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Original Article

INHIBITORY EFFECT OF *EURYCOMA LONGIFOLIA* EXTRACT AND EURYCOMANONE ON HUMAN CYTOCHROME P450 ISOFORMS

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ABSTRACT

Objective: The aim of this study was to evaluate the inhibitory effect of *E. longifolia* extract on five human cytochrome P450 isoforms.

Methods: The inhibitory effect of *E. longifolia* standardized extract (TAF-273) on five human cytochrome P450 isoforms, namely CYP1A2, CYP3A4, CYP2C9, CYP2C19 and CYP2D6, was determined using a luminescence method. The effect of eurycomanone on cytochrome P450 isoforms CYP2C9 and CYP2C19 was examined using the same method. The final concentrations of *E. longifolia* extract tested were 0.01, 0.1, 1, 10, 100 and 1000 µg/mL, and eurycomanone was tested at concentrations ranging from 0.002 to 500 µM.

Results: The extract decreased CYP2C9 and 2C19 activities, with IC_{50} values of 710.20 µg/mL and 739.05 µg/mL, respectively. The IC_{50} of TAF-273's inhibitory action on CYP2D6, CYP3A4 and CYP1A2 could not be determined because at the highest concentration tested (1000 µg/mL) the activity of each enzyme was still greater than 50 %. Eurycomanone (a marker compound in TAF-273) showed IC_{50} values of 47.33 µg/mL and 167.88 µg/mL in inhibiting the activities of CYP2C9 and 2C19.

Conclusion: This inhibitory study showed that a standardized extract of *E. longifolia* was not a potent inhibitor towards CYP2C9, CYP2C19, CYP2D6, CYP3A4 and CYP1A2. On the other hand, eurycomanone (the marker compound) was a moderate inhibitor for CYP2C9 with an IC_{50} of 47.33 µM. The data indicated that *E. longifolia* extract did not potently inhibit any of the CYP isoforms, while eurycomanone was a moderate inhibitor of CYP2C9.

Keywords: *Eurycoma longifolia* jack, Inhibitory effect, Human cytochrome P450.

INTRODUCTION

Eurycoma longifolia Jack (*E. longifolia*) is one of the popular medicinal plants in Southeast Asia, including Indonesia and Malaysia. *E. longfolia* has many local names: in Brunei it is known as tungat ali, langsia siam or pasak bumi; in Cambodia it is known as antoung sar or antong sar; in Thailand it is known as plaalai phuenk, hae phan chan or phiak; in Laos it is known as tho nan; in Vietnam it is known as Cay ba binh; in Indonesia it is known as beseng, bidara laut or pasak bumi; and in Malaysia it is known as bedara merah, bedara putih or tongkat ali [1, 2].

In Malaysia, Tongkat Ali (*E. longfolia*) has been claimed to improve the stamina of men during sexual activity, increase vitality and restore erection. As such, it is reputed to be an aphrodisiac. The herb is commonly taken as a decoction of roots in water. Nearly 200 Tongkat Ali products are available on the domestic Malaysian market, either in combination with other herbs or as a single preparation [3]. Many publications have revealed the aphrodisiac activity of *E. longifolia*, which has been used to enhance male virility during sexual activity [4, 5, 6, 7 & 8]. Many activity studies have been performed with regard to the many constituents that are contained in *E. longifolia* [9, 10, 11, 12, 13, 14, 15 & 16]. Hitherto, only a few studies have been published on herb-drug interactions. One study by Salman *et al* (2010) [17] about the effect of a *E. longifolia* water-based extract on the bioavailability of propranolol showed that the extract decreased C_{max} (42%), reduced AUC (29%) and prolonged the T_{max} (86%) of propranolol. The effect of a standardized extract of *E. longifolia* on aminopyrine metabolism in male and female rats has also been examined. The extract significantly increased aminopyrine metabolism in male and female rat hepatocytes and there was a different effect at the molecular level on aminopyrine metabolism in male rats compared to female rats [18, 19]. Purwantiningsih *et al*. (2011) [20] studied the effect of *E. longifolia* extract on rosiglitazone metabolism. The results showed that the extract increased rosiglitazone metabolism significantly, especially in aged normal and diabetic male rats.

