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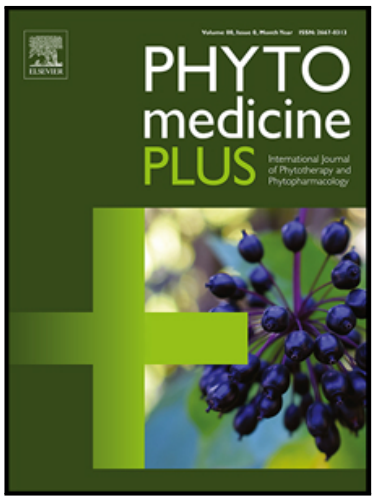

Evaluation of Southern African Wild Edible Plants for Potential Herb-Drug Interactions Through Ex Vivo P-Glycoprotein and In Vitro Cytochrome P450 3A4 Inhibitory Effects

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Evaluation of Southern African Wild Edible Plants for Potential Herb-Drug Interactions Through *Ex Vivo* P-Glycoprotein and *In Vitro* Cytochrome P450 3A4 Inhibitory Effects

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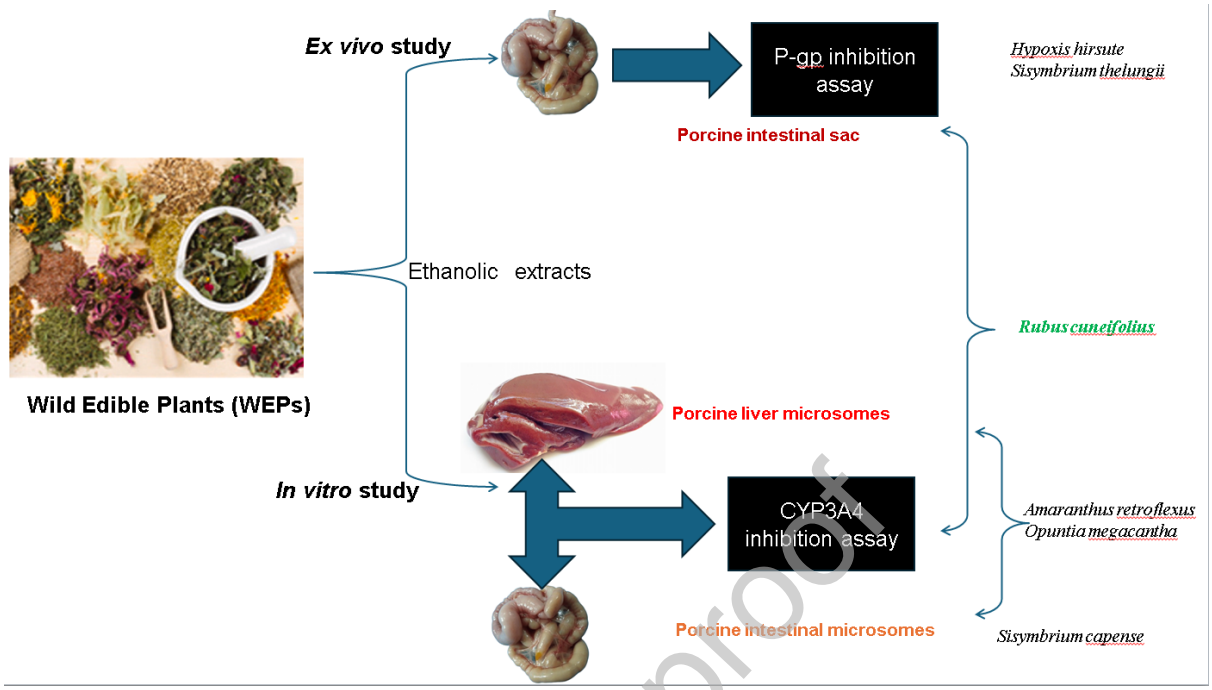
Graphical abstract



ABSTRACT

Background: Wild edible plants (WEPs) including herbs provide staple foods as well as income for local communities on the African continent. However, these commonly used plant materials interact with orthodox or conventional drugs through both p-glycoprotein (P-gp) and cytochrome P450 enzymes inhibition. Hence, it is vital to explore the possibility of herb-drug interactions when concomitantly taking conventional drug dosage forms with some of the WEPs. P-gp and CYP3A4 show analogous substrate specificities and work together to establish an intestinal absorption barrier against xenobiotics. This study investigated the *ex vivo* p-glycoprotein inhibition and the *in vitro* inhibition of cytochrome P450 3A4 (CYP3A4) isoezyme by selected wild edible plants to identify potential food/herb-drug interactions. **Methods:** Ethanolic extracts of 30 wild edible plants sourced from Lesotho, Eswatini and the Limpopo province of South Africa were used for the study. Drug transport studies using propranolol, a P-glycoprotein substrate, across porcine intestinal tissue were conducted using

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intestinal sacs with test solutions in Krebs-Ringer bicarbonate buffer (pH 7.4) under a 95% O2/5% CO2 atmosphere. For the *in vitro* luminescent-based CYP3A4 inhibition assay, both porcine liver and intestinal microsomes were extracted from their respective tissues by homogenization, followed by high-speed centrifugation and standardization before use.

Results: *Sisymbrium thelungii* O. E. Schulz significantly (p<0.05) inhibited P-gp with an effective apparent permeability coefficient ($P_{app\ eff}$) of (7.53±0.33)×10^{-6} cm/s and effective flux (J_{eff}) of 1.38±0.01 µg/cm^2/min which was similar to sodium orthovanadate (positive control), indicating potential for herb/food-drug interaction possibly by absorption enhancement of poorly permeable P-gp substrates by this plant. Additionally, both *Rubus cuneifolius* Pursh and *Hypoxis hirsuta* (L.) Coville had significant (p<0.05) p-glycoprotein inhibitory effects with $P_{app\ eff}$ of (6.60±0.52)×10^{-6} cm/s and J_{eff} of 1.11±0.08 µg/cm^2/min, and $P_{app\ eff}$ of 6.58±0.67 ×10^{-6} cm/s and J_{eff} of 1.19±0.12 µg/cm^2/min, respectively. Significantly (p<0.05), *Rubus cuneifolius* at a concentration of 0.2 mg/mL exhibited the strongest CYP3A4 inhibition in liver microsomes suggesting its potential as a bioavailability enhancer of CYP3A4 substrates. Meanwhile, *Wahlengergia androsacea* A.DC exhibited the most significant (p<0.05) CYP3A4 inhibitory effect at the same concentration in intestinal microsomes.

Conclusion: The observed inhibitory effects of certain wild edible plants on P-gp-mediated drug efflux and CYP3A4 drug metabolism indicate potential food/herb-drug interactions challenges for clinical practice. The herb/food-drug interactions also present opportunities for discovery of new functional excipients for enhancing the permeability of poorly absorbed orally administered drugs that are substrates of P-gp and CYP3A4.

