

Research Article



Evaluation of Pharmacological Potentialities of Polar and Nonpolar Fractions of *Justicia aurea* Extract

A. N. M. Shofi Uddin, Md. Iqbal Ahmed, Utpal Kumar Karmakar, Nripendra Nath Biswas, Bishwajit Bokshi*, Samir Kumar Sadhu
Pharmacy Discipline, Life Science School, Khulna University, Khulna-9208, Bangladesh.

*Corresponding author's E-mail: bokshi06@pharm.ku.ac.bd

Received: 30-06-2022; Revised: 22-08-2022; Accepted: 28-08-2022; Published on: 15-09-2022.

ABSTRACT

This study aims to explore analgesic, antidiarrheal, neuropharmacological, and antibacterial activities of petroleum ether and water fractions of *Justicia aurea* (*J. aurea*). The results of analgesic activity showed that both nonpolar (petroleum ether) and polar (water) fractions of *J. aurea* extract at 250 and 500 mg/kg significantly suppressed the writhing reflex dose-dependently. Here, the potentiality of petroleum ether fraction was higher than that of water fraction. In the castor oil-induced diarrheal mice, both fractions of *J. aurea* extract, at 250 and 500 mg/kg, significantly delayed the onset of diarrhea and lessened the total number of feces in a dose-dependent manner. Of the two fractions, the nonpolar fraction was found to exhibit a better effect. Both fractions appreciably reduced the number of squares crossed by the mice at both 250 and 500 mg/kg. At 500 mg/kg, petroleum ether fraction reflected a slightly better effect than that of water fraction. In disc diffusion antibacterial assay, both fractions showed minor effects against *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*), however, only the water fraction exhibited low activity against *Salmonella enterica* (*S. enterica*). In conclusion, the nonpolar fraction of *J. aurea* showed better analgesic, antidiarrheal, and CNS depression effects.

Keywords: *Justicia aurea*, Acanthaceae, analgesic, antidiarrheal, neuropharmacological, and antibacterial.

QUICK RESPONSE CODE →

DOI:
10.47583/ijpsrr.2022.v76i01.012



DOI link: <http://dx.doi.org/10.47583/ijpsrr.2022.v76i01.012>

INTRODUCTION

Natural resources, especially terrestrial plants, have been used by mankind in every society since antiquity¹. 80% of the people in underdeveloped nations still rely on conventional plant-based medicine for their main healthcare requirements despite recent developments in medical research, and a similar percentage of people in developed countries have at least tried it once due to its lower risk of side effects, time-tested effectiveness, and affordability^{2,3}. A true innovation in drug development has been enabled by knowledge of the medical ideas found in the traditional system of medicine and its application. According to published research, approximately one third of currently available prescription medications come from plants or were synthesized using chemicals that were initially derived from plants utilized in diverse ethnomedical usage¹. The plant *Justicia aurea* (*J. aurea*) is a member of the Acanthaceae family, which contains 600 species primarily habitat in tropical and pantropical regions. Analgesic, antidiarrheal, neuropharmacological, and antibacterial activity are the most notable biological properties associated with the species of this family⁴⁻⁷. *J. aurea* also referred to as Yellow Jacobinia, is an evergreen

suburb plant with ovate leaves. It is between two and three feet wide and over ten feet tall. It blooms with beautiful yellow flowers from mid-summer to late summer and during the wet season (Figure 1). It is ideal to grow this plant in moist, well-drained soils⁸. Upon literature survey, no work has been reported on the analgesic, antidiarrheal, neuropharmacological, and antibacterial activities of *J. aurea*. Therefore, this project aimed to evaluate the mentioned activities along with the phytochemical screening of polar and nonpolar fractions of crude extract and make a comparative feature in continuation of our previous research work on this plant⁹.



Figure 1: Photo of *J. aurea* plant

MATERIALS AND METHODS

Plant collection and identification

In March 2016, the whole plant of *J. aurea* was collected from Chalna, Khulna. The collected plant was identified by the experts of Pharmacy Discipline, Khulna University, Bangladesh⁹.