Herb-drug interactions follow modern pharmacological principles and are divided into pharmacodynamic and pharmacokinetic interactions [21, 22]. Herb-drug pharmacokinetic interactions appear through the influence of an herb on the absorption, distribution, metabolism or excretion of drugs. Concurrent use of herbal medicines may alter a drug's concentration by either inhibiting or inducing intestinal and hepatic drug-metabolizing enzymes, especially cytochrome P450s (CYP), and also drug transporters like P-glycoprotein (PgP) [23, 24 & 25]. An herb may modulate the metabolic clearance of drug by inducing or inhibiting a specific CYP enzyme [26]. Many assessments have been performed to evaluate the effect of medicinal plants on drug interactions and their effects on cytochrome activities [27]. This study examined the effect of a standardized *E. longifolia* extract on human cytochrome P450 (CYP450).

MATERIALS AND METHODS

Materials

The standardized extract of *E. longifolia* (TAF-273) was provided by Prof. Chan Kit Lam from the School of Pharmaceutical Sciences, Universiti Sains Malaysia. The extract was made according to Chan *et al.* (2004) [10] and a voucher specimen of the plant was deposited, with Reference No. 785-117, at the Penang Botanical Garden [1]. The *E. longifolia* extract was standardized with euryomanone as a marker compound, using a MICROTOF-QII LC-MS (Bruker ®, USA) and the eurycomanone content in the extract was found to be 15.89 % ± 0.95 SD. A luminescence assay was carried out as described in the P450-GLO™ Screening System by Promega®, USA and was performed in a 96-well microtiter plate (Thermo Scientific®, Finland). The screening system included the five sub-types of CYP450 (CYP2C9, CYP2C19, CYP2D6, CYP3A4 and CYP1A2) and 5 different substrates used in these experiments were purchased from Promega®, USA: luciferin 6' methyl ether (luciferin-ME) for CYP1A2, 6' deoxyluciferin (luciferin-H) for CYP2C9, an ethylene glycol ester of 6'deoxyluciferin (luciferin-H EGE) for CYP2C19, and an ethylene glycol ester of luciferin 6' methyl ether

(luciferin-H EGE) and luciferin 6' benzyl ether (luciferin-BE) for CYP2D6 and CYP3A4, respectively. Each system contained the luciferin detection reagent, CYP membranes and control membranes, solution A and B of the NADPH regeneration system, 1 M potassium phosphate buffer and luciferin-free water.

Enzyme assay

A standard curve of CYP-P450 activity was prepared using a 2 mM D-luciferin stock solution. Then, a 4X serial dilution series of the D-luciferin standard (8 µM, 1.6 µM, 0.32 µM and 0.064 µM) was prepared to obtain standard solutions with final concentrations of 2 µM, 0.4 µM, 0.08 µM and 0.016 µM. The 4X CYP1A2, CYP3A4, CYP2C9, CYP2C19 or CYP2D6 reaction mixture, 4X control reaction mixture and 2X NADPH regeneration system were prepared before the assay was performed. Then, 12.5 µL of 4X D-luciferin standard was added into a 96-well white opaque plate in the appropriate wells and 12.5 µL of luciferin-free water was added to the 0 µM D-luciferin wells. Then 12.5 µL of the 4X control reaction mixture was added and the plate was shaken by tapping the plate. The reaction was pre-incubated for 10 minutes at 27 ºC and then 25 µL of the 2X CYP NADPH regeneration system was added into each well. The plate was tapped and incubated at 27 ºC for 30 minutes. Then, 50 µL of the reconstituted luciferin detection reagent was added to each well, mixed briefly and incubated for 20 minutes at 27 ºC to stabilize the luminescent signal. The signal was recorded using a plate-reading luminometer (Hidex Plate CHAMELEON®, Hidex Oy, USA). Values were displayed as relative light units (RLU). To perform the test compound assay, 4X concentrations of the test compounds: *E. longifolia* solution or eurycomanone (12.5 µL) were added to the wells of a microtiter plate. In the untreated wells, the test compound was replaced with 12.5 µL of luciferin-free water. Then, 12.5 µL of the 4X control reaction mixture was added, the plate was shaken by tapping and the reaction was continued as mentioned above.

