Available online at www.banglajol.info/index.php/BJSIR

Bangladesh J. Sci. Ind. Res. **59(4)**, 195-204, 2024

BANGLADESH JOURNAL OF SCIENTIFIC AND INDUSTRIAL RESEARCH

ISSN. 0304-9809

Antibacterial, antioxidant, anti-inflammatory and anti-diarrheal potentiality evaluation of whole plant extract of *Phyllanthus niruri* L.

M. S. Hossain[1], H. K. Shaha[1], S. J. Shipa[2], M. S. Islam[3], F. Ahmed[4] and N. Saqueeb[1]*

[1]*Department of Clinical Pharmacy and Pharmacology, Faculty of Pharmacy, University of Dhaka, Dhaka-1000, Bangladesh*
[2]*Department of Pharmacy, East West University, Dhaka-1212, Bangladesh*
[3]*Pharmaceutical Sciences Research Division, BCSIR Dhaka Laboratories, Bangladesh Council of Scientific and Industrial Research (BCSIR), Dhaka-1205, Bangladesh*
[4]*Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Dhaka, Dhaka-1000, Bangladesh*

ARTICLE INFO

Received: 16 July 2024

Revised: 06 October 2024

Accepted: 09 October 2024

DOI: https://doi.org/10.3329/bjsir.v59i4.74975

Abstract

Traditional medicine has utilized *Phyllanthus niruri* L. (Vhui-amla), a plant belonging to the Euphorbiaceae family, for treating a large number of diseases worldwide. In this study, the whole plant extract was subjected to antibacterial, antioxidant, anti-inflammatory, and anti-diarrheal assays. In the disc diffusion assay, the ethyl acetate fraction (400 µg/disc) and the chloroform fraction (400 µg/disc) produced broad-spectrum antibacterial properties. Both fractions produced the highest antimicrobial potency (zone of inhibition: 25 mm) against *Staphylococcus paratyphi*. The chloroform fraction in the DPPH scavenging activity assay exhibited strong antioxidant efficacy with an IC_{50} value of 22.01 µg/mL, whereas the IC_{50} value of the standard ascorbic acid was 6.39 µ g/mL. The ethyl acetate fraction at 400 mg/kg dose exhibited potent anti-inflammatory properties in the carrageenan-induced hind paw edema method, with 30.97% ($p < 0.01$), 36.55% ($p < 0.01$), 44.78% ($p < 0.001$), and 53.45% ($p < 0.001$) paw edema inhibition after 1 hour, 2 hours, 3 hours, and 4 hours, respectively. A notable anti-diarrheal effect was also produced by the ethyl acetate fraction at a dose of 400 mg/kg, which caused 40.68% ($p < 0.01$) fecal inhibitory effects in the castor oil-induced diarrhea method (standard loperamide showed 61.02% ($p < 0.001$) fecal inhibitory results. *P. niruri* has been found to be a potential reservoir of antibacterial, antioxidant, anti-inflammatory, and anti-diarrheal substances that necessitate its further phytochemical screening.

Keywords: *Phyllanthus niruri*; Antibacterial; Antioxidant; Anti-inflammatory; Anti-diarrheal

Introduction

Antimicrobial resistance refers to the ability of microorganisms to resist the therapeutic efficacy of medications that have previously proven effective against them. Human diseases are becoming more difficult to treat because of resistant microorganisms (Ahmed *et al.* 2024). Although it is a naturally occurring condition, human activity has significantly accelerated and worsened its course in recent years. This phenomenon is regarded as one of the biggest risks to world health in the twenty-first century (Huang and Eze, 2023). It is also thought that we are in the perilous post-antibiotic era; even small injuries have the potential to become fatal once more due to a lack of effective antimicrobials. A greater dependence on newer antibiotics, which are more costly and hazardous, has resulted from the resistance of first-line antibiotics (Smith and Coast, 2013). By 2050, ten million people worldwide might pass away due to diseases linked to antibiotic resistance (Huang and Eze, 2023). It is a matter of concern that the situation is getting worse due to the inadequate advancement in the manufacturing of new antibacterial drugs. Effective antimicrobials are immensely required for managing infectious diseases. But the process of creating a new antibiotic takes years, and it is expensive. Since 1987, there has been no successful

*Corresponding author's e-mail: nsaqueeb@du.ac.bd

discovery of newer antibiotic agents, indicating a discovery void of more than three decades (Debono *et al.* 1987). The scientific community is searching for a remedy for this threat, and as a source of antibacterial compounds, medicinal plants are being investigated robustly (Saha *et al.* 2022; Vaou *et al.* 2021).