Keywords p-glycoprotein, cytochrome P450 3A4 isoenzyme, microsomes, porcine intestinal tissue, wild edible plants

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Abbreviations: BCRP, breast cancer resistance protein; CYP3A4, cytochrome P450 3A4; KRB, Krebs-Ringer bicarbonate; MRPs, multidrug resistance proteins; P-gp, p-glycoprotein; WEP, wild edible plants.

1. Introduction

Wild edible plants (WEPs), which are defined as plants that grow spontaneously or not cultivated and can exist independently of direct human intervention, contribute to the diet of many communities in Africa (Kheoane et al., 2016; Maundu et al., 1999). For millennia, humans have relied on these plants to meet many of their basic medical needs. In many ancient and modern cultures, WEPs are an integral part of herbal medicines and have been used to treat many diseases. Nowadays, some of the WEPs are used to formulate botanical dietary supplements that people consume as a source of essential nutrients to maintain a healthy lifestyle. They provide staple and supplementary foods as well as cash income to local communities thus favouring food security (Al-Fatimi, 2021). WEPs have substantial potential for the development of new crops through domestication and provide a genetic resource pool for hybridization and selection (Al-Fatimi, 2021). The most common myth is that people consume these WEPs under the impression that they are completely safe because they are of natural origin.

However, the co-administration of allopathic medicines with herbs and/or wild edible foods causes many herb/food-drug interactions in clinical situations (Kar et al., 2021). The major challenge with herb/food-drug interactions is the associated alteration of the pharmacokinetics and pharmacodynamics of the drug by inhibiting or inducing drug transporters such as P-glycoprotein (P-gp) and drug-metabolizing enzymes mainly cytochromes P450 (CYPs) to affect drug transport and metabolism, respectively. P-gp and CYPs are expressed differently, but work in a coordinated manner to orderly eliminate and metabolize xenobiotics, which reduces intracellular drug concentrations (Li et al., 2016). Moreover, most drugs that interact with herbs are dual substrates for P-gp and CYP3A4 as both proteins are functionally synergistic and substantially share substrate specificity (Husain et al., 2023). Apart from being functionally synergistic, P-gp and CYP3A4 modulate pregnane X-receptor (PXR) activities and thus play crucial roles as intestinal absorption barriers against xenobiotics (e.g. drugs) (Watanabe et al., 2013).

P-glycoprotein (P-gp) is one of the most-characterized transport proteins in drug discovery history known to pump substrates out of cells using an ATP-dependent mechanism (Aashish et al., 2023). P-gp is responsible for modulating the plasma concentration level of the drug and regulates drug bioavailability by limiting its intestinal absorption. P-gp has broad substrate specificity and plays a significant role in the pharmacological and toxicological profile of its substrates (Kido et al., 2024). Meanwhile, the drug-metabolizing enzyme, CYP3A4 plays a significant role in the first-pass metabolism of several intestinally delivered drugs as it catalyzes numerous processes for drug activation or inactivation (Wang et al., 2023). It is highly expressed in the liver and small intestine and is the most important of the family of drug-metabolizing cytochrome P450 enzymes. In addition to its activity in the liver, CYP3A4 participates in the metabolic biotransformation of drugs and performs biosynthetic processing and drug oxidation in tissues such as the gastrointestinal tract and the brain (Wang et al., 2023).

Medicinal herbs, including WEPs, have the potential to improve the permeability and metabolism of drugs in the absorptive direction by modulating these secretory transporters and metabolic enzymes. For instance, *Hypericum perforatum* (St. John's wort), which is used to treat mild depression, plays a significant role in human drug disposition and hepatic drug metabolism by upregulating the expression of P-gp and numerous CYP isoforms including CYP3A4, respectively. As a result, *H. perforatum* reduces the efficacy of conventional drugs such as warfarin, cyclosporine, digoxin, indinavir, irinotecan, midazolam, and others that are substrates for P-gp and CYPs by decreasing their bioavailability (Elmeliegy et al., 2020; Husain et al., 2023; Nicolussi et al., 2020).

Conversely, plants such as *Zanthoxyli fructus* and St John’s Wort have demonstrated intestinal P-gp inhibitory effects *in vitro* and one of the mechanisms of action could be by increasing the accumulation of P-gp substrates within cells leading to enhanced bioavailability (Džubák et al., 2006; Yoshida et al., 2005). Ethyl acetate fractions of *Zanthoxyli fructus* showed a significant inhibitory effect of P-gp with an IC_{50} value of 166 μg/mL. Multiple oral administrations of St John's wort increased duodenal P-gp expression

by 1.4-fold in healthy volunteers. Also, St John's wort caused 18 % decrease of digoxin exposure after a single oral dose of digoxin (0.5 mg) (Durr et al., 2001). Moreover, previous reports on the modulation of P-gp by grapefruit juice to cause herb-drug interactions are well-documented (Elmeliegy et al., 2020; Husain et al., 2023; Lü et al., 2022). Similarly, grapefruit juice inhibits the pre-systemic metabolism of drugs such as cyclosporine and felodipine by inhibiting the catalytic activity of intestinal CYP3A4, which alters their pharmacokinetics and pharmacodynamic profiles to enhance their oral bioavailability (Bailey et al., 2013; Fuhr et al., 2023). Plant extracts of 13 WEPs, including *Curcuma longa*, *Piper nigrum*, and *Zingiber officinale* showed strong inhibitory CYP3A4 effects with IC_{50} values ranging from 1.3 and 10 μg/mL (Husain et al., 2023).

In terms of assay choice, *ex vivo* and *in vitro* studies are cheaper, faster, more ethical, and less labor-intensive options to evaluate drug absorption compared with *in vivo* studies (Majid et al., 2021). Studies have shown that experimental techniques using the gut of different animal models including rats, rabbits, pigs (porcine), dogs, and monkeys are useful to study the intestinal absorption of drugs. Among these animal models, pig or porcine intestines are preferred to study the intestinal passive absorption of drugs as there is a good correlation between the porcine and human intestines (Lunney et al., 2021).

P-gp and CYP3A4-mediated herb-drug interactions frequently occur in combined drug- and/or herb-based therapies (Abduraman et al., 2022; Elmeliegy et al., 2020). Since P-gp and CYP3A4 enzymes transport and metabolize a majority of therapeutically used medications, respectively, alterations in their activities by herbs may influence the pharmacokinetics of co-administered medicines, resulting in treatment failure or toxicity (Abduraman et al., 2022). However, there is a dearth in the P-gp and CYP3A4-mediated herb-drug interaction potential of many WEPs in Southern Africa.

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Therefore, the identification of WEPs that pose risks of pharmacokinetic herb-drug interactions through *ex vivo* and/or *in vitro* screening methods and the determination of their modulatory effects on drug efflux transporters and drug-metabolizing enzymes is important for their clinical and pharmaceutical formulation relevance. Hence, the present study investigated the *ex vivo* inhibition of P-gp and the *in vitro* inhibition of CYP3A4 by selected wild edible plants from the southern African region.

