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Research Article

PARTIAL PURIFICATION AND ANTINOCICEPTIVE INVESTIGATION OF EXTRACTS OF LEAVES OF *LABISIA PUMILA*

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ABSTRACT

Labisia *pumila, LP* (Myrsinaceae), is a popular herb among the women in Malaysia known locally as ''Kacip Fatimah". It has been used by many generations of Malay women to induce and facilitate child birth as well as a post partum medicine. This study was aimed to investigate the partially purified crude extracts of DCM from the leaves of *LP* for its antinociceptive and analgesic effects against two noxious stimuli; thermal (hot plate) and chemical (formalin). Partial purification of crude DCM extract resulted in 5 different fractions (A-E). All the fraction of crude DCM extract of LP showed significant antinociceptive and analgesic activity in both the hot plate test and formalin test. Phytochemical test showed the presence of flavanoids, tannins, saponins, alkaloids and steroids which have contributed to the results obtained in the hot plate and formalin study. Based on the results, LP seem to exhibit a central and peripheral analgesic properties and further studies are necessary to elucidate the mechanism of its traditional effects and its potential to treatment of pain.

Keywords: Antinocieptive, *Labisia pumila,* Hot plate, Formalin, Phytochemical

INTRODUCTION

Labisia pumila(LP) is a popular herb from the family Myrsinaceae .It is a small woody and leafy plant that grows and can be found widely in the lowland and hill forests of peninsular Malaysia at an altitude of 300–700 m and other countries like Indochina, Sumatra, Java and Borneo [1]. In Malaysia, this plant is locally known as "Kacip Fatimah" and is quite popularly known among the traditional folk practitioners or womenfolk to relief pain during menstruation [2]. Although this plant has not been subjected to any detailed pharmacological investigation, some of its biological activities have recently been documented. A water extract of *LP* was found to exercise uterotrophic action and regulate body weight by modulating secretion of leptin and resistin, expression of the adipokines in adipose tissues [3]. In 2010 [4], reported that the extract of the *LP* posses anti-photoaging caused by UVB radiation. Moreover, Norhaiza [5] claimed that this plant also have anti-oxidative properties. However, the pharmacological potential of this plant as antinociceptive (pain reliever) agent is still being explored. Thus the purpose of the present study was to evaluate the antinociceptive and analgesic effects of the partially purified extracts of leaves *LP.*

MATERIALS AND METHODS

Materials

The leaves of *LP* (Kacip Fatimah) were purchased from University Putra Malaysia, Serdang, Selangor Darul Ehsan. The plant was specifically identified by Mr. Shamsul Khamis, a research officer (plant taxonomy) from the Laboratory of Natural Products (NATPRO), Institute of Bioscience in University Putra Malaysia.

Preparation of extracts

The leaves were air-dried for almost 3 weeks and were then grounded into fine powder using a miller. An extraction with dichloromethane was carried out by successive maceration at room temperature for a week followed by filtration. The filtration process was repeated several times to make sure all the dirt and dust are completely removed [6]. The filtrate obtained after filtration was then concentrated by evaporation using a rotary evaporator at temperatures of 35℃until dryness to maximize the proportion of desired bioactive fractions contained in the dichloromethane [7]. The process of extraction, filtration and concentration was repeated several times until there were maximum yield of crude DCM extracts and the plant has worn out. Silica gel column chromatography was employed in this study to isolate the entire five bioactive components namely fraction A to fraction E from the DCM extracts of the plant *LP*.

Animals

Healthy young adult Sprague-Dawley rats of both sexes weighing 200 g that were purchased from IMR were used in this study. The rats were screened and housed in standard polypropylene cages (three rats per cages), maintained under standard laboratory conditions (*i.e.* 12:12 hour light and dark cycle; at an ambient temperature of 25 ± 5°C; 50-70 % of relative humidity); the animals were fed with standard rat pellet diet and water was made available at all times.

Drugs

The *LP* extracts (100mg/kg each) was suspended in vehicle [2% of tragacanth powder, 2 drops of glycerol and Tween®40 in saline solution for rats respectively]. Morphine sulphate (10mg/kg) was dissolved in saline solution and aspirin (100 mg/kg) was dissolved in 0.1 ml of absolute ethanol. These analgesic drugs were used as antinociceptive reference drug. Drugs were freshly prepared on the day of the experiment.

