Review Article

International Journal of Research in Pharmaceutical Sciences

Published by JK Welfare & Pharmascope Foundation Journal Home Page: www.ijrps.com

A review of the phytochemical compounds and pharmacological activities of *Eurycoma longifolia*

Insanu M, Pratiwi S N E*, Fidrianny I

Department of Pharmaceutical Biology, School of Pharmacy, Bandung Institute of Technology, Bandung-40132, Indonesia

Article History:

Received on: 20 Mar 2021
Revised on: 25 Apr 2021
Accepted on: 26 Apr 2021

Keywords:

Eurycoma Longifolia, Traditional Usage, Phytochemical Compound, Pharmacology Activity

Abstract

Eurycoma longifolia belongs to Simaroubaceae family. It is a tall tree and has different local name from many countries and this review consisted of their traditional usage, phytochemical compounds and pharmacological activity. A systematic review was conducted to study the scientific work of *E. longifolia* which published in the last 10 years and minimum 20 articles that published in the last 2 years, published in Pubmed, Scopus etc. also has a digital object identifier (DOI). *E. longifolia* was a popular traditional medicine. The leaves wasused as supplement for giving birth, its bark as anthelmintic and its roots as antimalaria, laxative, antidiabetic etc.Due to high demand of this plant there are various formula of *E. longifolia* available in health food market. Many studies have been performed to determine the active constituents and pharmacology activities of *E. longifolia*. Alkaloid, quassinoid, polyphenols, flavonoid, polysaccharide, triterpenoid were found in *E. longifolia*. Quassinoids is a major phytochemical compound in *E. longifolia* which has various types like eurycumanone, eurycomanol, eurycomadilactone and eurylactone. Quassinoids has bitter taste and often found in Simaroubaceae family.Pharmacological activities of *E. longifolia* such as anti-inflammatory, analgesic, antioxidant, antimicrobial, antidiabetic, anti-osteoporotic activities. The literature review results showed that *E. longifolia* can be considered as medicinal plant for human. In the future, further studies on mechanism of pharmacology activity of *E. longifolia* are warranted.

*Corresponding Author

Name: Pratiwi S N E
Phone: +62-82218747914
Email: sausanpratiwi17@gmail.com

ISSN: 0975-7538

DOI: https://doi.org/10.26452/ijrps.v12i2.4716

Production and Hosted by

IJRPS | www.ijrps.com

INTRODUCTION

Eurycoma longifolia is one of the species in Eurycoma genus and belong to Simaroubaceae family. This plant is originated from the Southeast Asia so can be found in Malaysia, Laos, Cambodia, Myanmar, Indonesia, Vietnam and Thailand. *E. longifolia* also called as bidaralaut and pasakbumi (Indonesia), tongkatali, lempedupahit, bedaramerah (Malaysia), Ian-don, piak and tung saw (Thailand), Cay babenh and bbabinh (Vietnam), tho nan (Laotian) (Jiraungkoorskul and Khanijo, 2016). *E. longifolia* is a slender, shrubby, tall and slow-growing tree andwell known as herbal medicine (Chen *et al.*, 2019). Since old time, *E. longifolia* has been used as traditional medicine and nowadays this plant has been sold in the market with various form. Their leaves, barks and roots have been used as traditional medicine for different diseases.

Many studies have been performed to determine the active constituents and pharmacology activities of *E. longifolia*. Several parts of *E. longifolia* have been used to treat some diseases.The most commonly used part is the roots because it has many active constituents. The extract also has been studied and proved that contain many active substances to treat various diseases (Li *et al.*, 2013). Active substances such as quassinoids, alkaloids, squalene derivatives, triterpenes were found in *E. longifolia*. The major active substance of *E. longifolia* was quassinoids with various type and biological activities.Quassinoids has bitter taste and often found in Simaroubaceae family (Ebrahimi *et al.*, 2016). Eurycomanone is the most bioactive of quassinoids found in *E. longifolia* (Nhan and Loc, 2017). Some of pharmacological activities of *E. longifolia* were antimicrobial, antidiabetic, cytotoxicity against cancerous cells, drug for sexual insufficiency, immunomodulator, anti-inflammation, etc. (Abubakar *et al.*, 2018). In this article review, we aimed to examine the chemical substances and pharmacological activities of *E. longifolia*.

MATERIALS AND METHODS

The article review was conducted by collecting the scientific work about chemical compound, and pharmacology activity of *E. longifolia* which published in the last 10 years and minimum 20 articles that published in the last 2 years, published in Pubmed, Scopus etc., also has a DOI. The scientific work search by using keyword "*Eurycoma longifolia*".

RESULTS AND DISCUSSION

Traditional Uses

In Malaysia, decoction of *E. longifolia* have been used to treat depression, high blood pressure, malaria and fatigue (Talbott *et al.*, 2013). Based on cultural beliefs, the traditional uses of *E. longifolia* have been passed from generation to generations. The taste of the decoction is bitter and it is assumed more bitter give better efficacy (Ma *et al.*, 2017).