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2. Materials and methods

2.1 Selection of plant materials

Thirty wild edible plants were sourced from Lesotho, Eswatini, and the Limpopo province of South Africa, based on their frequent use by local communities and availability or peak season (Kheoane et al., 2016). The plant materials were identified by a taxonomist and voucher specimens were deposited in the Herbarium at the Department of Pharmaceutical Sciences, Faculty of Science, Tshwane University of Technology, Pretoria, South Africa.

2.2 Methods

2.2.1 Preparation of crude plant extracts

Wild edible fruits were dipped and liquidized in 200 mL of distilled water using a Kenwood Kitchen food blender, then frozen and lyophilized (VirTis Benchtop K, Model 2KBTES, SP Industries (Tarirai et al., 2012), while roots and leaves were shredded using a knife, air-dried at room temperature for 7 days and ground with a coffee grinder and subsequently size controlled through an aluminium sieve of (250 µm).

2.2.2 Extraction

Ethanolic extracts, containing a wide range of polar and non-polar compounds present in the WEPs, were prepared according to the modified methods (Khader et al., 2010; Mbara et al., 2022). Briefly, 150 g of the dried powder samples were soaked in 500 mL of 50% v/v ethanol and left to mix through magnetic stirring at room temperature for 3 days under sterile conditions until a slurry of uniform consistency was formed. The samples were later spun at 3000 rpm for 10 min and then filtered through a Whatman No. 1 filter paper. The filtrates were concentrated using a rotary evaporator (Model R-200 Buchi Rotavapor, Netherlands) at 200 rpm at 50 ºC for 20 minutes and freeze-dried at -50 ºC for 18 hours.

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2.2.3 Preparation of porcine intestinal sacs

The effects of wild edible plants on p-glycoprotein-mediated propranolol efflux were determined using a modified intestinal sac method described by Joubert et al. (2017). Intestinal tissues from pigs aged between 15-17 weeks old and weighing 70 kg on average, were generously donated by R & R Abattoir (Pretoria North, South Africa). Immediately after slaughter, approximately a 60 cm length of the small intestine was quickly excised and flushed several times with ice-cold Krebs-Ringer bicarbonate (KRB) buffer (pH 7.4). The intestinal tissue was preserved by immersing it in ice-cold KRB buffer (pH 7.4) in a cooler box for transportation. In the laboratory, approximately 4 cm lengths of the intestinal segment were excised and stripped of the outer serosal muscle under continuous lavage with cold KBR buffer.

2.2.4 Drug permeability studies across porcine intestinal tissue

Propranolol transport studies were done according to a modified version of a previously described method (Wuyts et al., 2015). Each 4 cm length of the porcine intestinal sac was ligated on one end and mounted in a 50 mL conical tube which was clamped onto an Ussing chamber water bath-controlled block heater. The sacs were filled with 7 mL of KRB buffer (pH 7.4) then equilibrated to the environment by submersion in 7 mL of KRB buffer (pH 7.4) in the conical flask at 37°C for 20 min before commencement of the drug transport study. Throughout the experiment, a mixture of 5% carbon dioxide and 95% oxygen was continuously bubbled into the conical flask to stir the contents and to more closely simulate intestinal conditions by preventing stagnant layer formation. The medium inside the intestinal sac (donor chamber, apical side of the intestinal sac) was replaced with 7 mL aliquots of either propranolol (50 µM); verapamil and sodium orthothanadate (positive controls, 400 µg/mL and 10 mM, respectively); or propranolol plus the WEP extracts, 2.5 mg/mL) and the

other end was then ligated; while the receiver chamber inside the conical flask (basolateral side of the intestinal sac) was refilled with fresh blank warm KBR buffer solution (pH 7.4 and 37 °C). A 300 µL volume of the sample was drawn from the receiver chamber inside the 50 mL conical tube (i.e. the basolateral side of each intestinal sac) at 20-minute time intervals for 2 hours and was immediately replenished with a 300 µL volume of KBR buffer solution (pH 7.4 and 37 °C). Propranolol concentrations were determined using a Spectrostar (BMG LabTech, Germany) microplate reader at 319 nm.

2.2.4.1 Data analysis

The concentrations of propranolol transported from the apical to basolateral side of each porcine intestinal sac were calculated using a propranolol standard curve equation. The percentage of propranolol transported from the apical-basolateral direction across the porcine intestinal sac tissue was calculated using equation (1):

$$\%\ drug\ transported = \left(\frac{Amount\ of\ drug\ transported}{Initial\ amount\ of\ drug\ added\ to\ the\ sac}\right) \times 100$$ [Equation 1]

The apical surface area, A (cm^2), of the porcine intestinal sac that was exposed to the test sample was derived from the equation (2):

$$A = 2 \times \pi \hat{r}^2 + (L \times W)$$

[Equation 2]

Where r̂ is the average radius of the intestinal cylinder at each open end, L is the length of the tube (4 cm), and W is the width (circumference) of the rectangle formed when the intestinal tube is dissected open.

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Since the intestinal sac was tied at both ends, the radius approached zero (r̂ →0). Therefore, equation (2) was simplified to equation (3):

$$A_{eff} = L \times W$$

[Equation 3]

Where A_{eff} is the effective interior surface area of the intestinal sac.

The effective apparent permeability coefficient ($P_{eff\ app}$), which indicates the apical-basolateral permeability of propranolol across the membrane, was calculated using equation (4):

$$P_{eff\ app} = \frac{dQ}{dt} \times \frac{1}{C_0 \times A_{eff}} \ (cm/s)$$ [Equation 4]

Where dQ/dt (µg/s) is the rate at which propranolol increased within the receiving chamber, A_{eff} (cm^2) is the surface area of the excised porcine intestinal sac lumen exposed to the test solution, and C_0 is the initial propranolol concentration (µg/cm^3) in the donor chamber (apical or luminal side) (Engdal and Nilsen, 2008; Hansen and Nilsen, 2008).

The effective flux (J_{eff}) of propranolol across the porcine intestinal sac was determined using equation (5):

$$J_{eff} = \frac{V(dC/dt)}{A_{eff}} \ (\mu g/cm^2/min)$$

[Equation 5]

Where V (mL) is the receiver chamber volume, dC/dt [(µg/mL)/min] is the change in propranolol concentration in the receiver chamber per transport time, and A_{eff} (cm^2) is the effective surface area of the porcine intestinal sac lumen that is in contact with the test solution (Engdal and Nilsen, 2008; Hansen and Nilsen, 2008).

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2.2.5 Extraction of microsomes from porcine liver and jejunum tissues

Porcine hepatic tissue (approx. 2 kg in weight) was donated by Pork Packers Irene (Centurion, South Africa) while porcine jejunum tissue of about 40 cm in length was received as donation from R & R Abattoir (Pretoria North, South Africa) collected immediately after pigs aged between 15-17 weeks old and weighing 70 kg on average, were slaughtered. Both hepatic and jejunum tissues were submerged in ice-cold Krebs ringer bicarbonate buffer, pH 7.4, in an ice cooler box for transportation (Tarirai et al., 2012).