Experimental Protocol

Hot plate test

In this method, a 24 cm diameter glass cylinder was placed on a hot plate with temperature set at 55 ± 0.5 °C. Latency of the rats was determined before and after the treatment. The latency was recorded at the time before and 15, 30, 45, and 60 minutes after intraperitoneal administration of the extracts or drugs [8]. Each rat was placed on the hot plate in order to obtained the animals response to heat-induce antinociceptive pain stimulus. Response was defined as licking, or biting of the paw, or jumping where all four paws leave the plate. Time taken for each response was noted and recorded in seconds [9]. A latency period of 30s was fixed as the cut off time to prevent tissue damage to the rats [10]. Eight group of rats (n=5) received dichloromethane extracts and fractions of *LP* (100mg/kg), 0.9% saline (1ml/kg) and morphine (10mg/kg) each.

Table 1: Effect of Hot plate test on rats and its Maximum Possible Effect (MPE) % versus treatment

Treatment	Dose (mg/kg)	Time (min) (MPE %)				
		0	15	30	45	60
Negative control (Saline)	-	3.45±0.072	3.63±0.211	3.62±0.245	3.59±0.228	3.57±0.227
Positive control(Morphine)	10mg/kg	5.37±0.489** (7.23)	6.58±0.309*** (11.19)	7.62±0.376*** (15.16)	9.06±0.472*** (20.71)	5.59±0.319** (7.64)
Fraction A	100mg/kg	3.97±0.238** (1.96)	4.37±0.135** (2.81)	4.55±0.172** (3.53)	4.86±0.068*** (4.81)	3.81±0.156 (0.91)
Fraction B	100mg/kg	4.05±0.188*** (2.26)	6.86±1.405*** (12.25)	8.58±0.754*** (18.80)	7.60±1.080*** (15.18)	4.02±0.215* (1.70)
Fraction C	100mg/kg	4.68±0.396** (4.63)	6.73±0.518** (11.76)	8.47±0.280*** (18.39)	9.80±0.275*** (23.51)	4.89±0.653** (4.99)
Fraction D	100mg/kg	4.52±0.634* (4.03)	5.97±0.344*** (8.87)	7.71±0.236*** (15.50)	6.02±0.138*** (9.20)	3.63±0.107 (0.23)
Fraction E	100mg/kg	4.31±0.195*** (3.24)	6.17±0.111*** (9.63)	7.49±0.076*** (14.67)	6.16±0.093*** (9.73)	4.78±0.146*** (4.58)
DCM Extract	100mg/kg	3.31±0.238 (-0.53)	4.48±0.105** (3.22)	5.72±0.110*** (7.96)	6.57±0.316*** (11.28)	3.74±0.164 (0.64)

Values given with respect to the mean ± SD, n=5 rats. Asterisks indicated significant difference from control. *p<0.05, **p<0.01, ***p<0.001 (ANOVA followed by paired t-test). MPE were calculated as percentage (%).

Table 2: Effect of Formalin test on rats and its Percentage of Inhibition

Group	Dose (mg/kg)	Number of licking			
		Early Phase (0-5min)	% inhibition	Late Phase	% inhibition (15-30)
Negative control (Saline)	-	44±3.16	0.00	58±5.29	0.00
Positive control(Aspirin)	100mg/kg	23±2.41***	47.73	21±1.00***	63.79
Fraction A	100mg/kg	21±3.58***	52.27	20±2.70***	65.52
Fraction B	100mg/kg	31±4.09**	29.55	20±3.96***	65.52
Fraction C	100mg/kg	40±3.21*	9.09	60±4.82	0.00
Fraction D	100mg/kg	31±5.26**	29.55	69±6.80	0.00
Fraction E	100mg/kg	37±2.74*	15.91	66±2.88	0.00
DCM Extract	100mg/kg	37±4.78*	15.91	24±3.51***	58.62

One hour after test drug administration (p.o), 2.5% formalin was subcutaneously injected to a hindpaw in volume of 50µl. Each data represent the mean number of licking time ± SD from 5 rats in the early phase (0-5 min) and late phase (15-30 min) after formalin injection. *p<0.05, **p<0.01, ***p<0.001 compared with the control group.

Formalin test

The method used was similar to what has been described by [11]Gonzalez, 2007. To induce nociception, rat were injected with 50 µl of 2.5% formalin in 0.9 % of saline solution into the subplantar surface of the left hind paw, 1 hour after the administration of 0.9 % saline, 100mg/kg of Aspirin and 100mg/kg of each extracts. Rats were then observed for 30 minutes and the time spent licking the paw was recorded in two phases. The data were express as total licking time in the early phase (0-5 min) and the late phase (15-30min) after formalin injection.