It has been commonly used in traditional medicine to treat dysentery and glandular swelling (Chinnappan *et al.*, 2019). The leaves of *E. longifolia* was used as herbal supplement for following child-birth and prevent gum disease (Ahmed *et al.*, 2020). Mostly the bark of *E. longifolia* is used as anthelmintic. Its roots extract was used as traditional medicine for malaria, aging, aches, constipation, fever, enhancing energy, osteoporosis, diabetes, anxiety, syphilis, stress, etc. (Rehman *et al.*, 2016). *E. longifolia* is also popular for its aphrodisiac effect that can enhancing libido due to its ability to stimulate the production of androgen hormones (Ezzat *et al.*, 2019a).

Due to high demand of this plant there are various formula of *E. longifolia* are available in health food market, in crude drug powder form or capsules which contain crude drug powder or *E. longifolia* extract (Effendy *et al.*, 2012). It can be found as health supplements in capsules, tablets and beverages (Fadzil *et al.*, 2018). Nowadays, root extract of *E. longifolia* used as pre-mixed in tea, coffee and carbonated drink which sales in commercial market (Low *et al.*, 2013).

Phytochemical Compounds

Several major phytochemical compounds have been determined in *E. longifolia* such as quassinoid, squalene-type triterpenes, canthin-6-one alkaloids, and tirucallane-type triterpenes (Bräuer *et al.*, 2019). Quassinoidswas the most phytochemical compound identified in *E. longifolia*, mostly C18-C22 quassinoids (Chua *et al.*, 2011). Besides that, polyphenols, high molecular weight polysaccharides and glycoprotein were also founded in root of *E. longifolia* (Tsai *et al.*, 2020). Polar to semi-polar saponins have been founded in *E. longifolia* roots (Chua *et al.*, 2019).

Flavonoids was discovered in ethyl acetate, chloroform, methanol and acetone extracts of stem and root of *E. longifolia*. Alkaloids was only detected in chloroform, ethyl acetate and petroleum ether extracts of the stem. Terpenoids was observed in all the extracts except acetone extract. All the extracts of *E. longifolia* stem except petroleum ether and chloroform have been discovered the presence of protein (Khanam *et al.*, 2015). Eurylophenolosides A and B, eurylolignanosides A and B which were four new phenolic acid along with 12 known isolates were detected from 70% ethanol extract of the roots (Ruan *et al.*, 2019).

Quassinoid has many various types with biological activity. Phytochemical studies of this *E. longifolia* have led to the discovery of quassinoids type which promising various biological activities (Yang *et al.*, 2020). Five quassinoids, eurycomanone, 13α(21)-epoxyeurycomanone, eurycomanol, eurycomanol-2-O-β-d-glucopyranoside and 13,21-dihydroeurycomanone were observed using electrospray ionization (ESI) and atmospheric pressure chemical (APCI) in positive and negative ion modes (Teh *et al.*, 2011). Other five new quassinoids, eurylactone E, eurylactone F, eurylactone G, eurycomalide D, and eurycomalide E were identified along with 10 known quassinoids (Park *et al.*, 2014). Meanwhile study by Meng *et al.*