Extraction and fractionation

J. aurea crude extract was prepared and fractioned into nonpolar (petroleum ether) and polar (water) fractions following the same procedure reported previously by our group⁹.

Experimental animal

Swiss-albino young mice were nurtured as recommended by International Centre for Diarrheal Disease Research, Bangladesh (ICDDR,B) and accepted standards set by standard laboratory protocols mentioned in our previous study. Before experiments, the animals were regularly examined⁹.

Analgesic activity evaluation

The acetic acid-induced writhing model was followed to carry out the analgesic effect of the two fractions of *J. aurea* extract¹⁰. By comparing the experimental group to the control group, the percentage of writhing inhibition was calculated using the formula:

$$\text{Writhing inhibition (\%)} = [(Wc - Wt) / Wc] \times 100$$

where, Wc = mean writhing number in the control group, Wt = mean writhing number in test groups

Antidiarrheal activity evaluation

Castor oil-induced diarrheal method in mice was followed to study the antidiarrheal activity of the fractions of *J. aurea* extract reported by Mahmud et al., 2017¹¹. The test groups of mice received the extract orally at 250 and 500 mg/kg. Mice of the positive control group and control group received loperamide at 3 mg/kg and 1% v/v tween 80 at 10 ml/kg, respectively. Each mouse received 0.5 ml of castor oil orally 30 minutes later to induce diarrhea. Blotting paper was placed under each mouse and kept them apart from each other. The first defecation and number of stools were counted for the following four hours. The following equation was used to record the defecation reduction:

$$\text{Defecation reduction (\%)} = [(Sc - St) / Sc] \times 100$$

where, Sc = average number of stools in the control group, St = average number of stools in the test group

Neuropharmacological activity evaluation

The open field model described by Nripendra et al., 2017 was used to assess the neuropharmacological behavior (CNS excitatory or depressive) of mice for the effect of the fractions of *J. aurea* extract¹². This experiment was conducted in a room with reduced noise. The test groups of mice received oral doses of 250 and 500 mg/kg of the extract of each of the two fractions. Diazepam was given to mice in the positive control group at a dose of 1 mg/kg, and 1% v/v tween 80 was given at a dose of 10 ml/kg. Each mouse was then placed in a corner square, and the number of squares crossed by the mice was recorded for 3 minutes from 0 to 120 minutes with 30 minutes intervals.

Antibacterial activity evaluation

The disk diffusion test method was used to assess the antibacterial activity of *J. aurea* extract¹³. To conduct the study, samples of two gram-positive (*S. aureus* and *B. subtilis*) and two gram-negative (*E. coli* and *S. enterica*) bacteria species were selected. The bacteria were twice cultivated using nutrient agar and nutrient broth media. The second culture was grown in separate petridishes containing nutrient agar media after being incubated in a nutrient broth medium. Levofloxacin at 30 µg/disk and fractions at 250 and 500 µg/disk were impregnated into the filter paper discs. The petridishes were incubated at 37 °C overnight to suppress bacterial growth, and the zone of inhibition of bacterial growth was determined by a calibrated scale.

RESULTS

Analgesic activity evaluation

The results of Table 1 showed that the water fraction of *J. aurea* extract at 250 and 500 mg/kg significantly inhibited the writhing reflex by 25.28% and 35.06%, respectively. The pattern of writhing inhibition by petroleum ether fraction was similar to water fraction, 29.89% and 43.68% at 250 and 500 mg/kg, respectively. In this study, the standard drug diclofenac Na was found to show writhing inhibition by 76.44% at 25 mg/kg dose. Overall, the analgesic potentiality of the petroleum ether fraction of *J. aurea* extract was higher than that of the water fraction (Table 1).

Table 1: Effect of fractions of *J. aurea* extract on acetic acid-induced writhing inhibition in mice

Group	Mean writhing	% Inhibition of writhing
Control (1% Tween 80 in water)	17.40	0
Standard, Diclofenac Na (25 mg/kg)	4.10	76.44±0.43 ^c
Water fraction (250 mg/kg)	13	25.28±0.78 ^b
Water fraction (500 mg/kg)	11.3	35.06±0.60 ^c
Petroleum ether fraction (250 mg/kg)	12.2	29.89±0.52 ^c
Petroleum ether fraction (500 mg/kg)	9.8	43.68±0.46 ^c

Values are expressed as mean ± standard error of the mean (n = 3); ^aP<0.05, ^bP<0.01, and ^cP<0.001 compared to control.

