Pharmacological Studies of Different Fractions of *Litsea monopetala* Roxb.

Israt Jahan Bulbul, Mohammad A. Rashid and Mohammad Rashedul Haque

Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Dhaka Dhaka-1000 Bangladesh

(Received: January 13, 2020; Accepted: January 28, 2020; Published: January 30, 2020)

Now-a-days formulations based on natural products getting attention because of their low or absence of toxicity, complete biodegradability, availability from natural sources and their low-cost compared to those of compounds obtained by total chemical synthesis (Abbott, 1995). In developing countries like Bangladesh, the study for biological activities from locally available plants have been raised significantly to reduce public health costs. From this perspective, a locally available medicinal plant of Bangladesh, was selected for this study to explore its pharmacological potential.

Litsea monopetala Roxb. (Synonym: *Litsea polyantha* Juss.) belonging to the family Lauraceae which is locally known as Bara Kukurchita, Mendaphuri, Sukurja, Uruijja (Chittagong), Akorma, Lalkhori (Dinajpur) and Huoria (Sylhet). It is distributed in the forests of Chittagong, Chittagong Hill Tracts, Sylhet and Sal forests of Gazipur, Madhupur, Dinajpur and also found in the villages throughout the country. *L. monopetala* is a small or medium sized evergreen tree with 7.5-23 cm long leaves, elliptic-oblong, usually rounded at both ends, pubescent at the beneath. Flowers are small, pale greenish yellow, sessile or subsessile, about 5-6 together in rounded umbellate heads, 1-1.3 cm across, solitary or clustered on dwarf side shoots. Fruit is 10 mm long, ovoid and black. Traditionally, water extract of the bark is given with sugar to treat diarrhea and dysentery while powder of the bark is applied to body for pains arising from blows or bruises or from hard work (Yusuf *et al*., 2009).

Different extracts from bark, aerial parts, leaves, flowers and fruits of the plant have been reported for its biological activities as well as the presence of phytochemicals. The extract of dried bark and aerial parts of *L. monopetala* has antidiarrheal and antidepressant activity (Poonia *et al*., 2007). Phenolic compounds extracted from *L. monopetala* bark have antioxidant activity (Arfan *et al*., 2008). The leaf extract possesses antimicrobial (Ahmad *et al*., 2012; Hasan *et al*., 2016), clot lysis, anti-inflammatory (Ahmad *et al*., 2012), analgesic (Ghosh and Sinha 2010) and antioxidant and antidiarrheal (Dutta, 1968) properties. The presence of eugenol, chalcone and its derivatives (Ghosh and Sinha 2010), β-sitosterol and actinodaphnine (Choudhury *et al*., 1997) in bark extracts, α-caryophyllene alcohol, pentacosane, caryophyllene oxide, humulene oxide and tricosane in the flower oil, decanal, nonanol and capric acid in fruit oil and tetradecanal, tridecanol, myristic acid and tridecanal in bark oil (Banerji *et al*., 1968) have been reported from *L. monopetala.* An arabinoxylanis reported from the mucilage of the leaves of *L. monopetala* (Bulbul *et al*., 2016).

As part of our continuing investigation on medicinal plants of Bangladesh (Bulbul *et al*., 2017; Kupchan and Tosu, 1973) the different fractions (petroleum, chloroform and ethylacetate soluble fractions) of *L. monopetala* leaves were studied for the preliminary phytoconstituents, antioxidant potential in terms of total phenolic content and free radical scavenging, antimicrobial, analgesic,

Correspondence to: Mohammad Rashedul Haque; E-mail: haquemr@du.ac.bd

DOI: https://doi.org/10.3329/bpj.v23i1.45322

hypoglycemic and CNS depressant activities and we here in report the results of one findings.

The phytochemical screening (Table 1) revealed that alkaloids, tannins, saponins, cardiac glycosides, anthraquinone glycosides are present in different partitionates of *L. monopetala* leaf, whereas carbohydrates and reducing sugar were absent in all the partitionates.

