IN VITRO SCREENING OF CYP450 3A4 INHIBITION EFFECT OF SELECTED MALAYSIAN ULAM

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Abstract: Introduction: CYP3A4 enzyme metabolises more than half of all marketed medicine and is regarded as a major enzyme as it is involved in majority of phase I metabolism. Apart from that CYP3A4 has also been implicated in food-drug interactions such as grapefruit-CYP3A4 and St John's wort-CYP3A4. Traditional vegetables termed "ulam" is widely consumed in Malaysia, either in raw form or cooked. This is especially due to the people's increasing awareness of healthy lifestyle which makes vegetable consumption a big part of their diet. Therefore, knowledge regarding inhibitory potentialof ulam constotuents toward the CYP3A4 is important so that informed choices could be made in order to manage the risk of food-drug interactions. Therefore, this study wants to investigate the potential of CYP3A4 inhibition by active constituents of ulam to determine the existence of ulam-drug interaction. **Methods:** Four types of ulam was investigated that include *Melicope ptelefolia* ("tenggek burung"), *Cosmos caudatus* ("ulam raja"), *Oenanthe javanica* ("selom") and *Centella asiatica* ("pegaga"). The plant's active constituents were extracted using methanol and then its inhibition activity was determined using luminescence assay with ketoconazole as controls. **Results:** The enzyme CYP3A4 was found to be inhibited by ulam extracts and *Centella asiatica* (pegaga) was found to have the highest inhibition (IC50 value 23.99 µg mL-1). Then *Melicope ptelefolia* (IC50 value 34.67 µg mL-1), *Cosmos caudatus* (IC50 value 52.48 µg mL-1), and *Oenanthe javanica* (IC50 value 107.15µg mL-1). **Conclusion:** Ulam was found to have CYP3A4 inhibitory properties, especially *Centella asiatica*, *Melicope ptelefolia* and *Cosmos caudatus*. Therefore consumption of ulam may have potential for food-drug interactions and caution may be exercised when consuming ulam together with CYP3A4-metabolised drugs.
What this paper adds: Ulam is a popular traditional food. This paper investigates the food-drug interaction that may be affect the outcome of CYP 3A4-metabolised drugs and found possible interaction in case the ulam is consumed excessively and taken together with the CYP 3A4-metabolised drugs.

Keywords: traditional vegetable, ulam, CYP 3A4, enzyme inhibition, food-drug interaction

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INTRODUCTION

Malaysia is a tropical country rich with diverse flora and fauna due to its year-round climate of rain, elevated temperature and high humidity. This sets favourable conditions for the growth of various species of plants, among which is a group of traditional Malaysian vegetables collectively known as ulam (Bachok et al., 2014). This group consist of 120 or more species from various plant families originating from Southeast Asia (Mohamed et al.,2012). Ulam vegetables are consumed raw or cooked and are consumed as part of with rice-based meals and also may be prepared with a blend of sauces, aromatic herbs or spices. The tasty flavor encourages its consumption not only among the native Malay but also among other Malaysian ethnic groups (Bachok et al., 2014).

Fresh vegetables are known as important sources of vitamins, nutrients and fiber and universally regarded as an important component of healthy diets (Slavin et al., 2012). The demand of fresh vegetables has increased as people nowadays are more concerned about healthy lifestyle and balance diet (Loo et al., 2013). Application of a healthy diet that incorporates edible antioxidants neutralise free radicals that cause damage to cells such as nitric oxide and the alkoxyl radicals. Vegetables are rich source of edible antioxidants that includes ascorbic acid, tocopherols, carotenoids and phenolic compounds. For instance, *Centella asiatica* was shown to decelerate age-related cognitive decline and improve moods among the elderly. Ulam also has medicinal benefits such as those demonstrated by *Ocimum basilicum*'s antiviral and antiparasitic properties and *Oenanthe javanica's* antihepatitis properties (Loo et al., 2013).

