IJP (2022), Vol. 9, Issue 6 **(Research Article)**

Received on 10 December 2021; received in revised form, 24 June 2022; accepted, 28 June 2022; published 30 June 2022

ANALGESIC, ANTIDIARRHOEAL AND ANTIMICROBIAL ACTIVITIES OF *LIMNOPHILA POLYSTACHYA* BENTH (SCROPHULARIACEAE) LEAF EXTRACTS

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Keywords:

Analgesic activity, Antibacterial, Antidiarrhoeal, *Limnophila polystachya*

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ABSTRACT: Phytopharmacology is always a vital way for drug discovery. Antibiotic resistance is rising as a challenging threat day by day. Diarrhea and pain are always common problems. The present study was designed to evaluate the Analgesic, Antidiarrhoeal, and Antibacterial activities of ethanol extracts of leaf of *Limnophila polystachya* Benth by different methods. The analgesic activity of the samples was studied using the acetic acid-induced writhing model in mice. The Thomas method observed castor oil-induced antidiarrheal activity, and antimicrobial activity was monitored by the disc diffusion method. At a higher dose (500 mg/kg), Limnophila polystachya Benth inhibited 65.79 % and 63.16 % writhing of stem and leaf acetone extracts, respectively, compared to standard drug Diclofenac sodium inhibited 78.07 %writhing. Further study is needed to find possible active ingredients and mechanisms of action as well. At a higher dose (500 mg/kg) of the acetone stem and leaf extracts, significant inhibition of 50.58 and 52.87 % of characteristic diarrhoeal feces was observed, respectively, and at a lower dose (250 mg/kg) of both extracts, inhibition 41.37 and 40.22 %. A moderate zone of inhibition was observed at 250µg/disc and 500µg/disc compared to the zone of inhibition at an average 38mm of ciprofloxacin at 50µg/disc. From the above results, it is clear that Limnophila polystachya Benth showed significant pharmacological potentiality in different study models. So, it will be very much a possible source for an isolating lead compound for curing numerous disorders.

INTRODUCTION: Plant secondary metabolites play an important role in medical care for a good percentage of the world population.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.IJP.9(6).127-31

Article can be accessed online on: www.ijpjournal.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.IJP.9(6).127-31

Thus, emphasis is now given to the standardization of herbal medicines by a screening of biological activities of medicinal plants and isolation of active principles from them [1].

Diarrhoea is a major problem for developing countries, especially in infants and children, causing morbidity and mortality [2]. Distribution of the entire world diarrhoea accounts for more than 5-8 million deaths each year who are below 5 years old [3]. Diarrhea is a changing normal bowel

movement which characterized by increased frequency of bowel sound and movement, watery stool and abdominal pain [4]. Diarrhoea could be responsible for dehydration and electrolyte imbalance through loss of fluids [5]. Analgesics are agents which selectively relieve pain by acting in the CNS and peripheral pain mediators without changing consciousness. The study of pain in animals raises ethical, philosophical, and technical problems. Both peripheral and central pain models are included to make the test more evident for the analgesic property of the plant [6]. In antique times mankind invented the existence of microbes; the idea indicated certain plants had healing potential, indeed, that they contained antimicrobial properties [7]. Medicinal plants represent an abundant origin of antimicrobial agents [8, 9]. Antimicrobial is the term that refering to a group of drugs such as antibiotics, antifungals, antiprotozoals, and antivirals [10, 11].

Limnophila polystachya Benth belongs to the family Scrophulariaceae is an herb and rare trees, mostly autotrophic and less often hemiparasitic. Stipules are absent; leaves are alternate, opposite, whorled or basally opposite and apically alternate (Zipcode Zoo.com). The plant species are widely used in traditional Indian medicine to treat pestilent fever, dysentery, elephantiasis, dyspepsia, antipyretic, expectorant, lactogogue and also exhibits significant antibacterial antifungal and antineoplastic activities [12]. The *Limnophila polystachya* has been reported for its antitumor promoting agent, anti mutagenic [13], antimyco bacterial and antioxidant activity [14, 15].

The plant genus of species Limnophila polystachya has not been reported in any phytochemical investigation, and further logistic pharmacological actions further it's no more revealed that any work in this clinical investigation. In the past studies, the photo chemical studies reported that Limnophila indica resulted in the isolation of 5-hydroxy-6, 8-dimethoxy-3',4'-methylenedioxyflavone 16 5,8-dihydroxy-6,7,4'-trimethoxyflavone [6]. The essential oil has been isolated from *Limnophila rugosa* [17]. Two novel flavonoids have been reported on Limnophila *indica* [18, 19].