Statistical Analysis

The antinociceptive data were expressed as mean values + standard deviation. The results were analyzed by one-way analysis of variance (ANOVA) and paired *t-test* using statistical package for social science (SPSS) computer program version 18. Values were considered statistically significant when $p < 0.05$, highly significant difference when p<0.01 and very highly significant difference when p<0.001.

Phytochemical Analysis

Simple chemical test were performed to identify the possible bioactive fractions present in the effective fraction of the *LP*. The bioactive fractions that were tested for are flavonoids, alkaloids, tannins, steroids and saponins

RESULTS AND DISCUSSION

It is believed that current analgesia-inducing drugs such as opioids and non-steroidal anti-inflammatory drugs are not useful in all cases because of their side effects and low potency. As a result, searches for other alternatives medicinal plant became necessary and beneficial [12]. In this work we have studied the effect of all the partially purified fraction A-E and DCM crude extract from leaves of *LP* on two models of antinociceptive (hot plate and formalin). The hot-plate test is one of the widest used experimental methods to evaluate nociception in rats. It is based on the use of short duration nociceptive thermal stimulus. The nociceptive response in the hot plate model seems to results from direct activation of nociceptor such as opioid receptor and is inhibited by drugs which act mainly at central sites [13]. The hot plate test was selected to investigate central antinociceptive activity because it had several advantages, particularly the sensitivity to strong antinociceptives and limited tissue damage [14, 15]. *LP* extract given intraperitoneally at 100 mg/kg exhibited significant increase in the hot plate reaction time. The results showed that the partially purified fraction A-E and DCM crude extract showed a highly significant (p<0.001) antinociceptive activity (MPE) when compared with the negative control (saline, 100ml/kg). The MPE was measured at 45 minutes. Fraction C (23.51 %) has the highest MPE effect whereas fraction A (4.81 %) has the lowest MPE. Interestingly, the effect of fraction C (100mg/kg) was comparable to the reference drug morphine (10mg/kg). In formalin test, the formalin injection into the rat hind paw results in biphasic pain-related behaviors (such as licking, flinching and biting of the injured paw) that seems to involve two distinct mechanisms. The first phase (acute pain) appears immediately following formalin injection lasting only few minutes and is believed to be driven by primary afferent nociceptor activity [16]. The second phase (tonic pain) is observed 15 min after formalin injection, lasts at least for 60 min, and is thought to arise from nociceptive spinal neuron hyperactivity. In this second phase various mediators operate in a sequence to produce an inflammatory response and has been

correlated with the elevated production of prostaglandin (PG), induction of cyclo-oxygenase (COX) and release of nitric oxide (NO) [17], [18]. *LP* extract given orally at 100 mg/kg inhibited both the early and late phases in the formalin test. The results showed that the partially purified fraction A-E and DCM crude extract from the plant *LP* has a potent antinociceptive ($p<0.001$) effect against the chemical stimuli provoked by the formalin subplantar injection when compared with the negative control. The partially purified fraction A shows a better percentage of inhibition in the early and late phase, 52.27 % and 65.52 % respectively and the percentage of inhibition in both phases are higher than that of aspirin. Thus, fraction A ($p<0.001$) has higher potency of pain inhibition when compared to aspirin and can be assumed to be mimicking the action of aspirin in the inhibition process. These results suggest that the partially purified fraction A-E and DCM crude extract exhibited centrally mediated effect and peripheral mediated effect. Preliminary phytochemicals screening of the *Labisia pumila* extracts indicated the presence of flavonoids, tannins, saponin, alkaloids and steroids. Most of these of these phytochemical have been reported to have antinociceptive effect [19], [20], [21].

CONCLUSION

The partially purified fraction A-E and DCM crude extract of the leaves of *LP* showed a significant antinociceptive effect, as observed through hot plate (thermal stimulus) and formalin test (chemical stimulus). From these result, the partially purified fraction A-E and DCM crude extract has a centrally mediated effect and peripherally mediated effect. The antinociceptive effect may also be due to the presence of the phytochemical such as flavonoids, steroids, saponin, alkaloids and tannins which have been reported to possess antinociceptive effect.

