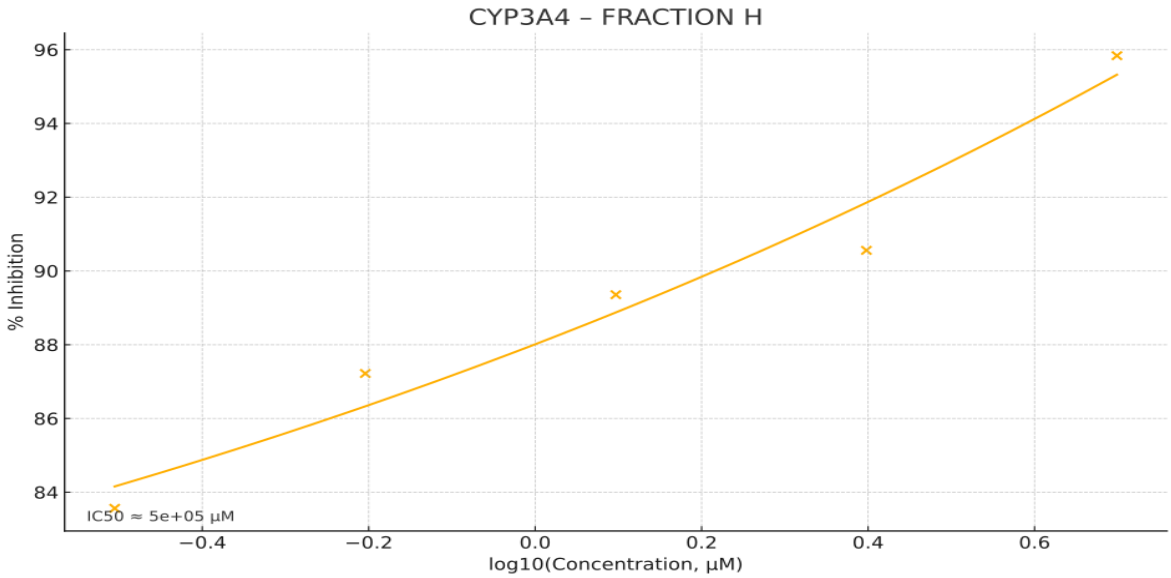

Figure 9.3g: Dose-response curve for CYP3A4 inhibition by Fraction H. Where: 5e+05 µm= 5×10^{5} µM