Data analysis

The activity was presented as a percentage of the activity of the control. Then, the IC_{50} was calculated to determine the potency of the *E. longifolia* extract in inhibiting cytochrome P450 enzyme activities.

RESULTS AND DISCUSSION

The inhibitory effects of the *E. longifolia* extract on CYP2C9 and CYP2C19 activities are displayed in Fig. 1. The *E. longifolia* extract decreased CYP2C9 and CYP2C19 activities significantly, with an IC_{50} of 710.20 µg/mL and 739.05 µg/mL, respectively; while eurycomanone (the marker compound) showed IC_{50} values of 47.33 µM and 167.88 µM against CYP2C9 and CYP2C19 activities (Fig. 2).

The inhibitory effect of the *E. longifolia* extract on the activity of CYP2D6, CYP3A4 and CYP1A2 are presented in Fig. 3. The IC_{50} of TAF-273 could not be determined because, even at the highest concentration tested (1000 µg/mL), the activities of the enzymes were still more than 50 %.

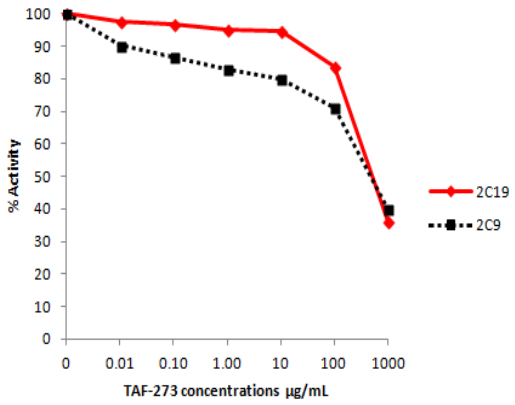

Fig. 1: Inhibition effect of *E. longifolia* standardized extract (TAF-273) on CYP2C9 and CYP2C19

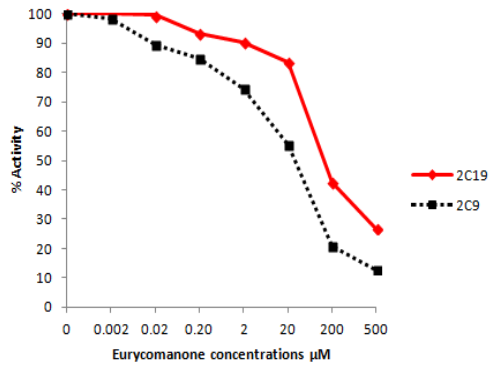

Fig. 2: Inhibition effect of eurycomanone on CYP2C9 and CYP2C19

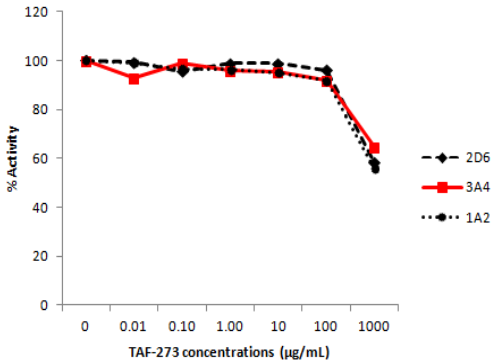

Fig. 3: Inhibition effect of *E. longifolia* standardized extract (TAF-273) on CYP2D6, CYP3A4 and CYP1A2

Cytochrome P-450 enzymes have an important role in the catabolism of endogenous substrates and the metabolism of most drugs and xenobiotics [28]. A drug may have a complex profile, i.e. it may be a substrate for an inducer and an inhibitor at the same time. For example, quinidine is known as a potent inhibitor of CYP2D6, but is mainly metabolized by CYP3A4 [29]. All CYPs may be inhibited or induced by exposure to a wide variety of xenobiotics, including various herbs.