Antidiarrheal activity evaluation

Both water and petroleum ether fractions of *J. aurea* extract, at 250 and 500 mg/kg, significantly prolonged the onset of diarrhea and lessened the total number of stools dose-dependently. Inhibition of defecation at 250 and 500



mg/kg for water fraction was 18.01% and 26.08% and for petroleum ether fraction was 32.80% and 50.26%, respectively. Standard loperamide at 3 mg/kg showed a significant increase in latent period and inhibition of defecation (Table 2). Of the two fractions, the nonpolar fraction was found to show a better effect.

Neuropharmacological activity evaluation

The water and petroleum ether fractions of *J. aurea* extract significantly decreased the number of squares crossed by the mice at 250 and 500 mg/kg compared to the control. The decrease of locomotors was revealed at 30 min and continued up to 90 min, indicating the decrease in locomotor activity (Table 3).

Antibacterial activity evaluation

Both the polar fraction and nonpolar fractions reflected very low antibacterial activities against the bacterial species tested even at the higher dose of 500 µg/disk (Table 4).

Table 2: Effect of fractions of *J. aurea* extract on castor oil-induced diarrheal episode in mice

Group	Mean latent period (min)	% inhibition of defecation
Control, 1% tween-80 in water	33.00±2.55	-
Standard, Loperamide (3 mg/kg)	114.60±3.59 ^c	80.64±0.50 ^b
Water fraction (250 mg/kg)	44.25±2.87 ^b	18.01±1.75
Water fraction (500 mg/kg)	56.00±2.16 ^c	26.08±1.60 ^a
Petroleum ether fraction (250 mg/kg)	58.25±4.73 ^a	32.80±0.96 ^b
Petroleum ether fraction (500 mg/kg)	73.5±4.73 ^c	50.26±0.85 ^c

Values are expressed as mean ± standard error of the mean (n = 3); ^aP<0.05, ^bP<0.01, and ^cP<0.001 compared to control.

Table 3: Effect of fractions of *J. aurea* extract on open field model in mice

Group	Number of square crossed by the mice				
	0 min	30 min	60 min	90 min	120 min
Control	109.6±1.5	102.6±1.4	96.8±1.2	90.4±2.7	84.8±4.3
Positive control, Diazepam (1mg/kg)	112.4±6.4	40.2±1.4 ^c	31.8±0.9 ^c	26.2±1.0 ^c	32.8±1.4 ^c
Water fraction (250 mg/kg)	124.8±2.7 ^c	87.4±1.5 ^c	73±1.2 ^c	69.2±1.1 ^c	74.2±1.7 ^a
Water fraction (500 mg/kg)	125.2±2.4 ^c	80.4±1.1 ^c	70.6±1.5 ^c	65.8±1.2 ^c	76.8±1.9
Petroleum ether fraction (250 mg/kg)	128.4±3.7 ^c	96.6±3.3	81.4±4.4 ^c	69.6±3.9 ^c	82.8±3.2
Petroleum ether fraction (500 mg/kg)	121±3.7 ^a	88.6±2.4 ^b	70.6±2.7 ^c	54.6±2.3 ^c	79±2.4

Values are expressed as mean ± standard error of the mean (n = 3); ^aP<0.05, ^bP<0.01, and ^cP<0.001 compared to control.