The total phenolic content of the extractives of leaves of *L. monopetala* was found in the range of 3.35 ±0.5 to 4.96±0.23) mg of GAE/g of extractives, with the highest amount of phenolics (4.96±0.23) being observed in the petroleum ether soluble fraction (Table 2). In the DPPH free radical scavenging assay, the petroleum ether soluble fraction of leaves of *L. monopetala* showed maximum free radical scavenging activity having IC_{50} value of 59.76 ±0.71 µg/ml while the standard ascorbic acid showed IC_{50} value of 51.54 ±0.17 µg/ml (Table 2).

Table 1. Preliminary phytochemical analysis of different partitionates of *L. monopetala* leaf.

Sl. No	Test	Reagent	PESF	CSF	EASF
1	Alkaloids	Mayer's, Hager's, Wagner's, Dragendorff's	+	+	+
2	Tannins	Lead acetate	+	+	+
3	Saponins	Water + shake	+	+	+
4	Cardiac glycosides	Glacial acetic acid + Ferric Chloride + Conc. Sulphuric acid	+	+	+
5	Anthraquinone glycosides	Brontrager's reagent	+	+	+
6	Carbohydrates	Molisch's, Fehling's, Barfoed's reagents	-	-	-
7	Reducing sugars	Benedict's solution	-	-	-

Table 2. Total phenolic content, DPPH free radical scavenging activity of *L. monopetala.*

Plant sample/ standard	Total phenolic content (mg of GAE/gm of extract)	DPPH free radical scavenging activity (IC_{50}µg/ml)
PESF	4.96 ± 0.23	59.76 ± 0.71
CSF	3.35 ± 0.55	66.80 ± 0.12
EASF	3.93 ± 0.63	60.40 ± 0.15
Ascorbic acid		51.54 ± 0.17

PESF= Pet-ether soluble fraction; CSF= Chloroform soluble fraction; EASF= Ethyl acetate soluble fraction.

The antibacterial activity of different partitionates of *L. monopetala* has been summarized in table 3. The pet-ether soluble fraction (PESF) of *L. monopetala* showed moderate antimicrobial activity against *Bacillus subtilis* (13.67 mm), *B. megaterium* (13.00 mm) and *Vibrio parahemolyticus* (12.67 mm). The chloroform soluble fraction (CSF) revealed good activity against *B. subtilis* (16 mm) and *V. parahemolyticus* (15.33 mm). The CSF showed mild to moderate activity (9 to 12 mm) against the remaining organisms. The ethyl acetate soluble fraction (EASF) demonstrated good antimicrobial activity against *B. subtilis* (15.67 mm), *B. Megaterium* (16.33 mm), *B. cereus* (15.67 mm) and *P. aeruginosa* (15.33 mm) whereas mild to moderate activity against other organisms.

The leaf extract of *L. monopetala* showed significant peripheral ($p < 0.05$) analgesic activity at both doses of 100 and 200-mg/kg body weight with writhing inhibition of 33.89 and 38.98%, respectively (Table 4).

The effects of methanol extract of *L. monopetala* leaves on blood glucose level in alloxan induced diabetic rats are shown in table 5 which represents that the blood glucose level significantly decreased ($p<0.05$) on the 5th and 7th day of treatment after administration of both the 300 mg/kg/day and 500 mg/kg/day doses of the extract.

Table 3. Zones of growth inhibition (mm) showing antibacterial activity for three fractions of *L. monopetala*.