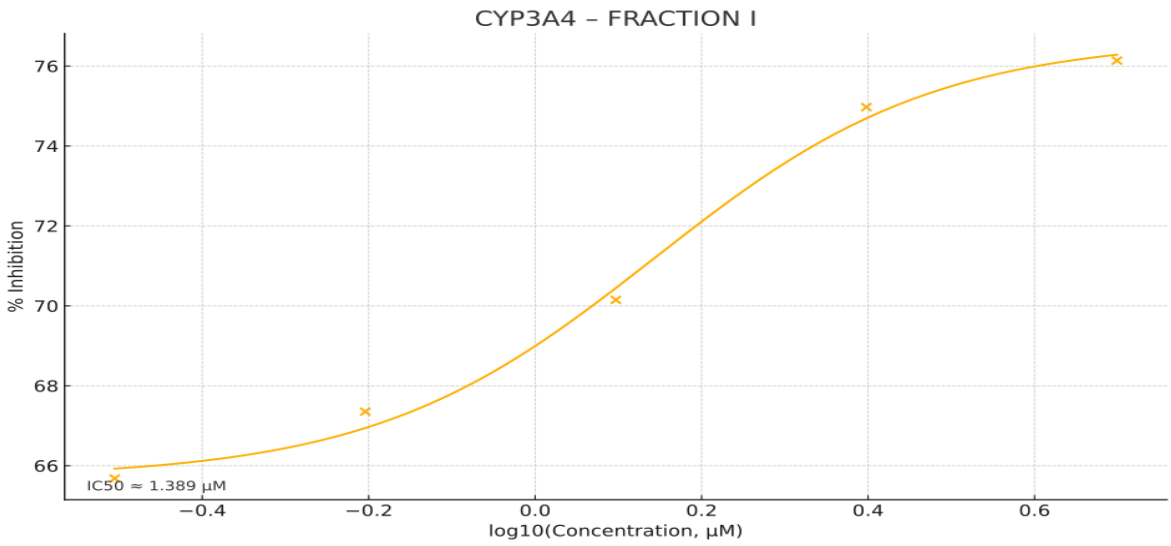

Figure 9.3h: Dose-response curve for CYP3A4 inhibition by Fraction I

CYP2D6 IC_{50}

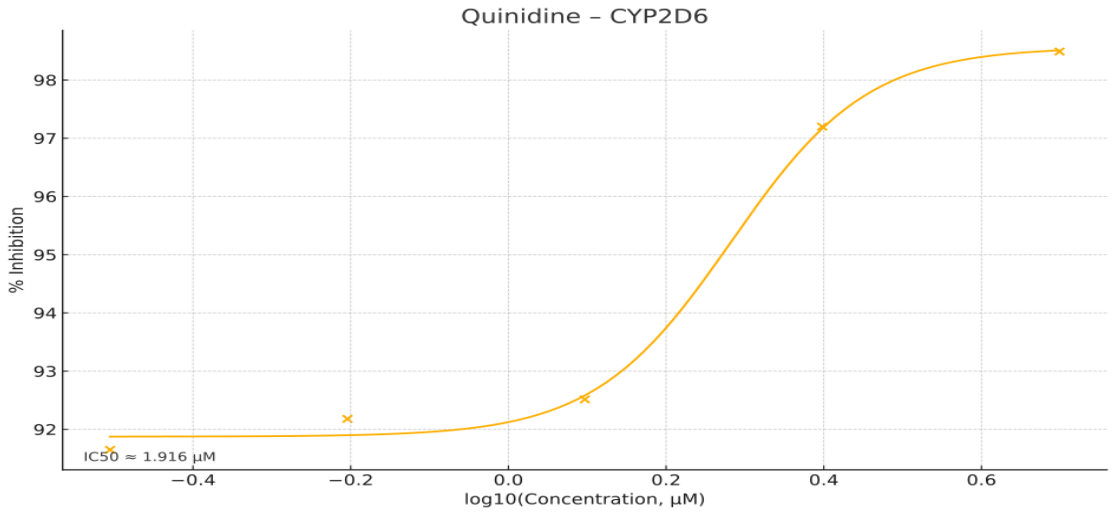

Figure 9.4a: Dose-response curve for CYP2D6 inhibition by the positive control quinidine