Table 4: *In vitro* antibacterial activity of fractions of *J. aurea* extract

Name of Bacterial strains	Type of Bacterial strains	Diameter of zone of inhibition					
		Control	Standard (Levofloxacin) 30 µg/disk	Water fraction (250 µg/disk)	Water fraction (500 µg/disk)	Petroleum ether fraction (250 µg/disk)	Petroleum ether fraction (500 µg/disk)
<i>S. aureus</i>	Gram(+)	0	60 mm	8 mm	8.9 mm	7.5 mm	8.2 mm
<i>B. subtilis</i>	Gram(+)	0	48 mm	0	0	0	0
<i>E. coli</i>	Gram(-)	0	47 mm	7	9	0	12
<i>S. enterica</i>	Gram(-)	0	45 mm	0	6	0	0

DISCUSSION

Numerous phytochemicals are found in abundance in every plant. The plant itself stores these phytochemicals as primary or secondary metabolites. The majority of secondary metabolites are rich in bioactive substances that have a variety of positive health impacts on both the plant and animal worlds¹⁴. In our previous article, the phytochemical screening showed the presence of

glycosides, flavonoids, phenolic compounds, tannins alkaloids, saponins, etc. which could be responsible for bio-activities. Furthermore, the previous study also reported that both the water and petroleum ether fractions were found to be non-toxic up to 2000 mg/kg⁹. Based on this toxicity report, up to 500 mg/kg was considered the safe dose for this study.



To investigate the analgesic activity, the writhing method was employed, which involves injecting acetic acid intraperitoneally into mice to produce pain of peripheral origin¹⁵. This nociceptive model is extremely successful for dosages where the analgesic and anti-inflammatory effects of medication would be ineffective in other pain models¹⁶. Peripheral nociception is activated through the direct induction of non-selective cationic channels or the indirect secretion of various endogenous mediators, such as prostaglandins, cytokines, and bradykinin, as well as elevated production of lipoxygenase and cyclooxygenase enzymes resulting in the activation of neurons sensitive to nonsteroidal anti-inflammatory drugs (NSAIDs)^{17, 18}. Therefore, this test could be used to investigate the antinociceptive activity of novel NSAIDs. Natural phenolic acids and flavonoids like rutin, quercetin, luteolin, etc. have been reported to have a primary role in analgesic activity upon targeting prostaglandins¹⁹. Both the polar and nonpolar *J. aurea* extract fractions had comparable antinociceptive potentialities in this test. The phytochemical assays of *J. aurea* extract described in our previous study revealed the presence of flavonoids and phenolics, which might contribute to analgesic effects⁹.

One of the most common gastrointestinal illnesses, diarrhea causes both morbidity and mortality in a large number of children and adults worldwide. The natural state of the stomach is deteriorated by increased gastrointestinal motility, consumption of unclean food, and gastrointestinal infections, which result in diarrhea⁵. A widely used laboratory procedure for determining the antidiarrheal activity of any sample is the castor oil-induced diarrheal technique in mice. Ricinoleic acid, the primary component of castor oil, can irritate and inflame the gastrointestinal mucosa and enhance motility and secretion²⁰. The polar and non-polar fractions of *J. aurea* extract enhanced the latent time of feces and decreased defecation in the castor oil-induced diarrheal test. Phytochemicals including alkaloids, tannins, flavonoids, and terpenes present in *J. aurea* extract may be responsible for the antidiarrheal effect²¹.

The most intricate and crucial system in the human body is the central nervous system (CNS). The primary regulatory component of the CNS consists of 86 billion neurons. Different neuropharmacological behaviors can be seen when these neurons are excited or depressed. The open field test is one of the common methods to measure the general locomotor activity levels, anxiety, and exploratory willingness in animals (usually rats). The frequency with which mice cross squares at regular intervals indicates whether their CNS is experiencing excitement or depression. The excitation effect on the CNS increases with the number of squares crossing, and *vice versa*²². In our open field model, two fractions of *J. aurea* extract showed us CNS depression effects over time. With the advancement of time, less number of squares were crossed by mice at both 250 and 500 mg/kg doses. Phytochemicals like glycosides, alkaloids, tannins, saponins, and flavonoids might be liable for this CNS depression^{23, 24}.