Intestinal and liver tissue microsomes were extracted according to a modified method (Damre et al., 2009). Small pieces of the tissues (approximately 1 g each) were washed with 0.154 M potassium chloride solution and homogenized in ice-cold homogenization buffer (50 mM Tri-HCl, 150 mM KCl, pH 7.4) using an IKA-T-18 Basic Homogenizer 115VAC (Cole-Parmer, South Africa). The homogenates were centrifuged at 15 000 x*g* (Optima™ XE, Beckman Coulter, South Africa) for 30 min at 4°C to extract the microsomes. The slurry was centrifuged at 105 000 x*g* (Optima™ XE, Beckman Coulter, RSA) for 60 min at 4°C to pellet down the microsomes. The microsomal pellets were resuspended in a storage buffer (0.1 M Tris-HCl, 20% glycerol, 1 mM EDTA, pH 7.4), homogenized, and stored at – 80°C for further use.

To standardise the quality of the microsomes, protein content of microsomes was determined using a Quick Start™ Bradford protein assay kit (Bio-rad Laboratories, USA) according to the manufacturer's guidelines. Linearity of the microsomal protein content versus their CYP3A4 activity was determined using luciferin-benzyl ether (luciferin-BE) as a CYP3A4 substrate. Different incremental protein concentrations of liver microsomes (0.5, 3.6, and 5 mg/mL) and intestinal microsomes (0.5, 4.6, and 6.0 mg/mL) were prepared using Luciferin-free water to dilute microsomes to achieve the different concentrations. CYP3A4 activity

assays of the liver and intestinal microsomes were carried out as described in the Promega P450-Glo^{TM} screening manual (Promega, USA). The ration of the substrate to CYP3A4 concentrations was kept constant by volume (0.94 µL of Luciferin-BE per 30 µL of CYP3A4).

2.2.6 CYP3A4 inhibition assay

Inhibitory effects of the WEP extracts (0.5 mg/mL and 1.0 mg/mL i.e. the recommended 5X working concentrations) on CYP3A4 activity were determined using a luminescence-based method adapted from the Promega P450-Glo™ CYP3A4 assays screening manual (Promega, USA), containing luciferin-isopropyl acetal (luciferin-IPA) and luciferin detection reagent. This assay measured the rate and extent of conversion of luciferin-isopropyl acetal (CYP3A4 substrate) to a luminescent product. Cimetidine was prepared at a concentration of 2.5 mg/mL and served as a positive control. Briefly, media containing 12.5 µL of the test compounds were added to each microsomes-containing microplate well except to the "untreated and minus-P450 control" wells, followed by the addition of 25 µL volume of the NADPH regeneration system to start the reaction. Fresh media containing 50 µL luciferin isopropyl acetal (Luc-IPA, a CYP3A4 substrate diluted 1:1000) was added to each well and incubated with 5% CO_2/95% O_2 at 37 °C for 30 minutes (compared to 5-10 min for CYP3A4 enzyme or 30-60 minutes for cell-based assays). Finally, 50 µL of reconstituted luciferin detection (P450-Glo^{TM}) reagent was added to the microsomal CYP assays and the control wells. The assays were equilibrated in excess (>20 min) for 30 minutes with luminescence (P450 activity) readings being recorded every 5 minutes using a Glomax Multi+ detection luminometer (microplate reader) system (Promega, USA).

2.2.6.1 Data analysis

Percentage inhibition of CYP3A4 by WEP extracts was calculated using equation (6):

$$\%\ inhibition\ of\ CYP3A4 = \left(\frac{(RLUb - RLUt)}{RLUb}\right) X\ 100 \qquad \text{[Equation 6]}$$

Where RLU (relative luminescence unit) is the luminescence obtained after subtracting background luminescence, b is the blank, and t is the test compound.

3. **Results**

3.1 P-gp inhibitory effects of wild edible plant extracts

The percentages of propranolol transported in the apical to the basolateral direction across the porcine intestinal sac tissue are shown in Figure 1. WEP that elicited ≤40% of propranolol transport were not included in the data in Figure 1. Three WEP extracts had significant ($p < 0.05$) P-gp inhibitory effects and thus improved the concentration of propranolol transported to the basolateral side of the intestinal sac tissue. Among these, *Sisymbrium thelungii* showed the highest percentage propranolol transport of 99.95 ± 0.52% after 2 hrs, followed by *Rubus cuneifolius* (96.74 ± 9.29%) and *Hypoxis hirsute* (94.76 ± 2.25%). Twenty-three of the 30 WEP extracts did not improve the absorption of propranolol from the apical to basolateral direction. *Sisymbrium thelungii* showed the highest P-gp inhibitory effects with $P_{app\ eff}$ of 7.53 ± 0.33 × 10^{-6} cm/s followed by *Rubus cuneifolius* with $P_{app\ eff}$ of 6.60 ± 0.52 × 10^{-6} cm/s and *Hypoxis hirsute* with $P_{app\ eff}$ of 6.58 ± 0.67 × 10^{-6} cm/s. The effective apparent permeability coefficient ($P_{app\ eff}$) and effective flux (J) data of propranolol from the apical to basolateral sides of the porcine intestinal sac are summarized in Table 1.

[Figure 1 and Figure 1 (cont'd) here]

[Table 1 here]

3.2 CYP3A4 inhibitory activity of wild edible plant extracts using porcine liver microsomes

The effects of WEP on CYP3A4 activity of liver tissue microsomes are shown in Figure 2. Different WEP demonstrated varying inhibition percentages on CYP3A4 activity ranging from 10% to 100% as compared to cimetidine (positive control). CYP3A4 inhibition was concentration dependent. The investigation of CYP3A4 activity in 30 WEP revealed that 20

plants inhibited liver microsomal CYP3A4 activity at a concentration of 0.1 mg/mL. Among these, 15 plants showed inhibitory effects at both 0.1 mg/mL and 0.2 mg/mL (Figure 2), while *Sonchus asper*, *Nemesia fruticans*, *Berkheya purpurea*, *Hypochaeris radicata*, and *Cyperus esculentus* only exhibited effects of 72%, 60%, 46%, 61% and 57%, respectively, at 0.1 mg/mL (data not shown in Figure 2). *Rubus cuneifolius* demonstrated the highest inhibition, reaching 100% at 0.2 mg/mL. In contrast, *Sisymbrium thelungii* had the lowest CYP3A4 inhibition, whereas *Sisymbrium capense* showed the strongest inhibition of 75% at both concentrations.

[Figure 2 here]

Other plants which showed CYP3A4 inhibition include *Opuntia megacantha*, *Sonchus dregeanus*, *Rorippa nudiuscula*, *Amaranthus retroflexus*, *Chenopodium album*, *Urtica dioica*, *Wahlengergia androsacea*, *Tragopogon porrifolius*, *Sonchus asper*, *Nemesia fruticans* and *Nasturtium officinale*. WEP which did not show any inhibitory effects on CYP3A4 and are not shown in Figure 2 include *Hypoxis hirsute*, *Sonchas integrifolius*, *Solanum retroflexum*, *Momordica foetida*, *Corchorus tridens*, *Amaranthus hybridus*, *Amarathus spinosus*, *Bidens pilosa*, *Momordica involucrate* and *Discorea minutiflora*.