Several studies related to herb interactions and CYP450 enzyme activities have been published [30]. Iwata *et al* (2004) [31] reported that *Schisandra* extract was a potent inhibitor of erythromycin N-demethylation mediated by CYP3A4. Some isolated components from *Schisandra* fruit have also been identified as inhibitors of N-demethylation activity. Grapefruit juice has shown an inhibitory effect on CYP3A4 activity and some furanocoumarins in grapefruit juice are indicated as the causative components of the inhibition [32, 33 & 34]. Inhibitory studies of seven components of danshen extract (*Salvia miltiorrhiza*) on CYP450 activity showed that each component had a different effect. Some components were potent inhibitors of CYP1A2, one component was a competitive inhibitor of CYP2C9 and the others were weak inhibitors of CYP2D6 and CYP3A4 [35]. Another study by Obach (2000) [36] demonstrated that St. John's wort extract inhibited CYP450 enzyme activities but the isolated biapigenin, hyperforin and hypericin presented different potencies. Biapigenin was a potent inhibitor of CYP2C9, CYP3A4 and CYP1A2. Hyperforin demonstrated strong inhibition of CYP2D6, CYP3A4 and CYP2C9 activities, while hypericin inhibited several CYP450 activities. These *in vitro* data suggest that St. John's wort remedies should be evaluated for their *in vivo* potential for pharmacokinetic herb-drug interactions because these remedies contain constituents that may potently inhibit major drug metabolizing enzyme activities in humans. Hanapi *et al.* (2010) [37] evaluated the effects of five Malaysian medicinal plants on CYP2C9, CYP2D6 and CYP3A4. They concluded that three of the five examined plants (*M. speciosa, O. stamineus and A. paniculata*) showed herb-drug interactions, especially when administered concurrently

with drugs that were metabolized by CYP2C9, CYP2D6 or CYP3A4. Inhibition or induction of CYP activities may influence drug metabolism and affect a drug's therapeutic effects. Thus, it may be beneficial or, on the other hand, cause adverse effects. The inhibitory effects of the major components of herbal remedies on CYP450 activities should be determined in order to predict interactions between drug and herbal remedies with regard to their metabolism. Their kinetics of inhibition and mechanism of action should also be identified [31]. Our results showed that *E longifolia* extract was not an inhibitor for CYP2D6, CYP3A4 and CYP1A2. The IC_{50} of the *E longifolia* extract's inhibitory activity could not be determined because the activity of the CYP isoforms were still greater than 50 % at the highest concentration tested. *E longifolia* extract is claimed to be an aphrodisiac by increasing testosterone levels [38, 39]. Increases in testosterone may increase the metabolic rate and oxidative phosphorylation. On the other hand, the inhibition studies of *E longifolia* extract on CYP2C9 and CYP2C19 activities indicate that *E longifolia* extract is not a potent inhibitor of CYP2C9 and CYP2C19. Moreover, eurycomanone (the major compound in *E longifolia* extract) showed only a weak inhibitory potency for CYP2C9 and CYP2C19 activities because the IC_{50} values were greater than 1 μM [35]. From the pharmacotherapy perspective, this lack of a CYP modulatory effect is beneficial to patients, since *E. longifolia* is often consumed concurrently with antidiabetics, antihypertensives and other agents that may be CYP substrates or inhibitors [40].

CONCLUSION

An inhibitory study on the effect of a standardized extract of *E. longifolia* on human cytochrome P450 showed that the extract was not a potent inhibitor towards CYP2C9, CYP2C19, CYP2D6, CYP3A4 and CYP1A2. On the other hand, eurycomanone (the marker compound) was a moderate inhibitor of CYP2C9 with an IC_{50} of 47.33 μM. This may beneficial for development of *E. longifolia* extract as a medicinal plant, since cytochrome P450 has an important role in drug metabolism and drug metabolism has a very close relationship with the therapeutic effects of drugs.