MATERIALS AND METHODS:

Collection of Plant Material: *Limnophila polystachya* Benth. (Scrophulariaceae) was collected from Samayapuram, Tiruchirappalli District in Tamil Nadu in September-November 2008 and identified by the taxonomists in Botanical Survey of India, Coimbatore, Tamil Nadu, India. The voucher specimen Number is BSI/ SC/5/23/08-09/Tech.275. After authentication, the fresh plant was collected in bulk washed, shade dried and pulverized in a mechanical grinder to obtain coarse powder.

Chemicals and Reagents: Diclofenac sodium, loperamide, ciprofloxacin, acetic acid, and castor oil were used.

Preparation of Ethanol Extract: At first, a clean flat flat-bottomed glass container was taken. About 300 gm of powdered leaves were added in a separate clean, flat-bottomed glass container. Then 1000 ml of ethanol was added into the container and soaked the powder into the methanol. Afterward, the container was sealed with its contents and kept for 14 days, accompanying occasional shaking and stirring. After that, the coarse part of the leaf was separated from the mixture by using white cotton. Then the liquid portion was also filtered three times with the help of white cotton. Then again, it was filtered through Whatman filter paper. Then the filtrate was kept in a Rotary evaporator machine, which separated the solvent, and desirable crude extract was obtained.

Experimental Animals: Swice albino mice (21-25g) were purchased from local market and their ages five to six weeks and were housed in animals' cages under standard environmental conditions (22-25°C, humidity 60-70%, 12 hr light: 12 hr dark cycle). The mice were feed with standard pellet diet. The animals used in this study were cared in accordance with the guidelines on animal experimentation of our institute. The animal ethical committee approval number is SBCP/2020-2021/CPCSEA/IAECI(1)/F16/154.

Test Microorganisms: Four pathogenic bacterial strains were used to evaluate antibacterial activity. Two were Gram-negative (Klebsiella Oxytoca, Escherichiacoli), and two were gram-positive (*Bacillus subtilis*, *Staphylococcus aureus*). All of the bacterial strains were collected from Microbiology Lab.

Analgesic Activity: For the analgesic test, all mice were divided into six groups. Each group comprises

4 mice. Control group (received 0.5% methylcellulose, per oral), Standard Group (received Diclofenac-Na 10mg/ kg intra-peritoneally), group III and IV were treated with Leaf extracts of *Limnophila polystachya* at the doses of 250 and 500 mg per kg of body weight, respectively. The analgesic activity of the samples was studied using acetic acid-induced writhing model in mice. Test samples and vehicle were administered orally 30 mins before intraperitoneal administration of 10ml/kg of 7% acetic acid, but Diclofenac-Na was administered intraperitoneally 15 min before the acetic acid injection; the mice were observed for specific contraction of body referred to as "writhing" for the next 10 min [20]. Percentage protection of acetic acid induced writhing was calculated by the formula.

$$\text{Percentage protection} = (Wc\text{-}Wt) / Wc \times 100$$

Where, wc is the mean value of control group, and Wt is the mean value of the treated group.

Castor Oil-Induced Diarrhoea: 24 mice were allowed to fast for 18 h and divided into six groups of four animals each. All groups received castor oil at a dose of 1 ml/animal orally (p.o.). 30 min after castor oil administration, group I (control group) received vehicle (1% CMC in distilled water), Group III and Group IV orally received the ethanol extract of leaf of *Limnophila polystachya* 250 mg/kg and 500 mg/kg doses, respectively. Group II received the reference drug, loperamide (3 mg/kg p.o.). Then the animals were placed separately in cages with filter papers underneath, which were changed every hour. The severity of diarrhoea was assessed each hour for 4 h and the characteristic diarrhoeal droppings were recorded [21].

Test of Antimicrobial Activity by Disc Diffusion Method: In this method-measured amount of the test, samples are dissolved in definite volumes of solvent to give solutions of known concentration (µg/ml). Then sterile material filter paper discs are impregnated with many test substances using a micropipette and dried. Standard antibiotic discs and discs on which the solvent used to dissolve the samples is adsorbed and dried are used as the positive and negative control. These discs are then placed in petri dishes (120 mm in diameter) containing a suitable agar medium seeded with the test organisms using a sterile transfer loop for antimicrobial screening. The plates are then kept at 40 °C to facilitate maximum diffusion. The test material diffuses from the discs to the surrounding medium. The plates are then kept in an incubator (37°C) for 12-18 hours to allow the microorganisms' grow. If the test material has any antimicrobial activity, it will inhibit microorganism's growth, giving a clear, distinct zone called "zone of inhibition". The antibacterial activity of the test agent is determined by measuring the diameter of the zone of inhibition in term of a millimeter. The experiments are carried out three times, and the mean of the reading are recorded [22].