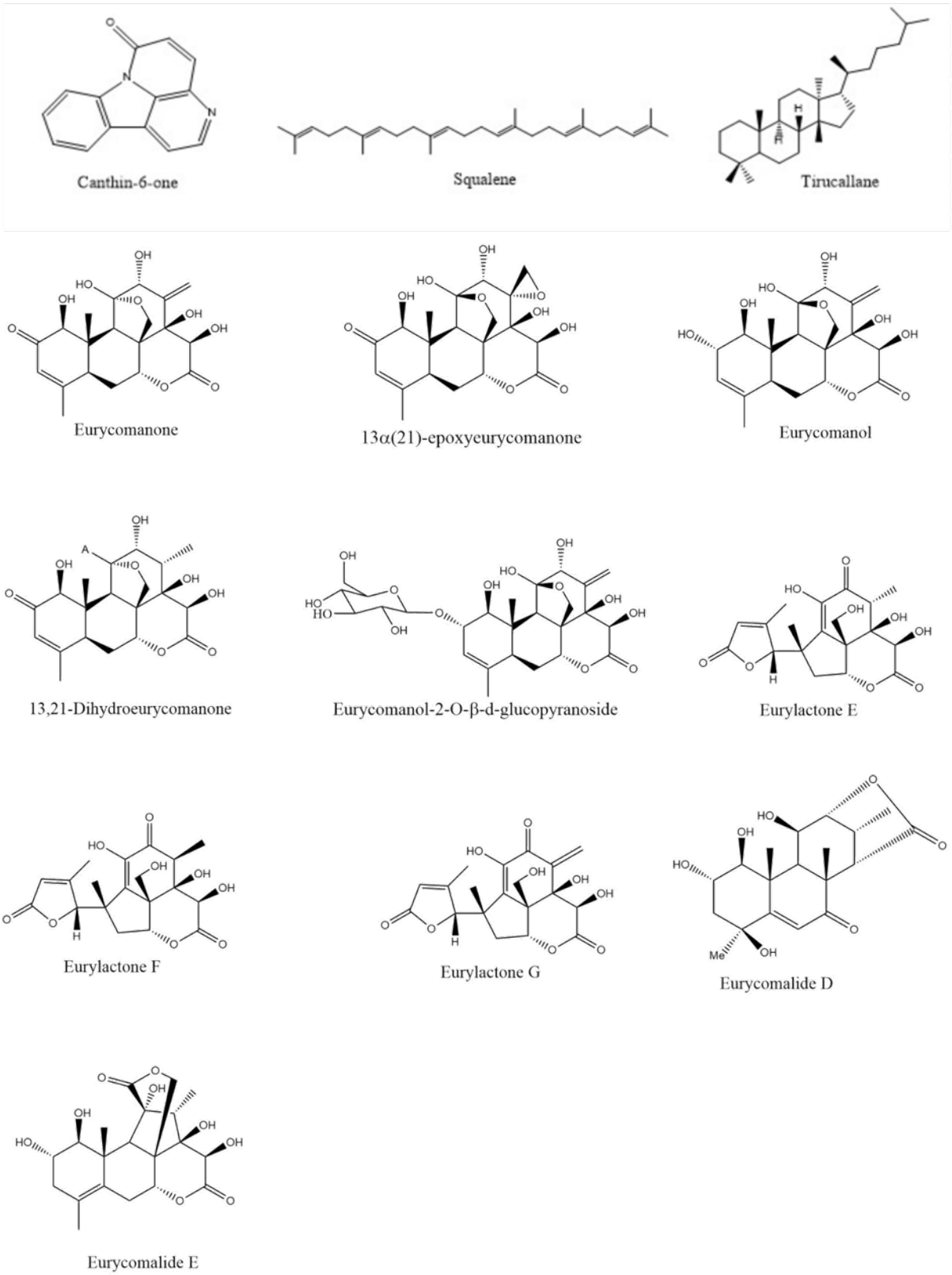

Figure 1: Structure of chemical compounds in *E. longifolia*

Table 1: Anti-inflammatory activity of isolated compound

Compound	IC_{50} (μM)	Ci_{95}(μM)
Eurycomalide C	18.4	16.9—20.1
Eurycomalactone	0.5	0.3—0.7
7α-hydroxyeurycomalactone	1.5	1.3—1.6
5,6-dehydroeurycomalactone	6.2	5.3—7.4
Eurycolactone E	3.8	3.2—4.5
Longilactone	4.7	3.7—5.9
14,15β-dihydroxyklaieanone	1.0	0.8—1.2
11-dehydroklaieanone	1.9	1.8—2.1
Eurycomanone	2.4	2.0—2.9
13,21-dehydroeurycomanone	0.7	0.6—0.9
1-methoxycarbonyl-β-carboline	29.3	20.3—42.4
9-hydroxycanthin-6-one	3.8	3.3—4.4
9-methoxycanthin-6-one	7.4	6.6—8.2
9,10-dimethoxycanthin-6-one	19.5	13.0—29.4
3,5,6,7,8,3′,4′-heptamethoxyflavone	23.3	18.4—29.4
Parthenolide (positive control)	1.5	1.3—1.8

Table 2: Antimicrobial activity of *E. longifolia*

Microorgan	Methanol extract		Acetone extract		Ethyl acetate extract		Chloroform extract		Petroleum ether extract	
	Stem	Root	Stem	Root	Stem	Root	Stem	Root	Stem	Root
E. coli	+	-	-	-	-	-	-	-	-	-
P. aerugi-nosa	-	-	-	-	+	-	-	-	-	-
S. Virchow	-	-	-	-	-	-	-	-	-	-
B. cereus	+	+	+	+	+	+	+	+	+	+
S. aureus	+	+	+	+	+	+	+	+	+	+
A. A. niger	-	-	-	-	+	-	-	-	-	-

Note: + = present; - = absent

(2014) exposed that Δ4,5,14-hydroxyglaucarubo, 5-iso-eurycomadilactone, eurycomadilactone, 13-epi-eurycomadilactone was found as four new quassinoids. The structure of chemical compounds in *E. longifolia* was shown in Figure 1.

Pharmacological Activities

E. longifolia had many pharmacological activities. The activities were described below:

Antioxidant Activity

Administration of 70% ethanol extract of *E. longifolia* root proved increase SOD (Super Oxidant Dismutase) levels and reducing MDA (Malondialdehyde) levels. *E. longifolia* contained flavonoid, alkaloid, phenols and glycoside which were known to have antioxidant activity (Triawanti *et al.*, 2020). Flavonoid has a role as free radical scavenger and stimulating antioxidant enzymes. Alkaloid has a role as scavenger of superoxide radical. The activity of antioxidant and SOD enzymes would increase but superoxide anion level decrease (Edyson *et al.*, 2019).

The other research by Oboh *et al.* (2018) presented that *E. longifolia* inhibited PDE-5, arginase and angiotensin-converting enzyme(ACE) as pro-oxidant-induced lipid peroxidation in a concentration dependent. The inhibitory activity showed with half maximum inhibitory concentration (IC_{50}). The result on PDE-5 (IC_{50} = 251.8 μg/ml), ACE (IC_{50} = 96.07 μg/ml) and arginase (IC_{50} = 48.28 μg/ml).

Anti-inflammatory and Analgesic Activity

All of the alkaloids from *E. longifolia* roots displayed potential nitric oxide(NO) inhibitory activities on lipopolysaccharide (LPS)-stimulated RAW264.7 cells. Many compounds such as 4,9-dimethoxy-5-

hydroxycanthin-6-one, 1-hydroxy-canthin-6-one, 4-methoxycanthin-6-one, 5-methoxy-canthin-6-one, 8-hydroxy9-methoxyycanthin-6-one, 4,9-dimethoxycanthin-6-one, 5,9-dimethoxycanthin-6-one and 9,10-dimethoxycanthin-6-one inhibited NO release from LPS-stimulated RAW264.7 (Zhang *et al.*, 2020).