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REFERENCES

1. Chan KL, Choo CY, Morita H, Itokawa H. High performance liquid chromatography in phytochemical analysis of *Eurycoma longifolia*. Planta med 1998;64(8):741-5.
2. Uji, T. Eurycoma longifolia Jack. Plant Resources of South-East Asia. In: de Padua LS, Bunyapraphatsara N, Lemmens RHMJ, editors. Medicinal and poisonous plants No. 12(1). Leiden, Netherlands: Backhuys Publisher; 1999. 274-75 p.
3. Cyranoski D. Malaysian researchers bet big on home-grown Viagra. Nat Med 2005;11(9):912.
4. Ang HH, Sim MK. *Eurycoma longifolia* Jack enhances libido in sexually experienced male rats. J Exp Anim Sci 1997;46(4):287-90.
5. Ang HH, Ngai TH. Aphrodisiac evaluation in non-copulator male rats after chronic administration of *Eurycoma longifolia* Jack. Fundam & clin pharmacol 2001;15(4):265-8.
6. Lin LC, Peng CY, Wang HS, Lee KW, Paulus WS, Pharm J. Reinvestigation of the chemical constituents of *Eurycoma longifolia*. Chin Pharm J 2001;53:97-106.
7. Ang HH, Lee KL. Effect of *Eurycoma longifolia* Jack on orientation activities in middle-aged male rats. Fundam Clin Pharmacol 2002;16(6):479-83.
8. Ang HH, Ngai TH, Tan TH. Effect of *Eurycoma longifolia* Jack on sexual qualities in middle-aged male rats. Phytomedicine 2003;10:590-93
9. Chan KL, Lee SP, Yuen KH, Ghazally I, Murtedza M, Laily D. Antipyretic Activity of Quassinoids from *Eurycoma longifolia* Jack1995. 219-24.
10. Chan K-L, Choo C-Y, Abdullah NR, Ismail Z. Antiplasmodial studies of *Eurycoma longifolia* Jack using the lactate dehydrogenase assay of Plasmodium falciparum. Journal of ethnopharmacology 2004;92(2-3):223-7.
11. Chan K-L, Choo C-Y, Abdullah NR. Semisynthetic 15-O-acyl- and 1,15-di-O-acyleurycomanones from *Eurycoma longifolia* as potential antimalarials. Planta medica 2005;71(10):967-9.
12. Kardono LB, Angerhofer CK, Tsauri S, Padmawinata K, Pezzuto JM, Kinghorn AD. Cytotoxic and antimalarial constituents of the roots of *Eurycoma longifolia*. Journal of natural products 1991;54(5):1360-7.
13. Jiwajinda S, Santisopasri V, Murakami A, Hirai N, Ohigashi H. Quassinoids from *Eurycoma longifolia* as plant growth inhibitors. Phytochemistry 2001;58(6):959-62.
14. Jiwajinda S, Santisopasri V, Murakami A, Sugiyama H, Gasquet M, Riad E, et al. In vitro anti-tumor promoting and anti-parasitic activities of the quassinoids from *Eurycoma longifolia*, a medicinal plant in Southeast Asia. J Ethnopharmacol 2002;82(1):55-8.
15. Ang HH, Ngai TH, Tan TH. Effects of *Eurycoma longifolia* Jack on sexual qualities in middle aged male rats. Phytomedicine : 2003;10(6-7):590-3.
16. Zakaria Y, Rahmat A, Pihie AHL, Abdullah NR, Houghton PJ. Eurycomanone induce apoptosis in HepG2 cells via up-regulation of p53. Cancer cell international 2009;9:16.
17. Salman SAB, Amrah S, Wahab MSA, Ismail Z, Ismail R, Yuen KH, et al. Modification of propranolol's bioavailability by *Eurycoma longifolia* water-based extract. J Clin Pharm Ther 2010; 35(6) :691-6.
18. Purwantiningsih, Hussin AH, Chan, KL. Phase I drug metabolism study of the standardised extract of Eurycoma longifolia (TAF-273) in rat hepatocytes. Int J Pharm Pharm Sci 2010; 2 suppl 3 2010:147-52.
19. Purwantiningsih, Hussin, AH, Chan, KL. Sex-Related Alterations of Aminopyrine Metabolism by Standardized Extract of Eurycoma longifolia (TAF-273) Alimen Hung. 2012;41(3):316-26.
20. Purwantiningsih, Hussin, AH, Chan, KL. Herb-Drug Interaction Study of Eurycoma longifolia Extract (TAF-273) on Antidiabetic Drug (Rosiglitazone) Metabolism, Asian J Pharm Clin Res 2011;4(2):88-92.
21. Izzo AA, Borrelli F, Capasso R. Herbal medicine: the dangers of drug interaction. Trends Pharmacol Sci 2002;23(8):358-91; author reply 9.
22. Izzo AA. Drug interactions with St. John's Wort (*Hypericum perforatum*): a review of the clinical evidence. Int J Clin Pharm Ther: 2004;42(3):139-48.
23. Wilkinson GR. The effects of diet, aging and disease-states on presystemic elimination and oral drug bioavailability in humans. Advanced drug delivery reviews 1997;27(2-3):129-59.
24. Evans AM. Influence of dietary components on the gastrointestinal metabolism and transport of drugs. Ther Drug Monit 2000;22(1):131-6.
25. Ioannides C. Xenobiotic Metabolis: an Overview. In Ioannides C, editor. Enzymes Systems The Metabolise Drugs and Other Xenobiotics. West Sussex: John Wiley & Sons, Ltd; 2002. 1-30.
26. Zhou S, Gao Y, Jiang W, Huang M, Xu A, Paxton JW. Interactions of herbs with cytochrome P450. Drug Metab Rev 2003;35(1):35-98.
27. Saxena A, Tripathi KP, Roy S, Khan F, Sharma A. Pharmacovigilance: effects of herbal components on human drugs interactions involving cytochrome P450. Bioinformation 2008;3(5):198-204.
28. Ortiz de Montellano PR. The Cytochrome P450 System. In Woolf TF, editor Handbook of Drug Metabolism. New York: Marcel Dekker Inc.; 1999. 109-30.
29. Guengerich FP, Hosea NA, Parikh A, Bell-Parikh LC, Johnson WW, Gillam EM, et al. Twenty years of biochemistry of human P450s: purification, expression, mechanism, and relevance to drugs. Drug Metab Dispos 1998;26(12):1175-8.
30. Ismail, MHM. Herb-Drug Interactions and Patient Counseling. Int J Pharm Pharm Sci 2009;58(6):648-53.
31. Iwata H, Tezuka Y, Kadota S, Hiratsuka A, Watabe T. Identification and characterization of potent CYP3A4 inhibitors in *Schisandra* fruit extract. Drug Metab Dispos 2004; 32(12): 1351-8.
32. Edwards DJ, Bellevue FH, Woster PM. Identification of 6',7'-dihydroxybergamottin, a cytochrome P450 inhibitor, in grapefruit juice. Drug Metab Dispos 1996;24(12):1287-90.
33. Bailey DG, Malcolm J, Arnold O, Spence JD. Grapefruit juice-drug interactions. Brit J Clin Pharmaco 1998;46(2):101-10.