3.3 CYP3A4 inhibitory activity of wild edible plant extracts using porcine intestinal microsomes

The effects of 20 wild edible plants on CYP3A4 activity in porcine intestinal microsomes are presented in Figure 3. Out of the 20 WEP tested, only 10 showed CYP3A4 inhibitory activity at a concentration of 0.1 mg/mL. Among these, *Rorippa nudiuscula*, *Urtica dioica*, *Wahlenbergia androsacea*, and *Lepidium capense* also exhibited inhibitory effects at 0.2 mg/mL achieving 52%, 44%, 85%, and 41% CYP3A4 inhibition, respectively, with *Wahlenbergia androsacea* demonstrating the strongest inhibition at this concentration.

However, *Wahlengergia androsacea* showed lower CYP3A4 inhibition of 23% at the concentration of 0.1 mg/mL while *Chenopodium album* had the lowest overall CYP3A4 inhibitory effects at both concentrations.

[Figure 3 here]

Rorippa nudiuscula showed the highest CYP3A4 inhibition at both concentrations of 0.1 mg/mL and 0.2 mg/mL. Other WEP which had CYP3A4 inhibitory effects were *Rubus cuneifolius*, *Opuntia megacantha*, *Sonchus dregeanus*, *Rosa rubiginosa* and *Amaranthus retroflexus*. However, these plants had CYP3A4 inhibitory effects at a lower concentration of 0.1 mg/mL, but did not show any CYP3A4 inhibitory effects at a higher concentration of 0.2 mg/mL. *Momordica foetida* showed little CYP3A4 inhibitory effects of 10% at higher concentration of 0.2 mg/mL.

Similarly, *Hypoxis hirsute*, *Sonchas integrifolius*, *Solanum retroflexum*, *Momordica foetida*, *Corchorus tridens*, *Amaranthus hybridus*, *Amarathus spinosus*, *Bidens pilosa* and *Discorea minutiflora* did not demonstrate any CYP3A4 inhibitory effects in the intestinal microsomes and are therefore excluded from Figure 3. Table 2 presents a summary of CYP3A4 activity inhibition by the wild edible plants.

[Table 2 here]

4. Discussion

WEP have played a significant role throughout human history as many people rely on them for sustenance and nourishment during times of food scarcity and to provide remedies for minor health issues. However, due to their rich phytochemical composition, there is a high probability of herb/food-drug interactions associated with their consumption or self-medication, which can mimic, increase, or reduce the pharmacokinetic and pharmacodynamic

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effects of allopathic medications when combined. Therefore, it is imperative to clarify whether WEP can alter the activity and expression of P-gp and CYP3A4, recognized as the representative drug transporter and/or metabolic proteins, respectively, to predict their interaction with prescription drugs.

The inhibition of efflux transporters such as P-gp may lead to adverse herb/food-drug interactions. Conversely, this could be an opportunity to improve the bioavailability of P-gp substrates such as propranolol through absorption enhancement of these drugs. In the present study, *Sisymbrium thelungii*, *Rubus cuneifolius*, and *Hypoxis hirsute* significantly ($p<0.05$) inhibited P-gp mediated propranolol efflux, indicating that the concomitant use of these plants with P-gp substrates could inhibit their efflux into the lumen, and thus improving their absorption and bioavailability. Moreover, these plants could serve as sources of raw materials from which active biomolecules or lead compounds, capable of inhibiting P-gp, may be extracted for use as drug absorption enhancer. *Sisymbrium thelungii* showed the highest P-gp inhibitory effects with $P_{app\ eff}$ of 7.53 ± 0.33 × 10^{-6} cm/s and flux $(J)_{eff}$ of 1.38 ± 0.01 µg/cm^2/min, which were similar to sodium orthovanadate (positive control) with $P_{app\ eff}$ of 7.87 ± 0.18 × 10^{-6} cm/s and flux $(J)_{eff}$ of 1.97 ± 0.00 µg/cm^2/min, followed by *Rubus cuneifolius* with $P_{app\ eff}$ of 6.60 ± 0.52 × 10^{-6} cm/s and flux $(J)_{eff}$ of 1.11 ± 0.08 µg/cm^2/min, and *Hypoxis hirsute* with $P_{app\ eff}$ of 6.58 ± 0.67 × 10^{-6} cm/s and flux $(J)_{eff}$ of 1.19 ± 0.12 µg/cm^2/min. This observation implies that *Sisymbrium thelungii*, *Rubus cuneifolius,* and *Hypoxis hirsute* could influence drug systemic exposure hence affect clinical outcomes and/or could be used as an alternative natural oral bioavailability enhancer for drugs that are substrates of the P-gp efflux system. WEP that decreased propranolol transport from the apical to basolateral side of the porcine intestinal sac were probably exerting competition for the efflux pump. The coadministration of propranolol with such WEP could lead to

therapeutic failure by inducing changes in drug oral bioavailability, resulting in changes in drug concentrations.

P-gp acts as an ATP-dependent efflux pump for drugs and/or xenobiotics. Sodium orthovanadate acts by inhibiting the P-gp ATPase activity (Kita et al., 2022; Sankaran et al., 1997). Given the closeness of permeability data of for sodium orthovanadate to those of *Sisymbrium thelungii*, *Rubus cuneifolius* and *Hypoxis hirsute* , these extracts possibly inhibit P-gp ATPase activity, which suggests the potential of WEP to directly interact with P-gp substrate binding. It has previously been reported that medicinal plants and their constituents allosterically modulate human P-gp on both drug-binding and ATPase-binding sites (Mondal et al., 2022; Najar et al., 2010). The phytochemical profile of *Sisymbrium thelungii*, *Rubus cuneifolius,* and *Hypoxis hirsute* merits further investigation to determine specific compounds which can be linked to their significant P-gp inhibitory effects.

Cytochrome P450 enzymes (like CYP3A4) can be found both in the liver and the intestines where they can be inhibited or enhanced by drugs and herbal materials (Gerges and El-Kadi, 2023). In the present study, the inhibitory effects of WEP on CYP3A4 in porcine liver and intestinal microsomes were preferable to rodent models for translational and clinical research applications due to similarity of pig and human physiology (Lunney et al., 2021). The inhibitory behavior of WEP on the liver and intestinal microsomes provides important information of their effects on first-pass metabolism of both orally and non-orally administered drugs. In the present study, the inhibitory effects of WEP on CYP3A4 activity in hepatic microsomes had almost the same results as CYP3A4 activity assays in intestinal microsomes (Table 2), which aligns with the emerging evidence that the small intestines can even play more significant role in CYP3A4-mediated first-pass metabolism as the liver (Basheer and Kerem, 2015).