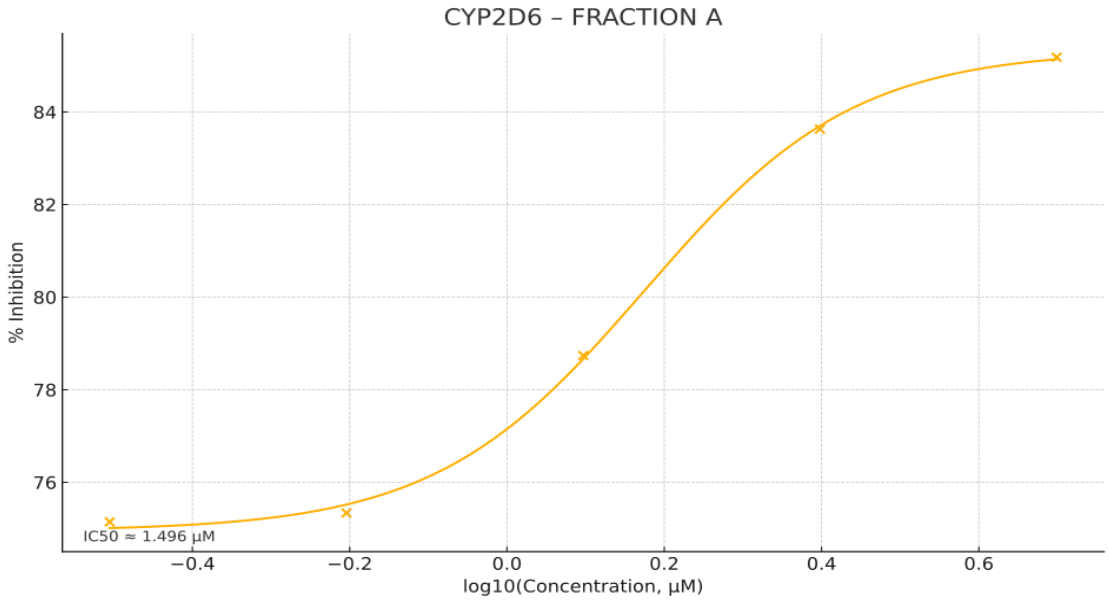

Figure 9.4b: Dose-response curve for CYP2D6 inhibition by Fraction A

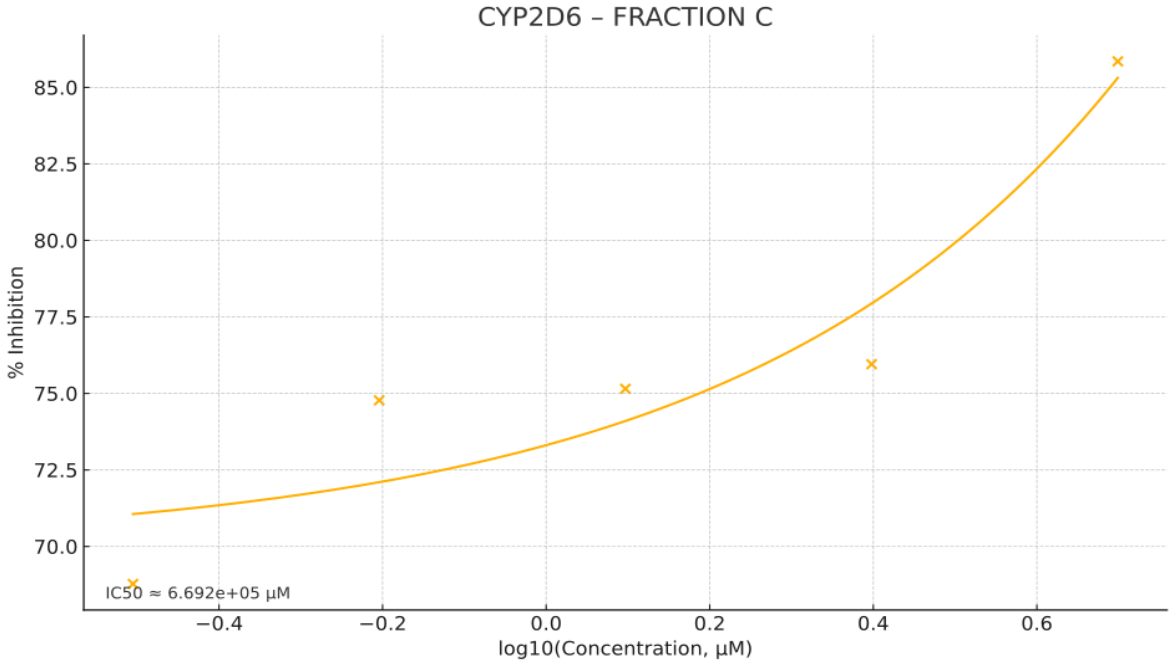

Figure 9.4c: Dose-response curve for CYP2D6 inhibition by Fraction C. Where: 6,692e+05 µm= 6,69× 10^5µM