Some of quassinoids and alkaloids showed the most potent NF-κB inhibitory effect with IC_{50} displayed in the Table 1 (Van Anh Tran *et al.*, 2014). Recent study was identified new anti-inflammatory β-carboline, 7-methoxy-(9Hβ-carbolin-1-il)-(E)-1-propenoic acid from *E. longifolia* hairy roots which had strong inhibitory effect on NO release and decrease cyclooxygenase-2 (COX-2) expression induced by LPS RAW264.7 (Ngoc *et al.*, 2016).

E. longifolia extract discovered anti-inflammatory activity on carrageenan-induced paw edema. It inhibits the expression of COX-2 induced by LPS through blocking the NF-κB translocation. The extract also showed analgesic effect on heat-induced (hot plate test) and chemical-induced pain (acetic acid induced writhing). The analgesic effects were time and dose dependent and the analgesic activity of 400 mg/kg *E. longifolia* was higher than aspirin at 2 and 3 h after administration in heat-induced pain but lesser at 1 h. Thus, the onset time of *E. longifolia* is slower than aspirin (Han *et al.*, 2016).

Immunomodulatory and Cytotoxic Activity

Polysaccharide from *E. longifolia* roots could activate macrophages by improving pinocytic and phagocytic abilities in concentrations <250 μg/ml, promote NO and cytokine secretion. The polysaccharide containeduronic acid and its backbone composed of β-1,4-xylose which enhanced its immunomodulatory activity (He *et al.*, 2019). sd

The compounds TAF273, F3 and F4 from methanolic extract of E. longifolia roots found had cytotoxic activity at IC_{50} 19$\pm$3, IC_{50} 55$\pm$2 and IC_{50} 62$\pm$7μg/ml.The TAF273, F3 and F4 were tested on K-562 cells (Al-Salahi *et al.*, 2014). The extract have been reported able to increase immunological parameters such as T cells, $CD4^+$ T cells and lymphocytes (George *et al.*, 2016).

Antitumor Activity

Extract of *E. longifolia* root enhanced the apoptotic level of adenocarcinoma cells. Thehigher the dose of *E. longifolia* extract showed the higher the apoptotic level of adenocarcinoma cells. After 24 h given the extract, the percentage of DNA damaged cells increased 39,4% and 43,5% after 48 h with highest concentration (100 μg/ml) (Rahman *et al.*, 2020).

The treatment with TAF273 compound induced apoptotic in K-562 cells in dose and time dependent. The TAF273 compound at concentration 25 and 50 μg/ml enhanced apoptotic index (AI) from 10% (untreated) to 30 and 41%. Eurycomanone at concentration of 6 and 12 μg/ml also enhanced the apoptotic index to approximately 28 and 39%. The apoptotic index were calculated from the mean from at least three experiments (Al-Salahi *et al.*, 2014).

Anti-osteoporotic

Shuid *et al.* (2011) revealed that *E. longifolia* had a potential in treating androgen deficient osteoporosis in men by alternative agent to testosterone replacement. Based on histomorphometry, quassinoids in *E. longifolia* extract effective as testosterone in the ORX + EL25, DGX + EL50, and DGX + EL100 groups to reduce degenerative changes of bone structure by enhancing bone volume and trabecular number. The extract also reduced percentage of osteoclast and increased percentage of osteoblast (Jayusman *et al.*, 2018). Other than that *E. longifolia* suppressed the high of C-terminal telopeptide of type I collagen to prevent the enhancement of bone resorption rate after orchiectomy (Chinnappan *et al.*, 2020). Based on recent study, *E. longifolia* showed anti-osteoporotic effect in six samples which improved fracture bones by increasing bone volume (Meng *et al.*, 2014).

Sexuality Drug for Men

Thu *et al.* (2017) demonstrated that *E. longifolia* was detected to increase male sexual libido, male fertility, penile erection and testosterone level. The conventional way to treat TDS (testosterone deficiency syndrome) is TRT (testosterone replacement therapy). *E. longifolia* showed improving sexual health by restoring testosterone levels and naturally used as TRT (George and Henkel, 2014).

Studies showed eurypeptides activate CYP17(17 α-hydroxylase/17,20 lyase), an enzyme that enhancing the conversion of pregnolone and 17-OH-pregnolone to produce more dehydroepiandrosterone and the metabolism of pregnolone and 17-OH-pregnolone to testosterone and 4-androstenedione (Erasmus *et al.*, 2012). Aqueous extract of *E. longifolia* found to inhibit ROCK-II which has a role for inhibiting smooth muscle contraction.Trans-coniferyl aldehyde, eurycomanone and scopoletin exhibited maximum inhibition of ROCK-II at 82.1$\pm$0.63, 78.3$\pm$0.38 and 77.1$\pm$0.11 % (Ezzat *et al.*, 2019b).