Drug metabolism is essentially the biochemical modification of drugs or xenobiotics via specialised enzymatic systems of the organism. It consist of two phases: phase I and II. Phase I drug metabolism involves redox (oxidation and reduction) and hydrolysis reactions and mostly mediated by Cytochrome P450 and flavin-containing monoxygenases (FMOs) in humans (Hanapi et al., 2010). Cytochrome P450 enzymes is a superfamily of heme-containing enzymes that are involved in the metabolism of both exogenous (e.g drugs) and endogenous compounds such as steroidal hormones (Esteves et al., 2021).

The CYP superfamily consist of 57 genes and 58 pseudogenes that are divided into 18 families and 44 subfamilies. Out of these, the CYP1A2, CYP2C9, CYP2D6, CYP3A4, and CYP3A5 enzymes metabolise approximately 72% of commercially available drugs (Esteves et al., 2021). CYP enzymes are mainly expressed in liver, but also found in the small intestine, lungs, placenta, and kidneys (Lynch et al., 2007).

Cytochrome P450 3A4 (CYP3A4) is a drug metabolizing enzyme with the highest level of expression in humans (Bibi et al. 2008). CYP3A4 metabolises 50% of commercial drugs and plays a major role in drug response by estimated involvement in 40% to 45% of all phase I metabolism (Baylon et al., 2013). Due to this, CYP3A4 highly influences the bioavailability and the effective plasma concentration of a wide range of drugs (Baylon et al., 2013). In addition to drugs, CYP3A is also involved in the oxidation of a variety of endogenous substrates such as steroids, bile acids and retinoic acid (Marcella et al., 2006).

Plants have long been used to treat diseases in humans, but the effects of traditionally used herbal products are less investigated in pharmacological studies. A concern is the risk of adverse interactions between herbal medicinal products and conventional therapies (Colalto, 2010). A drug interaction is refers to changes influenced by another exogenous chemical in the action of a particular drug. The exogenous chemicals could be in the form of drugs, herbs or even food items. The mechanisms for drug interaction could be either via pharmacokinetics and pharmacodynamics (Ismail et al. 2009).

The possibilities of drug interaction are infinite due to the availability of more than 30000 over-the-counter pharmaceutical products which incorporates more than 1000 types of chemical constituents (Ismail et al. 2009). As the number of drug products taken together is increased, so does the risk for drug interactions (Ismail et al. 2009).

Food-drug interactions has been defined as modulation a drug's pharmacokinetics or pharmacodynamics properties or an effect on individual's nutritional status due to interaction with a drug (Genser, 2008). Elderly patients need to be given special attention as they are the recipients of approximately 30% of all the prescription drugs on top of having higher probability of practicing polypharmacy due to multiple ailments (Genser, 2008; Dagli & Sharma, 2014). Inadequate detection and management of food-drug interactions can lead to non-favourable outcomes. For instance, the reduced absorption of oral antibiotics due to food-drug interactions may lead to suboptimal antibiotic concentrations at the site of infection (Genser, 2008).

Ulam consumption is increasing as many are making positive changes to their lifestyle by including more greens in their diet. However, information on potential inhibitory properties of ulam toward CYP3A4 is lacking. It is important to investigate the food-drug interaction of ulam with CYP3A4 to gauge whether there would be potential impact if ulam are taken concomitantly with drugs metabolised by CYP3A4, particularly because some ulam have been formulated into herbal teas and supplements. In an effort to answer this, the current study sets to investigate the CYP 3A4 inhibitory properties of selected ulam extracts namely *Centella asiatica* ("pegaga"), *Melicope ptelefolia* ("tenggek burung"), *Cosmos caudatus* ("ulam raja") and *Oenanthe javanica* ("selom").

MATERIALS AND METHODS

Sample collection

Samples of *Melicope ptelefolia* ("tenggek burung"), *Cosmos caudatus* ("ulam raja") and *Centella asiatica* ("pegaga") was sourced from Taman Pertanian, Universiti Putra Malaysia. *Oenanthe javanica* (selom) was obtained from Seremban Market. Authentication of samples were done by Institute of Bioscience, Universiti Putra Malaysia.