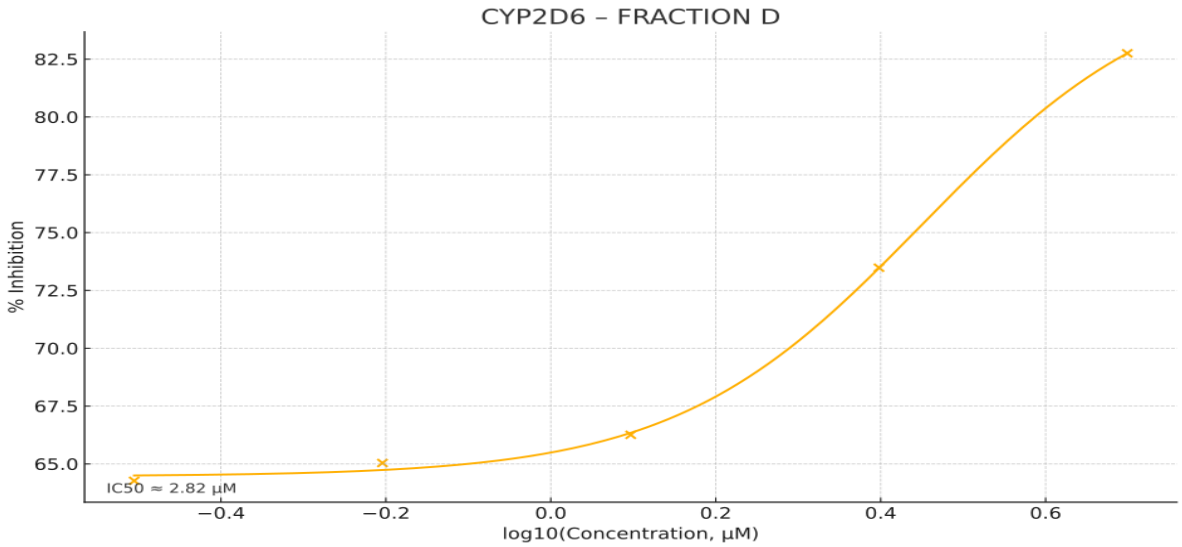

Figure 9.4e: Dose-response curve for CYP2D6 inhibition by Fraction D

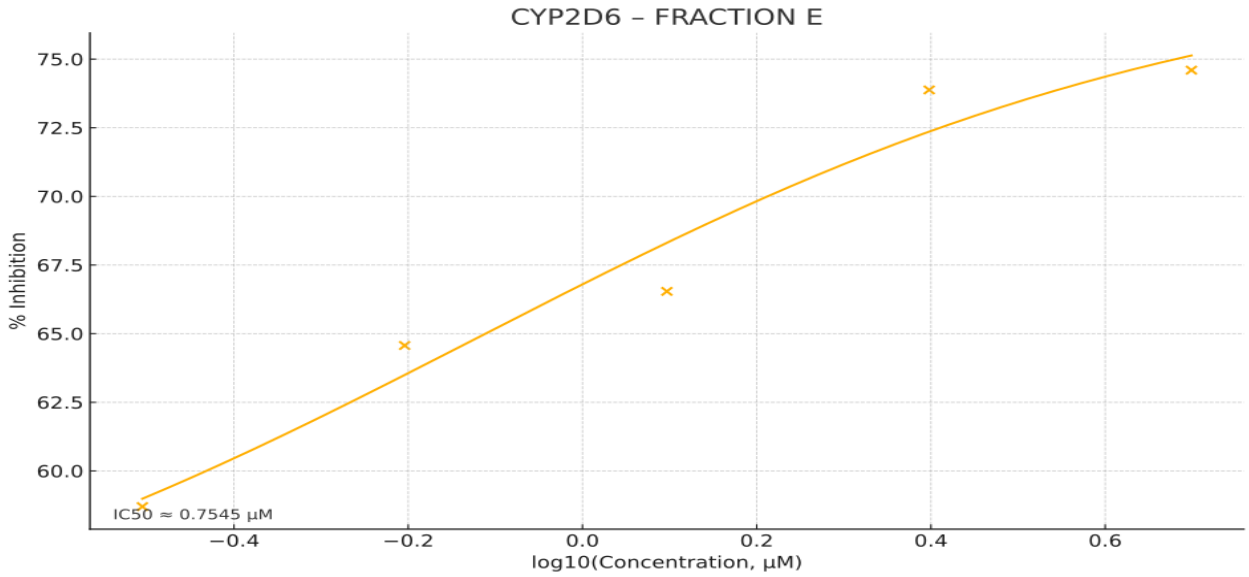

Figure: 9.4f: Dose-response curve for