Statistical Analysis: The results are presented as Mean ± SEM. Data were analyzed by one-way ANOVA followed by Dunnet's test and P values <0.001 were considered statistically significant.

RESULT AND DISCUSSION: *Limnophila polystachya* hindered 64.57 % writhing of leaf extracts, respectively, In the acetic acid-induced writhing test, the extract of showed a significant ($p<0.001$) reduction in the number of writhes compared to standard drug Diclofenac Na inhibited 75.06 % writhing. Strong prominent effects were observed with both extracts group (500 mg/kg) **Table 1**.

TABLE 1: ANALGESIC EFFECT OF *LIMNOPHILA POLYSTACHYA* LEAVE EXTRACTS ON ACETIC ACID-INDUCED WRITHING IN MICE

Treatment	Writhing counting (Mean ± SEM)	% Inhibition
Control	28.5±0.77	
Standard	6.25±0.22***	75.06
(Diclofenac Na)	10.25±0.43***	63.16
Leaf 250 mg	9.5±0.58***	64.57
Leaf 500 mg		

Values are presented as Mean ± SEM (n = 4), ***P < 0.001, which is significant compared with the control group (one-way ANOVA followed by Dunnett test).

Pain is an unpleasant sensation as well as emotional touching, which is linked to tissue damage. Its aim is to allow the body to react and prevent tissue damage as well. The cause of the pain may also be damage to the nervous system, the peripheral nerves, the brain, and the spinal cord. Pain can also be occurred without damaging tissues, although the patient refers to it (as psychogenic pain). The mode of pain is a perplexing phenomenon. Experience of

pain depends on the strength of the stimulus and individual resistance to pain. Pain receptors are sensitive to mechanical, thermal or chemical stimuli. The operation of harmful stimulus to these receptors results in the process into an electrical signal [23]. The extract at 250 and 500 mg/kg doses produced a dose-dependent decrease in the number of faecal matters passed by the mice in castor oil-induced diarrhoeal model **Table 2**. In superior dose (500 mg/kg) of the leaf extract, significant inhibition 51.67% of characteristic diarrhoeal faeces was observed, respectively, as well as in poorer dose (250 mg/kg) of the extract inhibition, 40.12 % of diarrhoeal faeces was found, respectively. The castor oil metabolite ricinoleic acid-activated intestinal and uterine smooth muscle cells *via* prostaglandin E2 receptor 3 (EP3) prostanoid receptors which stimulate peristaltic activity in the small intestine, leading to changes in the electrolyte permeability of the intestinal mucosa. That's why delayed diarrhoea induced with castor oil [24, 25]. The antimicrobial effects of ethanol leaf extract against different test organisms are shown in **Table 3**. Both extracts were showed moderate inhibitory activity against all of these organisms.

TABLE 2: EFFECT OF LEAF EXTRACT OF *LIMNOPHILA POLYSTACHYA* ON CASTOR OIL-INDUCED DIARRHOEA IN MICE

Treatment	No. of faecal droppings in 4 h	% Inhibition of defaecation
Control	21.75±1.18	-
Standard	7.0±0.37***	67.82
(Loperamide)	13.0±0.37***	40.12
Leaf 250 mg	10.25±0.43***	51.67
Leaf 500mg		

Values are presented as Mean ± SEM (n = 4), ***P < 0.001, which is significant compared with the control group (one-way ANOVA followed by Dunnett test).

TABLE 3: *IN-VITRO* ANTIBACTERIAL ACTIVITY OF LIMNOPHILA POLYSTACHYA LEAF EXTRACTS

Bacterial Strains	Type of bacteria	Blank	Ciprofloxacin (50µg/disc)	Leaf (250 µg/disc)	Leaf(500 µg/disc)
Klebsiella Oxytoca	Gram (-)	-	34mm	10mm	8mm
Escherichia coli	Gram (-)	-	36mm	14mm	16mm
Bacillus subtilis	Gram (+)	-	35mm	12mm	12mm
Staphylococcus aureus	Gram (+)	-	38mm	14mm	14mm

Gram (-): indicates Gram Negative Bacteria and Gram (+): Gram Positive Bacteria.

CONCLUSION: The results of this scientific research reflects that the ethanol leaf extract of *Limnophila polystachya* is safe and may provide possibilities of alleviation of diarrhoea along with being a potential wellspring of antioxidant and antimicrobial agents which can be considered as an alternate source for exploration of new medicinal products in near future. These activities support to the ethno pharmacological uses of this plant. This species can be promoted for the large scale cultivation and marketing for the benefit of the local communities.