Anticancer and Antiproliferative

Eurycomanone had ability to affect the expression of cellular protein that have multifunctional roles in proliferation and associated with cancer develop-

ment. This compound will affect cell replication that involving in p53 tumor suppressor gene, cancer cells with hnRNP A2/B1 markers, annexin I, prohibitin and inhibit lung cancer proliferation (Wong *et al.*, 2012). SQ40 contain 40% of the total quassinoids showed inhibitory activity against LNaP human prostate cancer cells. SQ40 detected to inhibit LNCaP cell growth at IC_{50} 5.97 μg/ml. In lower dose, SQ40 inhibited LNCaP cell growth and in higher dose SQ40 can cause cleavage in LNCaP cells (Tong *et al.*, 2015).

Antimicrobial activity

Methanol, acetone, ethyl acetate, chloroform and petroleum ether extract of E. longifolia roots were found active against Gram positive bacteria but inactive against Gram negative bacteria. The chloroform extract of the root exhibited highest activity among Gram positive bacteria with inhibition zone of 11.67 $\pm$ 1.53 mm against S. aureus followed by acetone extract with inhibition zone of 11.00 $\pm$ 1.00 mm at 200 μg/ml.

Stem extracts were found active against Gram positive bacteria and showed low to moderate activity against Gram negative bacteria with inhibition zone of 9.33 $\pm$ 0.58 mm against P*. aeruginosa* and 3.33 $\pm$ 5.77 mm against *E. coli*. The ethyl acetate exposed the highest activity among all tested extracts with inhibition zone of 11.00 $\pm$ 1.73 mm against *Aspergillusniger* at 200 μg/ml (Khanam *et al.*, 2015). The antimicrobial activity of *E. longifolia* root and stem were presented in Table 2.

Research by Lee *et al.* (2018) study regarding influence of *E. longifolia* against tuberculosis. Treatment tuberculosis with *E. longifolia* and rifampicin found increasing in TNF-α production by promoting autophagy through ERK1/2 and NF-Kb signal to suppress intracellular *Mycobacterium tuberculosis* growth. Pasakbumin play a role in this activity. It was also enhanced levels of pro-inflammatory cytokine and NO via ERK1/2 and NF-Kb.

Antidiabetic

E. longifolia root was capable in modulating glucose and lipid metabolism. It enhanced insulin sensitivity and suppressed lipid production in 3T3-L1 adipocytes simultaneously. Also increase uptake glucose up to more than 200% with a dose of 50 μg/mL (Lahrita *et al.*, 2015).

As said above, *E. longifolia* has been used to treat erectile dysfunction that one of the compilations cause by diabetes. More than half men having diabetic also experiencing erectile dysfunction led to impotency (Thorve *et al.*, 2011). *E. longifolia* 800 mg/kg showed reducing in omentum fat weight and body weight of 31,9% and 5,7%. Opponently the serum testosterone concentration increased by 30.2% (Solomon *et al.*, 2014).

Anti-obesogenic

E. longifolia root expressed strong potential lipolysis enhancement but low pancreatic lipase inhibitory and reduced lipid accumulation in 3T3-l1 adipocytes. The roots containedeurycomanone and 13β,21-epoxyeurycomanone which increased lipolysis with EC_{50} of 14.6 and 8.6 μM (Ruangaram and Kato, 2020). The standardized quassinoid and eurycomanone fractionwas found affected in 3Y3-L1 preadipocyte cells. Both compounds decreased body weight, epididymal and perianal fat. Besides that, standardized quassinoids-enriched fraction (SQEL) also increased glucose clearance, reduced elevated total cholesterol and serum triglycerides levels (Balan *et al.*, 2018).

Antigout

Quassinoids in *E. longifolia* hadhuman urate transporter 1 (URAT1) inhibition activity by reducing blood uric acid levels in induced hyperuricemia animal model (Bao *et al.*, 2019). Study by Liu *et al.* (2019) presented that *E. longifolia* reduced ankle swelling caused by gout in low and medium doses which indicated that *E. longifolia* has anti-gout effect.

CONCLUSION

This review summarized the traditional usage, phytochemical compound and pharmacology activity. Based on the literature review was reported the most compound that gave pharmacology activity on *E. longifolia* were quassinoids especially euricumanone. This review showed *E. longifolia* has potential as medicinal plant which can be developed into traditional medicine for human. In the future, further study about mechanism of pharmacology activity of *E. longifolia* is needed.

ACKNOWLEDGEMENT

The authors are thankful to Department of Pharmaceutical Biology, School of Pharmacy, Bandung Institute of Technology, Bandung, Indonesia, for providing the facilities.

Conflict of Interest

The authors declare that there is no conflict of interest for this study.

Funding Support

The authors declare that they have no funding support for this study.

REFERENCES

Abubakar, B. M., Salleh, F. M., Omar, M. S. S., Wagiran, A. 2018. Assessing product adulteration of Eurycoma longifolia (Tongkat Ali) herbal medicinal product using DNA barcoding and HPLC analysis. *Pharmaceutical Biology*, 56(1):368–377.

Ahmed, N. A. K., Lim, S. K., Pandian, G., Sugiyama, H., Lee, C. Y., Khoo, B. Y., Chan, K. L. 2020. Differentially expressed genes on the growth of mouse Leydig cells treated with standardised Eurycoma longifolia extract. *Molecular Medicine Reports*, 22(5):3645–3658.