CYP2D6 inhibition by Fraction E

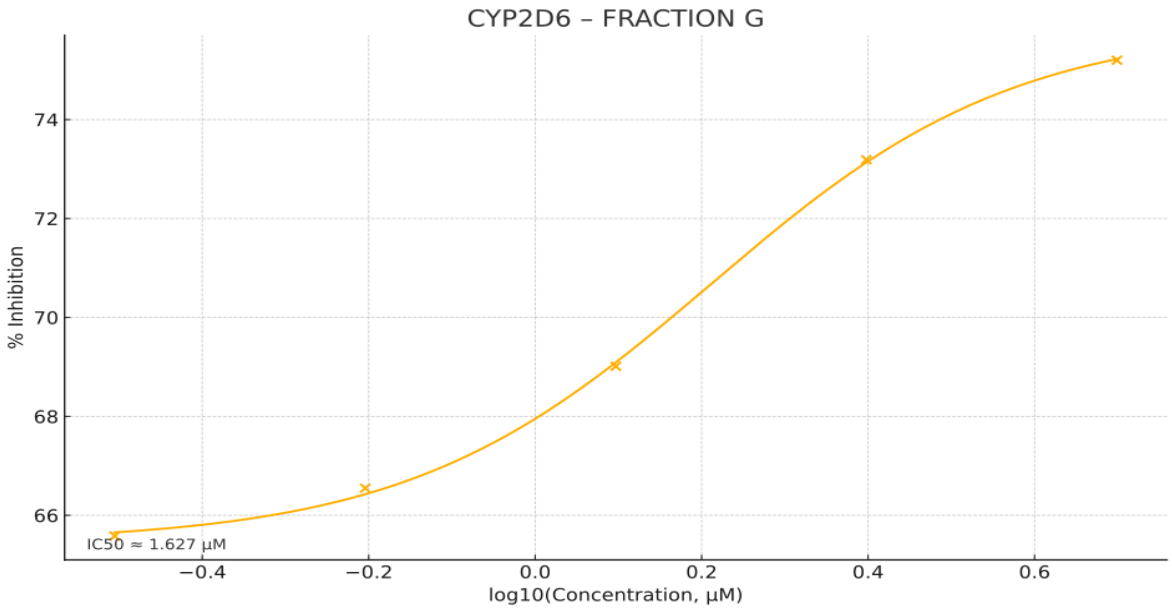

Figure: 9.4g: Dose-response curve for CYP2D6 inhibition by Fraction G

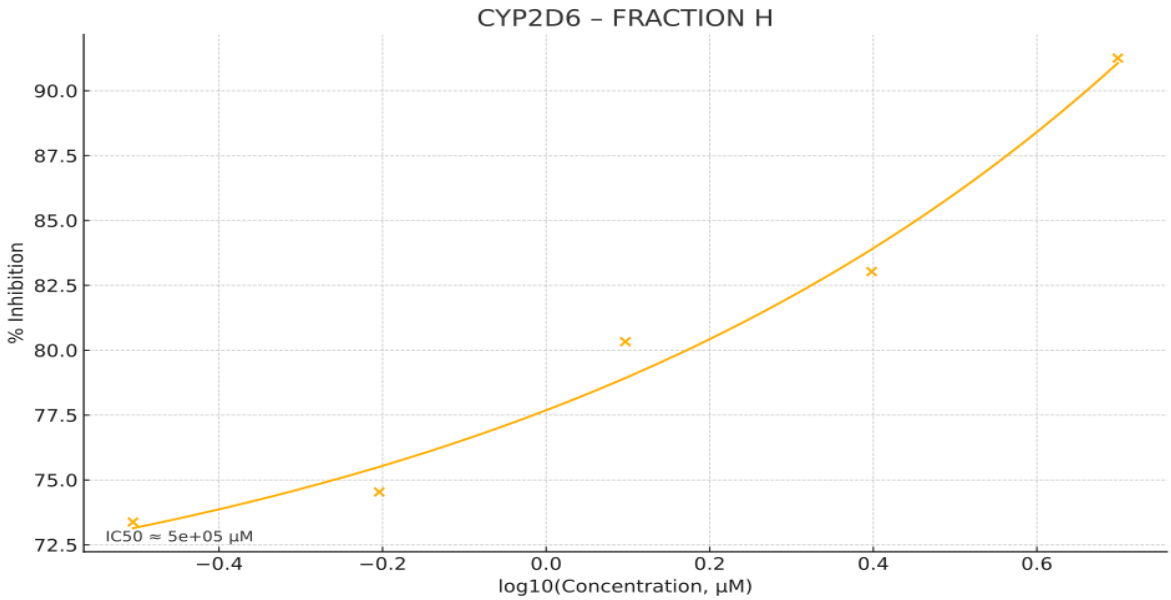