Preparation of ulam extracts

Air dried plant samples were grounded using electrical blender. Then, 40 g of sample were suspended in methanol (200 ml) and put in incubator shaker for 24 hours at room temperature. After filtering, solvent was removed under vacuum at 45°C using a rotary evaporator (Buchirotavapor R-210). Sample was then stored at -80 ºC overnight and then dried using a freeze dryer (Alpha 1-2LD) for 24 hours. The extracts were weighed and stored in a refrigerator at 4ºC. All plant extracts were then prepared as 1000 µg/ml in 70% methanol.

CYP3A4 inhibition assay

Two-fold serial dilution of triplicate samples were prepared in standard flat-bottomed white 96-well microplates. P450-Glo™ CYP3A4 Assay (Luciferin-PPXE) DMSO Tolerant Assay (Promega Corporation, Madison, USA) was performed using manufacturer's instructions. Control inhibitor used was ketoconazole and the positive control used is the human CYP 3A4 membrane preparation minus inhibitors. The effective concentration of extracts was determined prior to analysis.

The samples (12.5 µl) were mixed with the 4X CYP mixture (12.5 µl) and incubated at 37.5°C for 10 min. Then NADPH Regeneration Solution (25.0 µl) was added. After 10 min incubation, Luciferin Detection Reagent (50.0 µl) was added and incubated at room temperature for 20 min. Results recorded using Glomax® 96 microplate Luminometer. All samples are analysed in triplicates. Inhibition of CYP 3A4 was recorded and the IC_{50} calculated.

Statistical Analysis

The experiment statistical analysis was done by Statistic Package For Social Sciences Programme version 20.0 (SPSS 20.0, IBM Corporation,New York,USA) and Microsoft Excel 2010. Analysis of variance (ANOVA) followed by post hoc Tukey's and Hochberg's test were performed to determine the statistical significance of this study.

RESULTS

Ulam extracts

Effective concentration was determined by selecting the concentration that emits quantifiable luminescence to ensure that the concentration range chosen is sufficient and suitable to exhibit quantifiable activity. The results are shown in Table 1.

Table 1. Effective concentration of ulam extracts

Types of plants	Effective concentration
Centella asiatica (CA)	250 µg/ml
Melicope ptelefolia (MP)	250 µg/ml
Cosmos caudatus (CC)	500 µg/ml
Oenanthe javanica (OJ)	1000 µg/ml

Ulam extract inhibition toward CYP 3A4

Table 2 shows the inhibition of CYP 3A4 by each ulam extract. Enzyme inhibition of common concentrations across all ulam extracts are shown in Figure 1. The IC50 values were determined for the extracts and summarised in Table 3.As the results showed, the Centella asiatica (CA) had the strongest inhibition with an apparent IC50 value of 23.99 µg ml-1. The rank order of inhibition is CA > MP > CC > OJ.

Table 2. Inhibition of CYP 3A4 by ulam extracts

Concentration (µg/ml)	Enzyme Inhibition (%)			
	CA	MP	CC	OJ
7.8	25.9	6.2	-	-
15.6	37.0	20.0	14.7	-
31.3	57.5	50.4	42.1	24.4
62.5	75.1	82.04	51.9	38.8
125	87.9	87.35	74.7	50.8
250	96.3	95.6	95.6	73
500	-	-	99.4	83.4
1000	-	-	-	94.6