34. Sahi J, Reyner EL, Bauman JN, Gueneva-Boucheva K, Burleigh JE, Thomas VH. The effect of bergamottin on diazepam plasma levels and P450 enzymes in beagle dogs. Drug Metab Dispos 2002;30(2):135-40.
35. Qiu F, Zhang R, Sun J, Jiye A, Hao H, Peng Y, et al. Inhibitory effects of seven components of danshen extract on catalytic activity of cytochrome P450 enzyme in human liver microsomes. Drug Metab Dispos 2008;36(7):1308-14.
36. Obach RS. Inhibition of human cytochrome P450 enzymes by constituents of St. John's Wort, an herbal preparation used in the treatment of depression. J Pharmacol Exp Ther 2000; 294(1):88-95.
37. Hanapi NA, Azizi J, Ismail S, Mansor SM, J. Evaluation of selected Malaysian medicinal plants on phase I drug metabolizing enzymes, CYP2C9, CYP2D6 and CYP3A4 activities in vitro. Int J Pharm 2010;6(4):494-99.
38. Wibowo S. Taufiqurrachman, Effect of *Eurycoma longifolia* Jack root extract (*Eurycoma longifolia*) in elevation of testosterone levels, LH and FSH in Sprague Dawley male rats. Media Medika Muda 2000;35(2):81-6.
39. Low B-S, Das PK, Chan K-L. Standardized quassinoid-rich *Eurycoma longifolia* extract improved spermatogenesis and fertility in male rats via the hypothalamic-pituitary-gonadal axis. J Ethnopharmacol 2013;145(3):706-14.
40. Pan Y, Tiong KH, Abd-Rashid BA, Ismail Z, Ismail R, Mak JW, et al. Effect of eurycomanone on cytochrome P450 isoforms CYP1A2, CYP2A6, CYP2C8, CYP2C9, CYP2C19, CYP2E1 and CYP3A4 in vitro. J Nat Med 2013;67(3):794-98.