ACKNOWLEDGEMENT: Nil

CONFLICT OF INTEREST: Nil

REFERENCES:

1. Burkill HM: The useful plants of west tropical Africa. Families EI. Royal Botanic Gardens 1994; 2.
2. Mamoon S and Azam MG: Preliminary phytochemical screening and antidiarrhoeal activity of *Derris trifoliata* Lour. International Journal of Pharmaceutical Sciences 2012; 3(1): 97-100.
3. Akter R, Hasan SR, Hossain MM, Jamila M, Chowdhury SS, Mazumder ME and Rahman S: Antidiarrhoeal and antioxidant properties of *Curcuma alismatifolia* leaves. Australian J of Basic and Applied Scie 2010; 4(3): 450-6.
4. Majumder R, Jami MS, Alam ME and Alam MB: Antidiarrheal activity of *Lannea coromandelica* Linn. Bark extract. American–Eurasian Journal of Scientific Research 2013; 8: 128-34.
5. Singh A, Saharan VA, Ram V and Bhandari A: Evaluation of antidiarrhoeal activity of *Elytraria acaulis* extracts on magnesium sulphate-and castor oil-induced diarrhoea in wistar rats. Malaysian Journal of Pharmaceutical Sciences 2013; 11(2): 31.
6. NCBI Analgesic activity. Available from https://www.ncbi.nlm.nih.gov/pubmed/26939272. Accessed 2019.
7. Sewell RD and Rafieian-Kopaei M: The history and ups and downs of herbal medicines usage. Journal of Herb Med pharmacology 2014; 3.
8. Nasri H: Toxicity and safety of medicinal plants. Journal of Herb Med Pharmacology 2013; 2.
9. Rios JL, Recio MC: Medicinal plants and antimicrobial activity. Journal of Ethno Pharmacology 2005; 100(1-2): 80-4.
10. Srivastava JP, Lambert J and Vietmeyer N: Medicinal plants: An expanding role in development. The World Bank 1996; 30.
11. Medicine Net. Medical Definition of Antimicrobial. Available from

https://www.medicinenet.com/script/main/art.asp?articlekey=9621. Accessed 25 2019.
12. Ambasta SP: The Useful Plants of India, PID, CSIR, New Delhi 1986; 329.
13. Arunya S, Nuntavan B, Opa N and Hiroshi W and Thai J: Phytopharm 2004; 11.
14. Suksamrarn A: Arch Pharm Res 2003; 26: 816.
15. Kukongviriappan UA and Luangaram SA: Biol Pharm Bull 2003; 30: 661.
16. Mukherjee KS, Brahmachari G, Manna TK and Mukherjee P: Phyto-chemistry 1998; 49: 2533.
17. Bramachari F, Gorai D, Chatterjee D, Mondal S and Mistri B: Indian J Chem 2004; 43: 219.
18. Mukherjee KS, Bramhachari G, Manna TK and Mukherjee P: Phyto-chemistry 1999; 28: 1778.
19. Brahmachari G, Jash SK, Mandal LC, Mondal A and Roy R: Rasayan J Chem 2008; 1: 288.
20. Ahmed F, Selim MS, Das AK and Choudhuri MS: Anti-inflammatory and antinociceptive activities of *Lippia nodiflora* Linn. Die Pharmazie-An International Journal of Pharmaceutical Sciences 2004; 59(4): 329-30.
21. Shoba FG and Thomas M: Study of antidiarrhoeal activity of four medicinal plants in castor-oil induced diarrhoea. Journal of Ethnopharmacology 2001; 76(1): 73-6.
22. Wiart C, Mogana S, Khalifah S, Mahan M, Ismail S, Buckle M, Narayana AK and Sulaiman M: Antimicrobial screening of plants used for traditional medicine in the state of Perak, Peninsular Malaysia. Fitoterapia 2004; 75(1): 68-73.
23. Świeboda P, Filip R, Prystupa A and Drozd M: Assessment of pain: types, mechanism and treatment. Pain 2013; 2: 7.
24. Tunaru S, Althoff TF, Nüsing RM, Diener M and Offermanns S: Castor oil induces laxation and uterus contraction *via* ricinoleic acid activating prostaglandin EP3 receptors. Proceedings of the National Academy of Sciences 2012; 109(23): 9179-84.
25. Akter R, Hasan SR, Hossain MM, Jamila M, Chowdhury SS, Mazumder ME and Rahman S: Antidiarrhoeal and antioxidant properties of *Curcuma alismatifolia* leaves. Australian Journal of Basic and Applied Sciences 2010; 4(3): 450-6.

How to cite this article:
Jose BE, Jebaseelan S, Meera R and Kalirajan R: Analgesic, antidiarrhoeal and antimicrobial activities of *Limnophila polystachya* Benth (Scrophulariaceae) leaf extracts. Int J Pharmacognosy 2022; 9(6): 127-31. doi link: http://dx.doi.org/10.13040/IJPSR.0975-8232.IJP.9(6).127-31.