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However, due to seasonal scarcity and variability of plant extracts, only 20 WEPs were examined for their CYP3A4 activity using intestinal microsomes. The remaining 10 WEPs were unavailable for further research due to seasonal factors, and they were not included in the subsequent season's studies to prevent inconsistencies in phytochemical content that could arise from seasonal variations. Seasonal availability can significantly influence the chemical composition of plants, making it essential to conduct studies during consistent periods to ensure reliable results. For example, seasonal variations, including changes in temperature, rainfall, and humidity, can impact the levels of essential phytochemicals in plants, which may consequently influence their medicinal properties or nutritional value (Kabubii et al., 2023). Hence, 20 WEPs were considered for the study to maintain the integrity of their findings and avoid any potential variability that could compromise the study's outcomes. *Wahlengergia androsacea, Rorippa nudiuscula, Rubus Cuneifolius, Opuntia megacantha, Sonchus dregeanus, Rosa rubiginosa* and *Amaranthus retroflexus* had CYP3A4 inhibitory effects on both the liver and intestinal microsomes (Figures 2 and 3), suggesting that these herbs could decrease the metabolism of drugs which are metabolized by this enzyme, possibly leading to increased bioavailability and toxicity.

Previous *in vitro* studies have reported significant inhibitory effects of medicinal herbs on CYP3A4-mediated metabolism (Rombolà et al., 2020; Zhang and Lim, 2008). Moreover, methanol extracts of *Euterpe oleracea* Mart. (açaí) berries have been reported to induce CYP3A4-mediated botanical-drug interactions (Zhang et al., 2019. Inhibition of CYP3A4 by most of the extracts could be associated with the conformational flexibility of the enzyme and its ability to bind with a wide range of structurally diverse phytochemical components to increase their modulatory effects (Wright et al., 2019). Therefore, the concomitant use of these WEPs with CYP3A4 substrates should be cautioned and may require dose adjustment. However, from a different perspective, for drugs that are extensively metabolized by the

hepatic CYP450 enzymes, inhibition of these enzymes could lead to improved bioavailability of drugs that undergo the first-pass metabolism.

Porcine intestinal tissue is often utilized because it closely resembles human intestinal tissue (Schaaf et al., 2023), making it a valuable model for research. However, its use presents certain limitations. For instance, it does not perfectly replicate the conditions found in the human body, which can affect the clinical generalizability or extrapolation of the results. Additionally, variations in the composition of compounds in WEPs due to different geographical locations and climate conditions may also pose a limitation, potentially influencing the study's outcomes in another setting. Therefore, future research studies that involves specific pure phytochemicals from these WEPs and *in vivo* models would provide insights into how the findings of the present study translate to living organisms, potentially offering a more accurate representation of physiological processes. Thus, investigating the specific phytochemicals responsible for the observed effects could also help identify the active compounds and understand their mechanisms of action. This targeted approach could enhance the effectiveness and application of the research outcomes, providing a clearer picture of the therapeutic potential and safety of the compounds involved.

5. Conclusion

The present study demonstrates the inhibitory effects of ethanolic extracts of selected Southern African wild edible plants on P-gp and CYP3A4 activity. The extracts of *Sisymbrium thelungii*, *Rubus cuneifolius,* and *Hypoxis hirsute* showed inhibitory effects on p-glycoprotein-mediated propranolol efflux indicating potential herb/food-drug interaction. There is a correlation between the inhibitory effects of WEPs on CYP3A4 activity of liver and intestinal microsomes. CYP3A4 activity assays indicated that extracts of 20 WEPs considerably inhibited CYP3A4 activity in hepatic and intestinal microsomes with 11 of these

inhibiting the catalytic activity of CYP3A4 in a concentration-dependent manner. The observed inhibitory effects of WEPs on P-gp and CYP3A4 give credence to the claim that certain WEPs have the potential to affect the pharmacokinetics of conventional drugs.

STATEMENTS AND DECLARATIONS

Competing Interests

The authors hereby declare that they have no conflicts of interest.

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ETHICAL APPROVAL

Use of porcine tissue was approved by the Tshwane University Animal Ethics Committee under Ethics Ref#: AREC2018/05/001TariraiC and Ref#: AREC2019/09/003MokhethoKC.

CREDIT AUTHORSHIP CONTRIBUTION STATEMENT

PS Kheoane: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Visualization, Writing – original draft, Writing – review & editing. KC Mokhetho: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Visualization, Writing – original draft, Writing – review & editing. S Mokhele: Data curation, Formal analysis, Methodology. KC Mbara: Project administration, Validation,

Writing – review & editing. CM Leonard: Writing – review & editing, Supervision. HT Gadaga: Writing – review & editing, Supervision. C Tarirai: Conceptualization, Data curation, Formal analysis, Funding acquisition, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – review and editing

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Figure captions:

Figure 1: Propranolol transport in the apical to basolateral direction across the porcine intestinal sac (value = mean ± sd, n = 3; *indicates a statistically significant inhibition compared to sodium orthovanadate positive control, $p<0.05$)

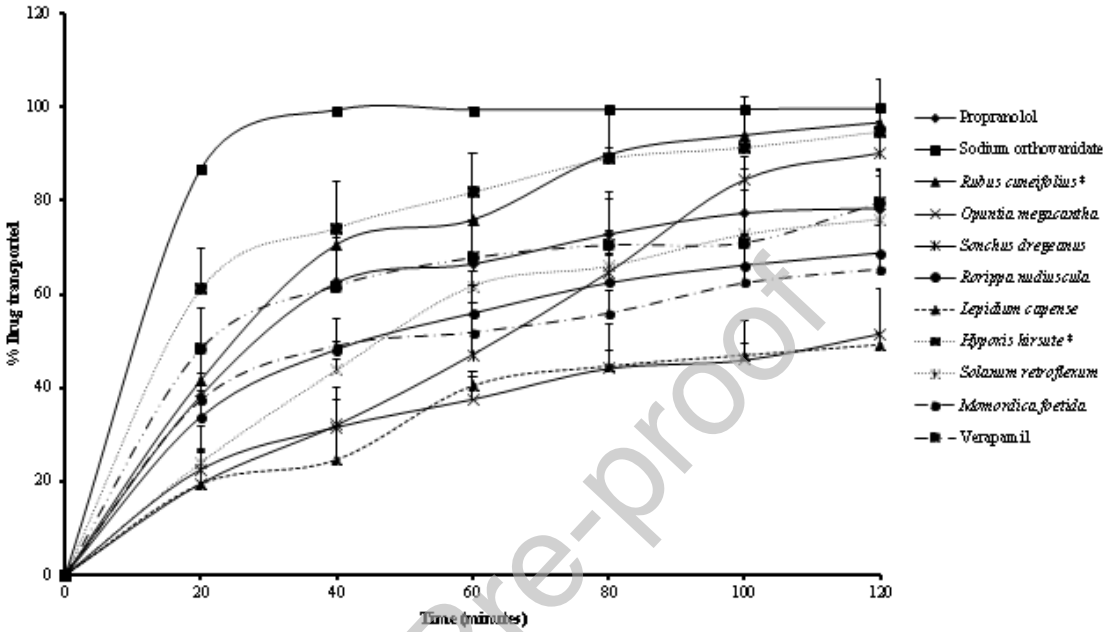

Figure 1 (cont'd): Propranolol transport in the apical to basolateral direction across the porcine intestinal sac (value = mean ± sd, n = 3; *indicates a statistically significant inhibition compared to sodium orthovanadate positive control, $p<0.05$)