Bacterial Strain	PESF	CSF	EASF	Kanamycin/Griseofulvin
Bacillus subtilis	13.67 ± 0.47	16.00 ± 0.82	15.67 ± 0.47	31.33 ± 0.94
B. megaterium	13.00 ± 0.82	11.33 ± 1.25	16.33 ± 0.47	32.00 ± 0.82
B. cereus	10.33 ± 0.47	9.67 ± 0.94	15.67 ± 0.94	30.67 ± 0.94
Staphylococcus aureus	8.33 ± 0.94	10.67 ± 0.94	11.00 ± 0.82	32.67 ± 0.47
Sarcina lutea	9.00 ± 0.82	12.33 ± 0.47	14.33 ± 0.47	30.33 ± 0.47
Escherichia coli	8.00 ± 1.41	11.00 ± 0.82	10.00 ± 0.82	29.33 ± 0.94
Vibrio mimicus	8.00 ± 0.82	11.67 ± 0.47	14.67 ± 0.47	30.33 ± 1.25
V. parahemolyticus	12.67 ± 0.47	15.33 ± 0.47	14.00 ± 0.82	28.33 ± 0.47
Pseudomonas aeruginosa	10.33 ± 0.47	11.00 ± 0.82	15.33 ± 0.47	31.33 ± 0.94
Salmonella paratyphi	11.67 ± 0.94	11.67 ± 0.47	12.67 ± 0.47	28.33 ± 0.47
Shigella dysenteriae	10.00 ± 0.82	11.33 ± 1.25	12.67 ± 0.94	30.67 ± 0.94
S. boydii	9.00 ± 0.82	9.67 ± 0.47	9.67 ± 0.47	30.33 ± 0.47
Candida albicans	8.67 ± 0.47	11.67 ± 0.47	14.33 ± 0.47	31.67 ± 1.25
Asperagillus niger	7.67 ± 0.94	11.33 ± 0.94	10.00 ± 0.82	29.67 ± 1.25
Sacharomyeces cereveceae	10.00 ± 0.82	11.00 ± 0.82	10.67 ± 0.47	28.67 ± 0.47

Values for zone of growth inhibition are presented as mean ± SD; disc diameter is 5.0 mm

Table 4. Peripheral analgesic activity of methanolic crude extract of *L. monopetala.*

Group	Treatment, dose &route	No. of writhing	% of inhibition
Group I (Control)	Saline water, tween 80, p.o	14.75 ± 3.21	-
Group II (Standard)	Indomethacin, 10 mg/kg, p.o	3.00 ± 1.20***	79.66%
Group III	*L. monopetala* 100 mg/kg, p.o	9.75 ± 3.21*	33.89%
Group IV	*L. monopetala* 200 mg/kg, p.o	9.00 ± 1.25*	38.98%

All values are expressed as mean ± SEM, (n=6); One way Analysis of Variance (ANOVA) followed by Dunnet's test. ***p<0.001, **p<0.01, *p<0.05, significant compared to control.

Table 5. Hypoglycemic Activity of methanolic crude extract of *L. monopetala*

Groups	Blood glucose level			
	1st Day	3rd Day	5th. Day	7th. Day
Control (Non-diabetic)	5.20 ± 0.17	5.01 ± 0.13	5.50 ± 0.35	4.87 ± 0.26
Control (Diabetic)	10.65 ± 0.22	10.16 ± 0.49	12.54 ± 0.32	11.93 ± 0.51
STD (Metformin HCl) 50 mg/kg/day	12.46 ± 0.67	5.53 ± 0.27**	4.46 ± 0.14**	4.26 ± 0.32**
MELM 300 mg/kg/day	9.8 ± 0.52	7.00 ± 0.62	6.55 ± 0.35*	6.00 ± 0.27**
MELM 500 mg/kg/day	11.2 ± 0.12	6.50 ± 0.62	5.25 ± 0.05**	5.00 ± 0.20**

All values are expressed as mean ± SEM, (n=6); One way Analysis of Variance (ANOVA) followed by Dunnet's test. ***p<0.001, **p<0.01, *p<0.05, significant compared to control.

In CNS depressant activity test, the extract showed a decrease in locomotion in the test animals. The number of crossing hole from one chamber to another by mice of the control group remained almost steady from 0 minute to 120 minutes (Table 6). But the three different fractionates at 500 mg/kg dose

showed significant and gradual decrease of movement from 0 to 120 min, which suggest the presence of CNS depressant potential in *L. monopetala*.

Table 6. CNS depressant activity of methanol extract of *L. monopetala* leaves.

Treatment	Doses	Number of Movements				
		0 min	30 min	60 min	90 min	120 min
1% tween 80 in saline water (Control)	10 ml/kg	13.50 ±1.19	14.00 ± 1.29	14.25 ± 0.85	14.00 ± 1.08	13.50 ± 0.29
Diazepam (Standard)	1 mg/kg	10.75 ± 0.48	6.75 ± 0.25*	4.00 ± 0.48*	2.75 ± 0.48	1.50 ± 0.29*
MESF	500 mg/kg	6.75 ± 0.99***	5.00 ± 0.82***	4.00 ± 1.05***	4.00 ± 0.82***	2.25 ± 0.73*

All values are expressed as mean ± SEM, (n=6); One-way Analysis of Variance (ANOVA) followed by Dunnet's test. ***p<0.001, **p<0.01, *p<0.05, significant compared to control.