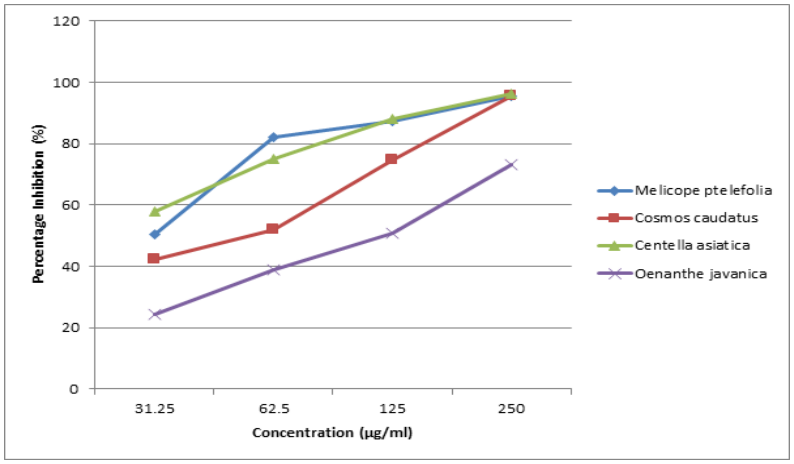

Figure 1. Percentage inhibition of CYP3A4 enzyme assay by ulam extracts

Table 3. IC50 of ulam extracts toward CYP 3A4

Type of plant extracts	IC_{50} (µg/ml)
Centella asiatica	23.99
Melicope ptelefolia	34.67
Cosmos caudatus	52.48
Oenanthe javanica	107.15

DISCUSSION

Consumption of traditional vegetables, ulam, has long been part of the diet of people of Southeast Asia. The ingested part of the plant may constitute shoots, leaves, and seeds of certain local vegetables (Arash et al., 2010). In addition, some ulam have been used traditionally as preventive or curative treatments. Five types of ulam that are often served locally include the leaves of Cosmos caudatus (Ulam Raja), Oenanthe javanica (Selom), Murraya koenigii (Curry Leaf), Centella asiatica (Pegaga) and the seeds of Parkia speciosa (Petai) (Reihani et al., 2012).

There is no guideline that limit in its consumption and information regarding its potential to influence drug therapy is lacking. The determination of potential food-drug interactions is important to be explored to safeguard the quality of life of the patients while assuring that effectiveness of drug therapy is maintained. D'Alessandro et al., (2022) reviewed the effect of food-drug interactions in management of renal patient therapy and determined that clear guide of limits in food consumption, appropriate scheduling of meals, and proactive modification of the drug dosing plans is needed to deal with potential food-drug interactions.

Bioavailability is an important pharmacokinetic parameter which is correlated with the clinical effect of most drugs (Bushra et al., 2011). In order to evaluate the clinical relevance of a food-drug interaction, the influence of the particular food toward clinical effect drugs has to be quantified. Induction or inhibition of enzymes may modulate the oral bioavailability of drugs. Widely known food-drug interaction is the role of grapefruit as a selective intestinal CYP3A4 inhibitor. The bioavailability of some drugs may be amplified by more than fivefold when taken with grapefruit juice, which increases the incidence of adverse reactions. Certain drugs may also impact the GI tract function that lead to a loss of electrolytes and fluid (Genser et al. 2008). Several fruits and berries have recently been shown to contain agents that affect drug-metabolizing enzymes (Bushra et al., 2011).

Many medicinal herbs and drugs could be therapeutic at one dose and toxic at another. Synergistic nature of medicinal herbs and drug may complicate the dosing of long-term medications as they influence each other. For instance, herbs traditionally used to lower glucose concentrations in diabetes patients could theoretically cause hypoglycaemia if taken in combination with conventional antidiabetic drugs. Thus, health care providers need to be aware of herb and supplement use by their patients (Ismail et al. 2009).

This study focuses on several species of plants traditionally used as ulam that includes *Cosmos caudatus* ("ulam raja"), Melicope ptelefolia ("tenggek burung"), *Centella asiatica* ("pegaga") and *Oenanthe javanica* ("selom"). Active

constituents of these traditional vegetables has also been investigated for its therapeutic value and developed as drugs or supplements. As an example, effectiveness of *Centella asiatica* for wound healing was investigated in clinical trials (Arribas-Lopéz et al., 2022). *Centella asiatica, Cosmos caudatus* and *Oenanthe javanica* has been investigated as alternative food intervention for vitamin A deficiency due to their high carotenoid content (Othman et al., 2017).