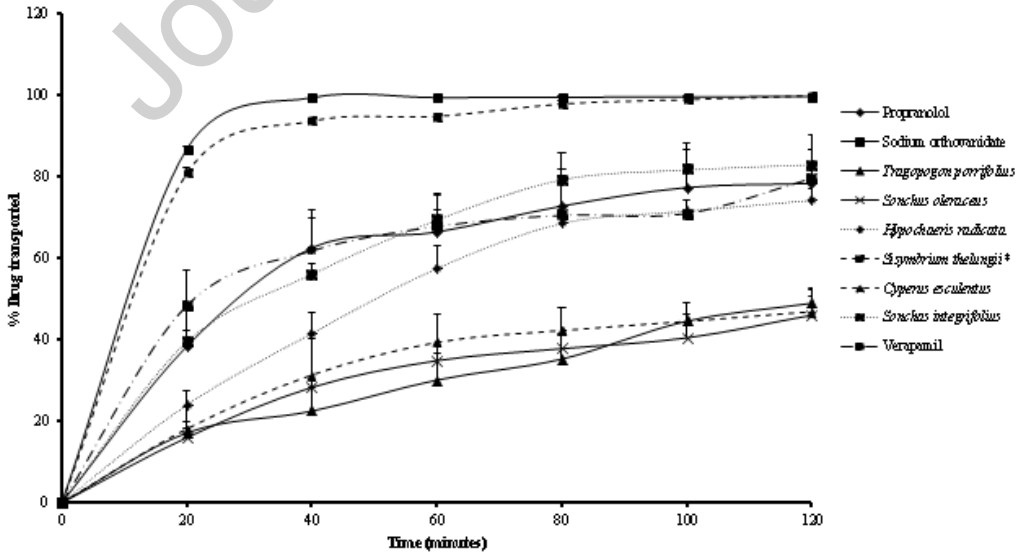

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Figure 2: CYP3A4 activity inhibition by WEP in porcine liver microsomes (value = mean ± sd, n = 3; *indicates a statistically significant inhibition compared to cimetidine positive control, $p<0.05$)

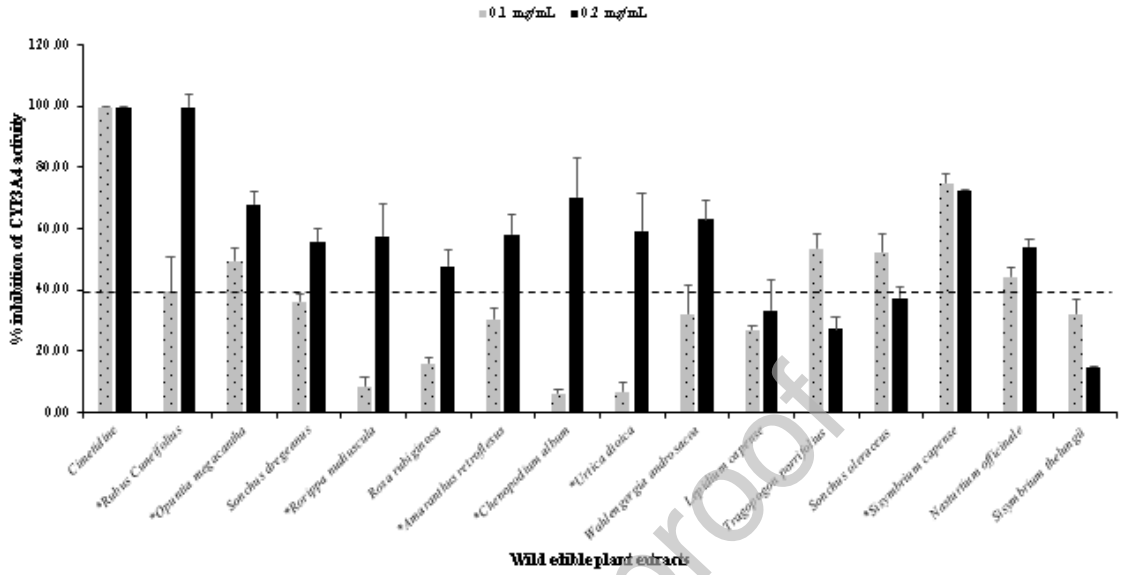

Figure 3: CYP3A4 activity inhibition by WEP in porcine intestinal microsomes (value = mean ± sd, n = 3; *indicates a statistically significant inhibition compared to cimetidine positive control, $p<0.05$)

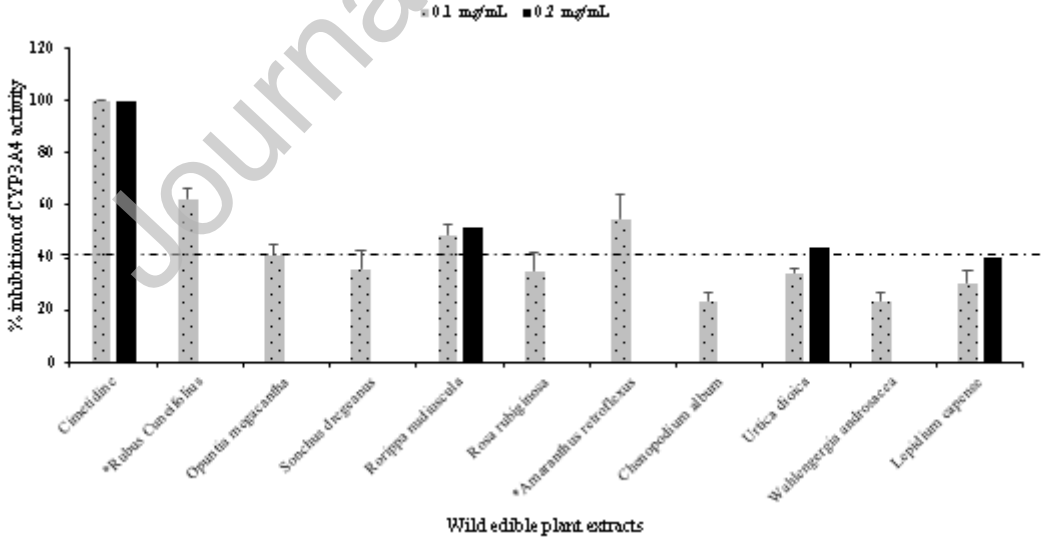

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Table headings:

Table 1: Effective apparent apical to basolateral permeability and flux data for propranolol in the presence of wild edible plant extracts