Al-Salahi, O. S. A., Ji, D., Majid, A. M. S. A., Kit-Lam, C., Abdullah, W. Z., Zaki, A., Din, S. K. K. J., Yusoff, N. M., Majid, A. S. A. 2014. Anti-Tumor Activity of Eurycoma longifolia Root Extracts against K-562 Cell Line: In Vitro and In Vivo Study. *PLoS ONE*, 9(1).

Balan, D., Chan, K.-L., Murugan, D., AbuBakar, S., Wong, P.-F. 2018. Antiadipogenic effects of a standardized quassinoids-enriched fraction and eurycomanone fromEurycoma longifolia. *Phytotherapy Research*, 32(7):1332–1345.

Bao, R., Liu, M., Wang, D., Wen, S., Yu, H., Zhong, Y., Li, Z., Zhang, Y., Wang, T. 2019. Effect of Eurycoma longifolia Stem Extract on Uric Acid Excretion in Hyperuricemia Mice. *Frontiers in Pharmacology*, 10:1–12.

Bräuer, P., Anielski, P., Schwaiger, S., Stuppner, H., Van Anh Tran, T., Vollmer, G., Zierau, O., Thieme, D., Keiler, A. M. 2019. In vitro metabolism of selected bioactive compounds of Eurycoma longifolia root extract to identify suitable markers in doping control. *Drug Testing and Analysis*, 11(1):86–94.

Chen, C. K., Ooi, F. K., Kasim, N. A. A., Asari, M. A. 2019. Effects of Eurycoma Longifolia Jack supplementation combined with resistance training on isokinetic muscular strength and power, anaerobic power, and urinary testosterone: Epitestosterone ratio in young males. *International Journal of Preventive Medicine*, 10(1):118.

Chinnappan, S. M., George, A., Ashok, G., Choudhary, Y. K. 2020. Effect of herbal extract Eurycoma longifolia (Physta®) on female reproductive hormones and bone biochemical markers: an ovariectomised rat model study. *BMC Complementary Medicine and Therapies*, 20(1):31–31.

Chinnappan, S. M., George, A., Krishnamurthy, P. T., Choudhary, Y., Vandana K. Choudhary, Ramani, Y., Dewangan, R. 2019. Corrigendum to "Nephroprotective Effect of Herbal Extract Eurycoma longifolia on Paracetamol-Induced Nephrotoxicity in Rats". *Evidence-Based Complementary and Alternative Medicine*, 2019:1–1.

Chua, L. S., Amin, N. A. M., Neo, J. C. H., Lee, T. H., Lee, C. T., Sarmidi, M. R., Aziz, R. A. 2011. LC–MS/MS-based metabolites of Eurycoma longifolia (Tongkat Ali) in Malaysia (Perak and Pahang). *Journal of Chromatography B*, 879(32):3909–3919.

Chua, L. S., Lau, C. H., Chew, C. Y., Dawood, D. A. S. 2019. Solvent Fractionation and Acetone Precipitation for Crude Saponins from Eurycoma longifolia Extract. *Molecules*, 24(7):1416.

Ebrahimi, F., Ibrahim, B., Teh, C., Murugaiyah, V., Lam, C. 2016. 1HNMR-Based Discriminatory Analysis of Eurycoma longifolia from Different Locations and Establishing a Profile for Primary Metabolites Identification and Quassinoids Quantification. *Planta Medica*, 83(01/02):172–182.

Edyson, P., Nugraha, A. M. E., Mashuri, H. G., Suhartono, E. 2019. In vivo antioxidant and UV-photoprotective of extract pasakbumi (Eurycoma Longifolia Jack. *AIP Conference Proceedings*, pages 20–029.

Effendy, N. M., Mohamed, N., Muhammad, N., Mohamad, I. N., Shuid, A. N. 2012. Eurycoma longifolia: Medicinal Plant in the Prevention and Treatment of Male Osteoporosis due to Androgen Deficiency. *Evidence-Based Complementary and Alternative Medicine*, 2012:1–9.

Erasmus, N., Solomon, M. C., Fortuin, K. A., Henkel, R. R. 2012. Effect of Eurycoma longifolia Jack (Tongkat ali) extract on human spermatozoa in vitro. *Andrologia*, 44(5):308–314.

Ezzat, S. M., Ezzat, M. I., Okba, M. M., Hassan, S. M., Alkorashy, A. I., Karar, M. M., Ahmed, S. H., Mohamed, S. O. 2019a. Brain Cortical and Hippocampal Dopamine: A New Mechanistic Approach for Eurycoma longifolia Well-Known Aphrodisiac Activity and Its Chemical Characterization. *Evidence-Based Complementary and Alternative Medicine*, 2019:1–13.

Ezzat, S. M., Okba, M. M., Ezzat, M. I., Aborehab, N. M., Mohamed, S. O. 2019b. Rho-Kinase II Inhibitory Potential of Eurycoma longifolia New Isolate for the Management of Erectile Dysfunction. *Evidence-Based Complementary and Alternative Medicine*, 2019:1–8.

Fadzil, N., Wagiran, A., Salleh, F. M., Abdullah, S., Izham, N. M. 2018. Authenticity Testing and Detection of Eurycoma longifolia in Commercial Herbal Products Using Bar-High Resolution Melting Analysis. *Genes*, 9(8):408.