Figure: 9.4h: Dose-response curve for CYP2D6 inhibition by Fraction H. 5e+05 µm= 5×10^{5}µM

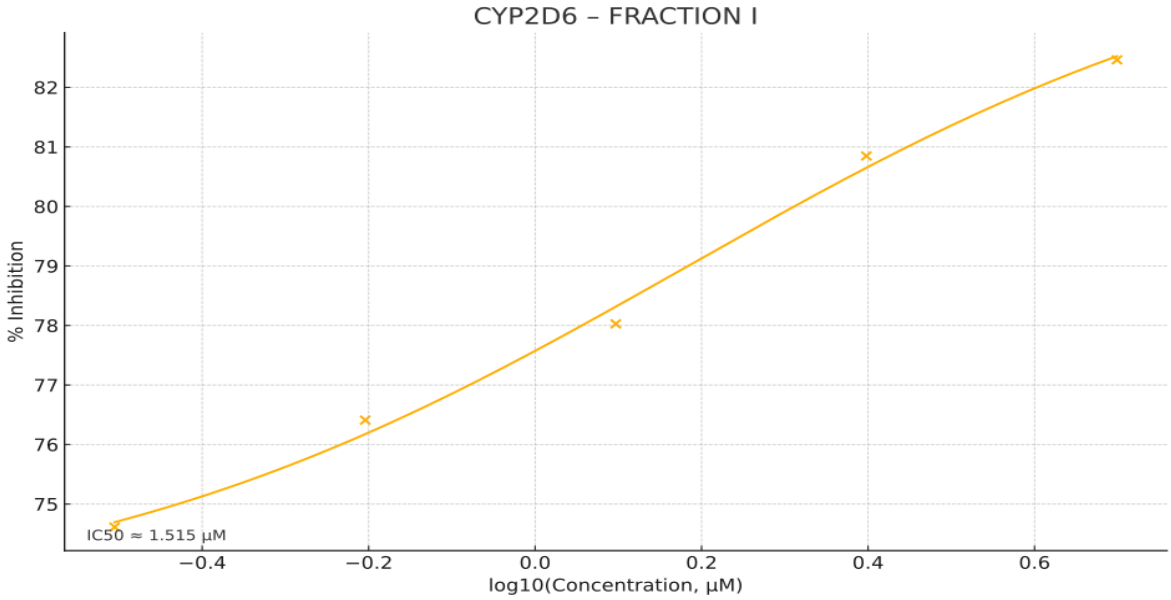

Figure: 9.4i: Dose-response curve for CYP2D6 inhibition by Fraction I