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REFERENCES

1. Manneras, L., Fazliana, M., Wan Nazaimoon, W.M., Lönn, M., Gu, H.F., Östenson, C.G., and Stener. V.E., 2010. Beneficial metabolic effects of the Malaysian herb Labisia pumila var. alata in a rat model of polycystic ovary syndrome. Journals of Ethnopharmacology, 127, 346-351.
2. Singh, A.P., 2006. PHCOG MAG.: Short Review Distribution of Steroid like Fractions in Plant Flora. Pharmacognosy Magazine, 2(6), 87-89.
3. Fazliana, M., Wan Nazaimoon, W.M., Gu, H. F and Östenson, C.G., 2009. Labisia pumila extract regulates body weight and adipokines in ovariectomized rats. Journals of Ethnopharmacology, 62, 91-97.
4. Choi, H., Kim, D., Kim, J.K., Ngadiran, S., Sarmidi, M.R., and Park, C.S., 2010. Labisia pumila extract protects skin cells from photoaging caused by UVB irradiation. Journal of Bioscience and Bioengineering, 109(3), 291-296.
5. Norhaiza, M., Maziah, M., Hakiman, M., 2009. Antioxidative properties of leaf extracts of a popular Malaysian herb, Labisia *pumila*. Journal of Medicinal Plants Research 3(4), 217-223
6. Das, K., Tiwari, R.K.S., Shrivastava, D.K., 2010. Techniques for evaluation of medicinal plant products as antimicrobial agent: Current methods and future trends. Journal of Medicinal Plants Research, 4(2), 104-111
7. Raaman, N., 2006. Phytochemical Techniques. 1st Ed., New Delhi; New India Publishing, pg 13-20
8. Gunn, A., Bobeck, E.N., Weber, C., Morgan, M.M., 2010. The Influence of Non-Nociceptive Factors on Hot-Plate Latency in Rats. The Journal of Pain, 1st Ed, Pg 1-6
9. Ojewle, J.A.O., 2005. Antinociceptive, anti-inflammatory and antidiabetic effected Bryophyllum pinnatum (Crassulaceae) leaf aqueous extract. Journal of Ethnopharmacology, 99,Page 13-19.
10. Heidari,M.R.,Azad,E.M., Mehrabani,M., 2006. Evaluation of the analgesic effect of Echium amoenum Fisch & C.A. Mey. extract in mice: Possible mechanism involved. Journal of Ethnopharmacology, 103(3), Pg 345-349
11. Gonz´alez, T.M.E., Pe˜na, E.I., Mart´ınez, A.L., Moreno, J., Guevara, F.P., D´eciga, C.M., L´opez, M.F.J., 2007. Evaluation of the antinociceptive effect of Rosmarinus officinalis L. using three different experimental models in rodents. Journal of Ethnopharmacology, 111, pg 476–482
12. Jaishree, V., Badami, S., Kumar, M., Tamizhmani, T., 2009. Antinociceptive activity of swertiamarin isolated from Enicostemma axillare. Journal of Phytomedicine , 16 , Pg 227–232
13. Palacios, E.F., Deciga, C.M., Mata, R., 2008. Antinociceptive, hypoglycemia and spasmolytic effects of *Brickellia veronicifolia.* Journal of Ethnopharmacology, 118, 448-454.
14. Kou, J., Ni, Y., Li, N., Wang, J., Liu, L., Jiang, Z.H., 2005. Analgesic and antiinflammatory activities of total extract and individual fractions of Chinese medicinal ants poly- rhachis lamellidens. Biol. Pharm. Bull. 28, 176–180.
15. Janssen, P., Neimemegeers, C.J.E., Dony, J.G.H., 1963. The inhibitory effects of fentanyl and other morphine like analgesics on the warm water induced tail withdrawal reflex in rats. Arzneimittel Forschung 13, 502–507.
16. Shibata, M., Okhubo, T., Takahashi, H., Inoki, R., 1989. Modified formalin test: characteristics biphasic pain response. Pain 38, 346–352.
17. Hama, A., Menzaghi, F., 2001. Antagonist of nicotinic acetylcholine receptors (nAchR) enhances formalin-induced nociception in rats: tonic role of nAchRs in the control of pain following injury. Brain Research 888,
18. Le Bars, D., Gozariu, M., Cadden, S.W., 2001. Animal models of nociception. Pharmacological Reviews 53, 597–652.
19. Dilipkumar, P., Sahoo, M., Mishra, A.K., 2005. Analgesic and anticonvulsant effects of saponin isolated from the stems of Opuntia vulgaris Mill. in mice. European Bulletin of Drug Research 13, 91–97
20. Kumar, V.S., Nemmani, P., 2002. Role of benzodiazepine–GABAA receptor complex in attenuation of U-50,488H-induced analgesia and inhibition of tolerance to its analgesia by ginseng total saponin in mice. Life Sciences 70, 1727–1740.
21. Farouk, L., Laroubi, A., Aboufatima, R., Benharref, A., Abder-rahman, C., 2008. Evaluation of the analgesic effect of alkaloid extract of Peganum harmala L. possible mechanism involved. Journal Ethnopharmacology 115, 449-454.