George, A., Henkel, R. 2014. Phytoandrogenic properties ofEurycoma longifoliaas natural alternative

to testosterone replacement therapy. *Andrologia*, 46(7):708–721.

George, A., Suzuki, N., Abas, A. B., Mohri, K., Utsuyama, M., Hirokawa, K., Takara, T. 2016. Immunomodulation in Middle-Aged Humans Via the Ingestion of Physta® Standardized Root Water Extract of Eurycoma longifolia Jack-A Randomized, Double-Blind, Placebo-Controlled, Parallel Study. *Phytotherapy Research*, 30(4):627–635.

Han, Y. M., Woo, S. U., Choi, M. S., Park, Y. N., Kim, S. H., Yim, H., Yoo, H. H. 2016. Antiinflammatory and analgesic effects of Eurycoma longifolia extracts. *Archives of Pharmacal Research*, 39(3):421–428.

He, P., Dong, Z., Wang, Q., Zhan, Q. P., Zhang, M. M., Wu, H. 2019. Structural Characterization and Immunomodulatory Activity of a Polysaccharide from Eurycoma longifolia. *Journal of Natural Products*, 82(2):169–176.

Jayusman, P., Mohamed, I., Alias, E., Mohamed, N., Shuid, A. 2018. The Effects of Quassinoid-Rich Eurycoma longifolia Extract on Bone Turnover and Histomorphometry Indices in the Androgen-Deficient Osteoporosis Rat Model. *Nutrients*, 10(7):799.

Jiraungkoorskul, W., Khanijo, T. 2016. Review ergogenic effect of long jack, Eurycoma Longifolia. *Pharmacognosy Reviews*, 10(20):139.

Khanam, Z., Wen, C. S., Bhat, I. U. H. 2015. Phytochemical screening and antimicrobial activity of root and stem extracts of wild Eurycoma longifolia Jack (Tongkat Ali). *Journal of King Saud University - Science*, 27(1):23–30.

Lahrita, L., Kato, E., Kawabata, J. 2015. Uncovering potential of Indonesian medicinal plants on glucose uptake enhancement and lipid suppression in 3T3-L1 adipocytes. *Journal of Ethnopharmacology*, 168:229–236.

Lee, H. J., Ko, H. J., Kim, S. H., Jung, J, Y. 2018. Pasakbumin A controls the growth of Mycobacterium tuberculosis by enhancing the autophagy and production of antibacterial mediators in mouse macrophages. *BioRxiv*, 14(3):1–19.

Li, C. H., Liao, J. W., Liao, P. L., Huang, W. K., Tse, L. S., Lin, C. H., Kang, J. J., Cheng, Y. W. 2013. Evaluation of Acute 13-Week Subchronic Toxicity and Genotoxicity of the Powdered Root of Tongkat Ali (Eurycoma longifolia Jack). *Evidence-Based Complementary and Alternative Medicine*, pages 1–11.

Liu, Y., Lai, L., Ju, Y., Liu, C., Meng, D. 2019. Chemical constituents and synergistic anti-gout studies on Eurycoma longifolia and potential mechanisms evaluation based on systemic analysis approach. *Bioorganic Chemistry*, 92:103302.

Low, B.-S., Choi, S.-B., Wahab, H. A., Das, P. K., Chan, K.-L. 2013. Eurycomanone, the major quassinoid in Eurycoma longifolia root extract increases spermatogenesis by inhibiting the activity of phosphodiesterase and aromatase in steroidogenesis. *Journal of Ethnopharmacology*, 149(1):201–207.

Ma, H.-Q., Ebrahimi, F., Low, B.-S., Khan, N. A. K., Chan, K.-L. 2017. Polar Quassinoids in Standardized Eurycoma longifolia Extract Formulated into a Lipid-Based Solid Dispersion to Improve Rat Sperm Count. *Phytotherapy Research*, 31(12):1875–1882.

Meng, D., Li, X., Han, L., Zhang, L., An, W., Li, X. 2014. Four new quassinoids from the roots of Eurycoma longifolia Jack. *Fitoterapia*, 92:105–110.

Ngoc, P. B., Pham, T. B., Nguyen, H. D., Tran, T. T., Chu, H. H., Chau, V. M., Lee, J. H., Nguyen, T. D. 2016. A new anti-inflammatory β-carboline alkaloid from the hairy-root cultures of Eurycoma longifolia. *Natural Product Research*, 30(12):1360–1365.

Nhan, N. H., Loc, N. H. 2017. Production of eurycomanone from cell suspension culture of Eurycoma longifolia. *Pharmaceutical Biology*, 55(1):2234–2239.

Oboh, G., Adebayo, A. A., Ademosun, A. O. 2018. Phenolic-rich extracts of Eurycoma longifolia and Cylicodiscus gabunensis inhibit enzymes responsible for the development of erectile dysfunction and are antioxidants. *Journal of Basic and Clinical Physiology and Pharmacology*, 29(6):689–696.

Park, S., Nhiem, N. X., Kiem, P., Van, Minh, C., Van, Tai, B. H., Kim, N., Yoo, H. H., Song, J. H., Ko, H. J., Kim, S. H. 2014. Five new quassinoids and cytotoxic constituents from the roots of Eurycoma longifolia. *Bioorganic & Medicinal Chemistry Letters*, 24(16):3835–3840.