It is clearly evident from the above findings that different partitionates of *L. monopetala* leaves contain alkaloids, tannins, saponins, cardiac glycosides, antraquinone glycosides and have significant free radical scavenging, moderate antimicrobial activities and significant peripheral analgesic. The plant also exhibited comparable hypoglycemic potential on the 5th and the 7th day of treatment. It has been reported that the leaves of *L. monopetala* are used to slow aging process, treat infectious diseases, pain, insomnia and other health disorders. Our findings rationalize some of the traditional uses of the plant species. Therefore, the plant is a good candidate for further chemical investigations to isolate the active constituents.

References

Abbott, A. 1995. Life sciences gain favour in patents. *Nature*. 375, 619.

Ahmad, A., Islam, M.T., Sultana, I., Mahmood, A., Hossain, J.A., Homa, Z., Ibrahim, M. and Chowdhury, M.M.U. 2012. Pharmacological and phytochemical screening of ethanol extract of *Litsea monopetala* (Roxb.) Pers. *IOSR J. Pharm.* **2**, 398-402.

Arfan, M., Amin, H., Kosinska, A., Karamac, M. and Amarowicz, R. 2008. Antioxidant activity of phenolic fractions of *Litsea monopetala* (Persimon-leaved Litsea) bark extract. *Pol. J. Food Nutr. Sci*. **58**, 229-233.

Banerji, N., Sarkar K.K. and Das, A.K. 1986. An arabinoxylan from the mucilage of the leaves of *Litsea polyantha*. *Carbohyd. Res.*, **147**, 165-168.

Bulbul, I.J., Fashiuddin S.B., Haque M.R., Sultan M.Z. and Rashid M.A. 2017. Anti-nociceptive and anti-inflammatory activities of *Crotalaria pallida* Aiton (Fam: Fabaceae) leaves. *Bangladesh Pharm. J.* **20**, 165-171.

Bulbul, I.J., Khan, M.F., and Rashid, M.A. 2016. Analgesic and central nervous system depressant activities of methanol extract of *Ziziphus rugosa* Lam. Leaves. *Afr. J. Pharm. Pharmacol.* **10**, 849-853.

Choudhury, S.N., Ghosh, A.C., Choudhury, M. and Leclercq, P.A., 1997. Essential oils of *Litsea monopetala* (Roxb.) Pers. *J. Essent. Oil Res.* **9**, 635-639.

Dutta, T., 1968. Investigations on *Litsea polyantha*, isolation and identification of actinodaphnine. *J. Indian Chem. Soc.* **45**, 987-991.

Ferdous, M.R, Ashrafudoulla, M., Hossain, M.S., and Bellah S.F. 2018. Evaluation of antioxidant, analgesic and antidiarrheal activities of methanolic extract of *Litsea monopetala* (Roxb.) leaves. *Clin. Pharmacol. Biopharm.* **7**, 1-6.

Ghosh, M. and Sinha, B.N. 2010. GC–MS studies on the bark extracts of *Litsea polyantha* Juss. *Middle-East J. Sci. Res.* **5**, 441-444.

Hasan, M.F., Iqbal, M.A. and Uddin, M.S. 2016. Antibacterial and antifungal activity of *Litsea monopetala* leaves on selected pathogenic strains. *Euro. J. Med. Plant.* **12**, 1-8.

Poonia, B.S., Sasmal D. and Mazumdar, P.M. 2007. Anti-diarrheal activity of methanol extract of *Litsea polyantha* bark in mice. *Fitoterapia* **78**, 1710-174.

Yusuf, M., Begum, J., Hoque, M.N. and Chowdhury, J.U. 2009. Medicinal Plants of Bangladesh (revised and enlarged). Bangladesh Council of Scientific and Industrial Research Laboratories, Chittagong, Bangladesh. p. 517.