Antioxidants are essential for mitigating the deleterious consequences of free radicals through their chemical neutralization. An imbalance between the body's antioxidant stores and the free radicals' production leads to oxidative stress (Sharifi-Rad *et al.* 2020). Multiple clinical conditions can be developed or worsened by oxidative stress (Taniyama and Griendling, 2003). Natural antioxidants (ascorbic acid, tocopherols etc.) are notable contributors while considering the prevention of the adverse effects of oxidative stress as well as repairing the harm. Because of the plants' antioxidant capabilities and related benefits to human health, the use of therapeutic plants has drawn more attention in recent years (Sharifi-Rad *et al.* 2020).

In addition, diseases linked to inflammation as well as diarrheal disease conditions are associated with significant morbidity and mortality cases, notably in the underdeveloped regions of the world. Conventional therapeutic agents for inflammatory diseases and diarrheal conditions also adduce several unfavorable events, like therapeutic resistance, negative health effects, and so on (Furman *et al.* 2019; Schiller, 2017). Safe, effective, and accessible medications are becoming necessary components of today's healthcare system to manage these diseases. Natural agents are gaining therapeutic potential in these research areas.

Phyllanthus niruri L., a plant of the Euphorbiaceae family, is locally called Vhui-amla in Bangladesh. This is a tiny, upright annual herb with a height of 30 to 40 cm (Samali A, 2012). The plant is also known as stonebreaker, Bhumyamalaki, pitirishi, etc. (Lee *et al.* 2016). Over 700 members belonging to the *Phyllanthus* genus are noticed in tropical and subtropical zones. Fifteen species belonging to the genus are reported to be commonly used in traditional Indian medicine (Mao *et al.* 2016). *P. niruri* is broadly used to serve therapeutic purposes all over the world. In South and south-east Asian regions, the plant is used to treat jaundice, dyspepsia, and renal stones etc (Lee *et al.* 2016). These uses are well established in Ayurvedic and Unani medicine system. Sometimes, the infusion made from the leaves is used to cure chronic diarrhea (Kamatnur and Chawan, 2013). It has long been used in China to treat liver damage caused by harmful substances (Venkateswaran *et al.* 1987). The plant's many parts are reported to be effective against bacterial and viral diseases, particularly diseases of the reproductive organs like syphilis and gonorrhea (Nisar *et al.* 2018).

Therefore, considering the large-scale usage of *P. niruri* all over the world, this study was conducted to determine the antibacterial, antioxidant, anti-inflammatory, and anti-diarrheal properties of the whole plant extract.

Materials and methods

Plant materials collection and authentication

The whole plant, *P. niruri*, was taken from the National Botanical Garden in Mirpur, Dhaka, Bangladesh, in the early summer of 2023. The voucher sample of the collected materials was placed in the National Herbarium of Bangladesh in Mirpur, Dhaka. The authenticity of the plant sample was verified (Accession number 90550) by a National Herbarium expert.

Plant materials preparation

The collected plant materials were thoroughly cleansed using distilled water. The sample was dried in the shade for a duration of three weeks to make it ready for grinding. With the help of a grinding machine, 2 kg of coarse powder was obtained from the sample. The powder was stored carefully until the beginning of extraction.

Crude extract preparation

About 2 kg of reserved coarse powder of *P. niruri* was drenched in 4 L of methanol. The whole contents were stored at room temperature (23 ± 0.5°C) for a duration of 20 days, with periodic stirring and shaking. The content was then subjected to filtration, and using a Buchi Rotary evaporator (Heidolph, UK), the filtrate was dried at a low pressure and 40°C temperature. In order to get a dry crude extract (about 30 g), the concentrated filtrates were then kept for further drying.

Fractionation of the crude extract

Following the protocols outlined in Kupchan *et al*. (1973), the fractionation of the crude extract of *P. niruri* was accomplished (Kupchan *et al.* 1973). The obtained extract was mixed with 10% (v/v) methanol to prepare a reserve solution. Then, it was successively extracted using n-hexane, chloroform, and ethyl acetate solvents. Thus, *P. niruri* yielded four distinct fractions.

Experimental animals

The *in vivo* assays of the study were conducted using Swiss-albino mice of either female or male sex (average weight of 28 to 30 gm). The animals were procured from Jahangirnagar University and kept at the animal house of the Institute of Nutrition and Food Sciences (INFS), University of Dhaka, to provide them with a proper housing period. In the animal housing, the recommended parameters (temperature: 24±1°C; light and dark cycles: 12 hours in sequence) were followed. They were supplied with standard rodent food and water. All other guidelines were rigorously followed while conducting the *in vivo* tests (Zimmermann, 1983). Following the submission of the detailed protocols for the *in vivo* assays, the authors obtained ethical approval for the use of animals in the study (Ref. No: CPP/DIU/EC/14).