Rahman, E. Y., Kusworini, K., Ali, M., Purnomo, B. B., Kania, N. 2020. The Cytotoxic Effect of Eurycoma longifolia Jack Root Extract on The Prostate Adenocarcinoma PC-3 Cells through Apoptosis Enhancement. *Open Access Macedonian Journal of Medical Sciences*, 8(A):317–322.

Rehman, S., Choe, K., Yoo, H. 2016. Review on a Traditional Herbal Medicine, Eurycoma longifolia Jack (Tongkat Ali): Its Traditional Uses, Chemistry, Evidence-Based Pharmacology and Toxicology. *Molecules*, 21(3):331.

Ruan, J., Li, Z., Zhang, Y., Chen, Y., Liu, M., Han, L., Zhang, Y., Wang, T. 2019. Bioactive Constituents from the Roots of Eurycoma longifolia. *Molecules*, 24(17):3157.

Ruangaram, W., Kato, E. 2020. Selection of Thai Medicinal Plants with Anti-Obesogenic Potential

via In Vitro Methods. *Pharmaceuticals*, 13(4):56.

Shuid, A. N., Bakar, M. F. A., Shukor, T. A. A., Muhammad, N., Mohamed, N., Soelaiman, I. N. 2011. The anti-osteoporotic effect ofEurycoma Longifoliain aged orchidectomised rat model. *The Aging Male*, 14(3):150–154.

Solomon, M. C., Erasmus, N., Henkel, R. R. 2014. In vivoeffects ofEurycoma longifoliaJack (Tongkat Ali) extract on reproductive functions in the rat. *Andrologia*, 46(4):339–348.

Talbott, S. M., Talbott, J. A., George, A., Pugh, M. 2013. Effect of Tongkat Ali on stress hormones and psychological mood state in moderately stressed subjects. *Journal of the International Society of Sports Nutrition*, 10(1).

Teh, C.-H., Murugaiyah, V., Chan, K.-L. 2011. Developing a validated liquid chromatography–mass spectrometric method for the simultaneous analysis of five bioactive quassinoid markers for the standardization of manufactured batches of Eurycoma longifolia Jack extract as antimalarial medicaments. *Journal of Chromatography A*, 1218(14):1861–1877.

Thorve, V. S., Kshirsagar, A. D., Vyawahare, N. S., Joshi, V. S., Ingale, K. G., Mohite, R. J. 2011. Diabetes-induced erectile dysfunction: epidemiology, pathophysiology and management. *Journal of Diabetes and its Complications*, 25(2):129–136.

Thu, H. E., Mohamed, I. N., Hussain, Z., Jayusman, P. A., Shuid, A. N. 2017. Eurycoma Longifolia as a potential adoptogen of male sexual health: a systematic review on clinical studies. *Chinese Journal of Natural Medicines*, 15(1):71–80.

Tong, K. L., Chan, K. L., AbuBakar, S., Low, B. S., Ma, H. Q., Wong, P. F. 2015. The In Vitro and In Vivo Anti-Cancer Activities of a Standardized Quassinoids Composition from Eurycoma longifolia on LNCaP Human Prostate Cancer Cells. *PLOS ONE*, 10(3):e0121752.

Triawanti, Sanyoto, D. D., Noor, M. S. 2020. The supplementation of pasak bumi (Eurycoma longifolia Jack.) in undernourished rats to increase spatial memory through antioxidant mechanism. *Clinical Nutrition Experimental*, 33:49–59.

Tsai, C. H., Fang, T. C., Liao, P. L., Liao, J. W., Chan, Y. J., Cheng, Y. W., Li, C. H. 2020. The Powdered Root of Eurycoma longifolia Jack Improves Beta-Cell Number and Pancreatic Islet Performance through PDX1 Induction and Shows Antihyperglycemic Activity in db/db Mice. *Nutrients*, 12(7):2111.

Van Anh Tran, T., Malainer, C., Schwaiger, S., Atanasov, A. G., Heiss, E. H., Dirsch, V. M., Stuppner, H. 2014. NF-κB Inhibitors fromEurycoma longifolia. *Journal of Natural Products*, 77(3):483–488.

Wong, P.-F., Cheong, W.-F., Shu, M.-H., Teh, C.-H., Chan, K.-L., AbuBakar, S. 2012. Eurycomanone suppresses expression of lung cancer cell tumor markers, prohibitin, annexin 1 and endoplasmic reticulum protein 28. *Phytomedicine*, 19(2):138–144.

Yang, W. Q., Shao, X. H., Deng, F., Hu, L. J., Xiong, Y., Huang, X. J., Fan, C. L., Jiang, R. W., Ye, W. C., Wang, Y. 2020. Unprecedented Quassinoids from Eurycomalongifolia : Biogenetic Evidence and Antifeedant Effects. *Journal of Natural Products*, 83(5):1674–1683.

Zhang, Y., Zhao, W., Ruan, J., Wichai, N., Li, Z., Han, L., Zhang, Y., Wang, T. 2020. Anti-inflammatory canthin-6-one alkaloids from the roots of Thailand Eurycoma longifolia Jack. *Journal of Natural Medicines*, 74(4):804–810.