The prevalent misconception is that herbal medicines or supplements are considered natural and therefore safe to be taken concomitantly with prescription drugs. However, in reality this does not make them safe, risk-free and devoid of any drug-drug interaction potential (Ekor, 2014). Natural compounds and herbal products such as St John's wort may cause pharmacokinetic interaction with drugs such as cyclosporine, indinavir and oral contraceptives when they are simultaneously administrated due to interactions with CYP P450 enzymes (Izzo, 2005). Although many drug–herbal interactions are likely to be negative in nature, it is important to understand that some interactions may have a beneficial effect on drug therapy. Example is the deliberate inhibition of CYP P450 enzymes to increase the bioavailability of anticancer drugs (Eisenmann et al., 2022).

CYP 3A4 is an important enzyme involved in the metabolism of endogenous compounds and xenobiotics but its expression and function has wide intra- and interindividual variability (Klein and Zanger, 2013). Its variability may be influenced by factors such as genetics, physiological attributes, environment, lifestyle and diet (Klein and Zanger, 2013). Furthermore, inhibition of CYP 3A4 may trigger adverse physiological consequences such as cholestasis (Chen et al., 2014). Therefore knowledge of potential CYP 3A4 inhibitors is valuable but data of plant-derived inhibitors is still lacking (Guttman & Kerem, 2022). Virtual screening of plant compounds with potential to inhibit CYP 3A4 revealed 115 compounds, out of which only 31 compounds were identified before (Guttman & Kerem, 2022). However, virtual screening needs to be supported by experimental data to confirm its predictions.

In this study, extracts of *Centella asiatica* was found to have the highest inhibition (IC_{50} 24 µg/ml) whereas *Oenanthe javanica* had the lowest inhibition (IC_{50} 107.2 µg/ml) towards CYP 3A4. Pan et al., (2010) found that ethanol and dichloromethane extracts of *Centella asiatica* showed negligible inhibition of CYP3A4, which is not the case in our study. The IC_{50} value of *Centella asiatica* was comparable to *Andrographis paniculata* (Hempedu bumi) (IC_{50} 27.6 µg/ml) which was found to have weak inhibition compared to ketoconazole (Hanapi et al., 2010). *Centella asiatica* has varying potency to inhibit other drug metabolizing enzymes such as CYP2C19, CYP2C9 and CYP2D6 (Pan et al., 2010a, 2010b). It is interesting to note that the effective concentration range for *Centella asiatica* is the same as *Melicope ptefolia*. Although the degree of inhibition is lower for *Melicope ptefolia*, more studies should be carried out to confirm the effects. Most of the plants used in this study contain many active constituents such as alkaloids, flavonoids, polyphenol and terpenoids. The presence of different active constituents in each plant extract may contribute to the inhibitory activity of CYP enzyme (Hanapi et al., 2010). This evidence of CYP 3A4 inhibition by ulam extracts should be used to inform choices in drug therapy as it may influence drug safety and effectiveness.

CONCLUSION

CYP3A4 was more susceptible to inhibitory effects of *Centella asiatica* extract compared to other plant extracts as it showed the highest inhibition of 96.28% (IC50 24 µg/ml). *Melicope ptelefolia* and *Cosmos caudatus* imply potential risk of interaction with CYP3A4 substrates. *Oenanthe javanica* show negligible inhibitory activity with IC50 value of 107.2 µg/ml. The results support that care should be taken when taking ulam as part of diet or supplements while on therapy with CYP3A4 metabolised drugs due to potential of interaction. But as the *ulam* plants are typically not consumed in high amount it is assumed the effects in food-drug interaction is negligible. However, in the event that these *ulam* are developed into supplements then care should be taken and more studies are warranted to gauge the effects *in vivo*.

DECLARATION OF INTEREST

The authors wish to declare that there are no conflicts of interest.

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