The emergence of microbes that are resistant to antibiotics has increased the urgency of hunting for new antibacterial substances. Numerous compounds with plant origins, including alkaloids, flavonoids, glycosides, terpenes, tannins, and polyphenols, have been found to have antibacterial activity. Many have also demonstrated synergistic benefits with already-available antibacterial medications²⁵. Flavonoids are either complexed with bacterial cell walls or bound to adhesions to demonstrate their antibacterial activities. Antibacterial activity of tannins was demonstrated either by binding to proteins, blocking enzymes, or rupturing bacterial cell walls. Alkaloids were intercalated into DNA and/or cell walls to demonstrate their antibacterial properties²⁶. In our study, the extract comprises a variety of phytochemicals, including flavonoids, terpenoids, tannins, and polyphenolic components. The varied zones of inhibition observed in our tests against the sensitive bacteria could have been caused by different diffusion rates of phytochemicals²⁷.

CONCLUSION

In summary, the nonpolar components of *J. aurea* demonstrated better analgesic, antidiarrheal, and CNS depression activities in comparison to the polar components. However, both parts showed little antibacterial activity. Further studies are required to isolate bioactive compounds from this plant and to identify the mechanisms of action that can help develop novel agents for the management of pain, diarrhea, and CNS disorders.

Acknowledgements: The authors express deep gratitude to the authority of Pharmacy Discipline, Khulna University for providing the laboratory facilities and chemicals requisite for carrying out this research.

REFERENCES

1. Richardson MA, Sanders T, Palmer JL, Greisinger A, Singletary SE. Complementary/alternative medicine use in a comprehensive cancer center and the implications for oncology. *Journal of clinical oncology*. 2000;18(13):2505-14.
2. Hoyle E, Martinez R, Mehta K, Nisonoff H, Boyd D. Beyond medical pluralism: characterising health-care delivery of biomedicine and traditional medicine in rural Guatemala. *Global public health*. 2018;13(4):503-17.
3. Baliga MS. Review of the phytochemical, pharmacological and toxicological properties of *Alstonia scholaris* Linn. R. Br (Saptaparna). *Chinese journal of integrative medicine*. 2012;11:1-14.
4. Lino C, Taveira M, Viana G, Matos F. Analgesic and anti-inflammatory activities of *Justicia pectoralis* Jacq and its main constituents: coumarin and umbelliferone. *Phytotherapy Research: An International Journal Devoted to Medical and Scientific Research on Plants and Plant Products*. 1997;11(3):211-5.
5. Mekonnen B, Asrie AB, Wubneh ZB. Antidiarrheal activity of 80% methanolic leaf extract of *Justicia schimperiana*. *Evidence-Based Complementary and Alternative Medicine*. 2018.