Table 2: Inhibition of CYP3A4 activity by wild edible plant extracts

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Table 1: Effective apparent apical to basolateral permeability and flux data for propranolol in the presence of wild edible plant extracts

Test sample	**P-gp inhibitory activity[#]**		**Conclusion**
	$P_{app\ (A-B)\ eff}$ **(x10^{-6} cm/s)**	$J_{(A-B)\ eff}$ **(ug/cm^2/min)**	
Propranolol (P-gp substrate)	5.74 ± 0.74	0.97 ± 0.14	P-gp substrate
Sodium orthovanidate (positive control)	7.87 ± 0.18	0.97 ± 0.00	P-gp inhibitor
Verapamil (negative control)	5.5 ± 3.57	0.99 ± 0.11	P-gp substrate
***Rubus Cuneifolius* Pursh [b]**	**6.60 ± 0.52**	**1.11 ± 0.08**	**P-gp inhibitor**
Opuntia megacantha	3.11 ± 0.51	0.55 ± 0.09	No inhibition
Sonchus dregeanus	3.97 ± 0.65	0.69 ± 0.12	No inhibition
Rorippa nudiuscula	4.67 ± 0.44	0.82 ± 0.08	No inhibition
Rosa rubiginosa	1.80 ± 0.49	0.29 ± 0.07	No inhibition
Amaranthus retroflexus	2.17 ± 0.74	0.39 ± 0.13	No inhibition
Chenopodium album	2.16 ± 0.47	0.37 ± 0.06	No inhibition
Urtica dioica	1.72 ± 0.41	0.30 ± 0.08	No inhibition

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Wahlengergia androsacea	2.18 ± 0.16	0.39 ± 0.02	No inhibition
Lepidium capense	3.26 ± 0.14	0.59 ± 0.02	No inhibition
Tragopogon porrifolius	2.45 ± 0.59	0.44 ± 0.09	No inhibition
Sonchus oleraceus	3.02 ± 0.68	0.51 ± 0.12	No inhibition
Sonchus asper	1.17 ± 0.36	0.20 ± 0.06	No inhibition
Sisymbrium capense	1.79 ± 0.48	0.29 ± 0.06	No inhibition
Nemesia fruticans	1.76 ± 0.35	0.29 ± 0.06	No inhibition
Nasturtium officinale	1.69 ± 0.60	0.29 ± 0.10	No inhibition
Berkheya purpurea	2.80 ± 0.34	0.45 ± 0.07	No inhibition
Hypochaeris radicata	4.91 ± 0.42	0.84 ± 0.08	No inhibition
Sisymbrium thelungii [a]	**7.53 ± 0.33**	**1.38 ± 0.01**	**P-gp inhibitor**
Cyperus esculentus	3.39 ± 0.77	0.57 ± 0.10	No inhibition
Hypoxis hirsute [b]	**6.58 ± 0.67**	**1.19 ± 0.12**	**P-gp inhibitor**
Sonchas integrifolius	5.77 ± 0.17	1.01 ± 0.03	No inhibition

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Solanum retroflexum	5.31 ± 0.18	0.90 ± 0.04	No inhibition
Momordica foetida	4.26 ± 0.69	0.76 ± 0.09	No inhibition
Corchorus tridens	1.61 ± 0.73	0.28 ± 0.12	No inhibition
Discorea minutiflora	2.86 ± 0.58	0.05 ± 0.01	No inhibition

[#] $P_{app\ (A\text{-}B)\ eff}$ = effective apparent drug permeability for apical to basolateral direction; $J_{(A\text{-}B)\ eff}$ = effective drug flux for apical to basolateral direction.

[a, b] Different letters indicate significant ($p<0.05$) statistical difference in P-gp inhibition compared to the positive control.

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Table 2: Inhibition of CYP3A4 activity by wild edible plant extracts

Plant botanical name	CYP3A4 inhibitory activity		Conclusion
	Liver microsomes	Intestinal microsomes	
Cimetidine (positive control)	Yes	Yes	CYP3A4 Inhibitor
***Rubus Cuneifolius* Pursh**[a]	**Yes**	**Yes**	**CYP3A4 Inhibitor**
***Opuntia megacantha* Salm-Dyck**[a]	Yes	Yes	CYP3A4 Inhibitor
Sonchus dregeanus DC.	Yes	Yes	CYP3A4 Inhibitor
Rorippa nudiuscula Thell.	Yes	Yes	CYP3A4 Inhibitor
Rosa rubiginosa L.	Yes	Yes	CYP3A4 Inhibitor
***Amaranthus retroflexus* (Richter & Loret) Thell. .**[a]	**Yes**	**Yes**	**CYP3A4 Inhibitor**
Chenopodium album L. [b]	Yes	Yes	CYP3A4 Inhibitor
Urtica dioica L. [b]	Yes	Yes	CYP3A4 Inhibitor
Wahlengergia androsacea A.DC.	Yes	Yes	CYP3A4 Inhibitor
Lepidium capense Thunb.	Yes	[#]Not tested	CYP3A4 Inhibitor
Tragopogon porrifolius L.	Yes	[#]Not tested	CYP3A4 Inhibitor
Sonchus asper[b]	Yes	[#]Not tested	CYP3A4 inhibitor

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***Sisymbrium capense* Thunb.** [a]	Yes	[#]Not tested	CYP3A4 inhibitor
Nemesia fruticans (Thunb.) Benth. [b]	Yes	[#]Not tested	CYP3A4 inhibitor
Nasturtium officinale R.Br.	Yes	[#]Not tested	CYP3A4 inhibitor
Berkheya purpurea (DC.) Mast	Yes	[#]Not tested	CYP3A4 inhibitor
Hypochaeris radicata L. [b]	Yes	[#]Not tested	CYP3A4 inhibitor
Sisymbrium thelungii O. E. Schulz	Yes	[#]Not tested	CYP3A4 inhibitor
Cyperus esculentus L.	Yes	[#]Not tested	CYP3A4 inhibitor
Hypoxis hirsute (L.) Coville	No	No	No inhibition
Sonchus integrifolius Harv.	No	No	No inhibition
Solanum retroflexum Dunal	No	No	No inhibition
Momordica foetida Schumach.	No	No	No inhibition
Corchorus tridens L.	No	No	No inhibition
Amaranthus hybridus L.	No	No	No inhibition
Amarathus spinosus L.	No	No	No inhibition
Bidens Pilosa L.	No	No	No inhibition
Momordica balsamina L.	No	No	No inhibition

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Discorea minutiflora Engl.	No	No	No inhibition

[#]Plant extract was not tested due to seasonal shortage.
[a,b] Different letters indicate significant ($p<0.05$) statistical difference in CYP3A4 inhibition compared to the positive control.

Competing Interests

The authors hereby declare that they have no conflicts of interest.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The author is an Editorial Board Member/Editor-in-Chief/Associate Editor/Guest Editor for *[Journal name]* and was not involved in the editorial review or the decision to publish this article.

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