6. Navarro E, Alonso S, Navarro R. Toxicity and neuropharmacological effects of efenine. Evidence-Based Complementary and Alternative Medicine. 2011.
7. Batool R, Salahuddin H, Mahmood T, Ismail M. Study of anticancer and antibacterial activities of *Foeniculum vulgare*, *Justicia adhatoda* and *Urtica dioica* as natural curatives. Cellular and Molecular Biology. 2017;63(9):109-14.
8. Corrêa GM, Alcântara AFdC. Chemical constituents and biological activities of species of *Justicia*: a review. Revista Brasileira de farmacognosia. 2012;22:220-38.
9. A. N. M. Shofi Uddin BC, Banani Mondal, Sarker Ramproshad, Md. Anisuzzman, Bishwajit Bokshi, Samir Kumar Sadhu. Phytochemical Screening and Pharmacological Activities of *Justicia aurea* Grown in Bangladesh. International Journal of Pharmaceutical Sciences Review and Research. 2022;75(2):130-4.
10. Medha MM, Devnath HS, Biswas B, Bokshi B, Sadhu SK. In silico profiling of analgesic and antihyperglycemic effects of ethanolic leaves extract of *Amischotolype mollissima*: evidence from in vivo studies. Saudi Journal of Biological Sciences. 2022:103312.
11. Mahmud I, Zilani M, Hasan N, Biswas NN, Bokshi B. Bioactivities of *Bruguiera gymnorrhiza* and profiling of its bioactive polyphenols by HPLC-DAD. Clinical Phytoscience. 2017;3(1):1-11.
12. Biswas NN, Acharzo AK, Anamika S, Khushi S, Bokshi B. Screening of Natural Bioactive Metabolites and Investigation of Antioxidant, Antimicrobial, Antihyperglycemic, Neuropharmacological, and Cytotoxicity Potentials of *Litsea polyantha* Juss. Ethanolic Root Extract. Evidence-Based Complementary and Alternative Medicine. 2017.
13. Bokshi B, Rahman SA, Sadhu SK, Muhammad A, Islam MA. Assessment of analgesic and antidiarrhoeal activities of different fractions of crude extract of *Stephania japonica* stem. International Journal of Pharmaceutical Sciences and Research. 2013;4(3):1233-41.
14. Kundu P, Debnath SL, Sadhu SK. Exploration of Pharmacological and Toxicological Properties of Aerial Parts of *Blumea lacera*, a Common Weed in Bangladesh. Clinical Complementary Medicine and Pharmacology. 2022;2(3):100038.
15. Gawade S. Acetic acid induced painful endogenous infliction in writhing test on mice. Journal of Pharmacology and Pharmacotherapeutics. 2012;3(4):348.
16. Nock DD, Abraham IG, Ahmad MH. Potential pharmacological properties of methanol leaves extract of *Culcasia angolensis* (Araceae): antinociceptive, anti-inflammatory and antipyretic activities in laboratory animals. The Journal of Basic and Applied Zoology. 2022;83(1):1-11.
17. Prabhu VV, Nalini G, Chidambaranathan N, Kisan SS. Evaluation of anti-inflammatory and analgesic activity of *Tridax procumbens* Linn against formalin, acetic acid and CFA induced pain models. Int J Pharm Pharm Sci. 2011;3(2):126-30.
18. Alabi AO, Ajayi AM, Omorogbe O, Umukoro S. Antinociceptive and anti-inflammatory effects of an aqueous extract of blended leaves of *Ocimum gratissimum* and *Psidium guajava*. Clinical Phytoscience. 2019;5(1):1-9.
19. Quibria T, Das BK, Hasan T, Uddin MA, Alam MM. Phytochemical screening and evaluation of analgesic activity of *Solanum surattense*. American Journal of Pharmaceutical Sciences and Nanotechnology. 2014;1(1):1-10.
20. Dash PR, Nasrin M, Raihan SZ, Ali MS. Study of antidiarrhoeal activity of two medicinal plants of Bangladesh in castor-oil induced diarrhoea. International Journal of Pharmaceutical Sciences and Research. 2014;5(9):3864-9.
21. Palombo EA. Phytochemicals from traditional medicinal plants used in the treatment of diarrhoea: modes of action and effects on intestinal function. Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives. 2006;20(9):717-24.
22. Denenberg VH. Open-field behavior in the rat: What does it mean? Annals of the New York Academy of Sciences. 1969;159(3):852-9.
23. Asif M. Mini review on important biological properties of benzofuran derivatives. J Anal Pharm Res. 2016;3(2):00050.
24. Datta B, Datta S, Chowdhury M, Khan T, Kundu J, Rashid M, et al. Analgesic, anti-inflammatory and CNS depressant activities of sesquiterpenes and a flavonoid glycoside from *Polygonum viscosum*. Die Pharmazie-An International Journal of Pharmaceutical Sciences. 2004;59(3):222-5.
25. Ncube N, Afolayan A, Okoh A. Assessment techniques of antimicrobial properties of natural compounds of plant origin: current methods and future trends. African journal of biotechnology. 2008;7(12):81-87.
26. Cowan MM. Plant products as antimicrobial agents. Clinical microbiology reviews. 1999;12(4):564-82.
27. Vila R. Flavonoids and further polyphenols in the genus *Thymus*. Thyme: CRC Press; 2002. p. 158-90.

Source of Support: The author(s) received no financial support for the research, authorship, and/or publication of this article.

Conflict of Interest: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

For any question relates to this article, please reach us at: globalresearchonline@rediffmail.com
 New manuscripts for publication can be submitted at: submit@globalresearchonline.net and submit_ijpsrr@rediffmail.com