Evaluation of antibacterial activity

The antibacterial properties of different extracts and fractions of *P. niruri* were examined using a well-established disc diffusion method (Bauer *et al.* 1966). In the assay, pure cultures of the bacterial strains were collected from the Biomedical Research Centre, University of Dhaka. Nutrient agar medium was prepared following the standard procedure (Haque *et al.* 2014). All the experimental organisms were shifted to the agar slants, and the subcultures were relocated to the sterilized Petridishes. The discs containing the test samples, the blank (negative control), and the standard antibiotic (ciprofloxacin) were placed on solidified agar plates. The plates were then refrigerated in order to provide enough diffusion. The plates were kept inverted to remove any remaining moisture from the agar medium. The zone of inhibition (mm) value was measured with the help of a clean, transparent scale.

Evaluation of antioxidant activity

The antioxidant efficacy of *P. niruri* was measured utilizing the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay (Brand-Williams *et al.* 1995). The free radical DPPH gives maximum absorption at 517 nm. When any antioxidant substance undergoes a reaction with DPPH, its free radical form is transformed into its reduced form, and the absorbance value becomes lower. This reduction in absorbance shapes the basis for measuring anti-oxidant activity. In the assay, ten separate solutions of different concentrations (500 µg/ml to 0.977 µg/ml) of the standard ascorbic acid were prepared. Solutions of the same concentrations were developed from all of the test fractions. A DPPH solution of 0.1 mM was made ready in an amber reagent bottle and preserved in a light-resistant box. In a dark environment, 2 ml of the prepared DPPH solution was mixed with every solution of the control (only methanol), standard, and test samples. After 30 minutes of addition, the absorbance of these preparations was measured at 517 nm. With the help of the equation, the percentage of the DPPH radical neutralizing activity was determined:

$$(I\%) = (1- A_{sample}/A_{control}) \times 100$$

The calculated data were analyzed to gather the IC_{50} values for the interpretation of antioxidant potency.

Evaluation of Anti-inflammatory Activity

Different fractions of *P. niruri* were subjected to the carrageenan-induced hind paw edema method to justify their anti-inflammatory properties (Winter *et al.* 1962). The fractions at 400 mg/kg and standard diclofenac sodium at 50 mg/kg body weight were administered orally to mice groups. After one hour, 1% carrageenan (0.1 mL) solution was injected into the subplantar surface of the right hind paw of each mouse in every group. With the help of a plethysmometer, hind paw volume was noted at 0, 1, 2, 3, and 4 hours of the administration. Paw edema at any point was calculated from the difference between paw volume at the respective time and at 0-hour values. The % paw edema inhibition results were employed for assessing the test samples' anti-inflammatory properties.

% paw edema inhibition = $\{1-(V_t-V_0)$ of test sample groups / (V_t-V_0) of control group$\} \times 100$

V_t = paw volume at t time

V_0 = paw volume at zero time

V_t-V_0 = paw edema

Evaluation of anti-diarrheal activity

In this assay, the castor oil was used to induce diarrhea in every experimental mouse in order to evaluate the test sample’s anti-diarrheal properties (Terefe *et al.* 2023). Six groups of five mice each were assembled from the animals. One group was treated orally with the control (0.9% NaCl solution), one group received the standard (loperamide), and the remaining four groups were treated with different fractions of *P. niruri* (test samples) using a feeding needle-equipped syringe. In order to make sure that the samples were fully absorbed, thirty minutes were given. Then, each mouse was fed an oral dose of 0.5 ml of castor oil. Over a four-hour observation period, the frequency of defecation and the consistency of fecal matter were measured. The fecal pellets of the mice were collected on clear absorbent paper. After completion, the upper half of the cage holding the piece of paper and the mice was raised to reveal the moist feces. Defecation inhibition as a percentage (%) was calculated applying the equation:

% Defecation inhibition = $(1 - F_s/F_c) \times 100$

F_s = mean number of defecations in test sample group

F_c = mean number of defecations in control group

Statistical Analysis

The statistical analysis involves results derived from the pharmacological experiments. Version 10.0 of Microsoft Excel was utilized to conduct the analysis. The data from the *in vivo* experiments were displayed as the mean ± SD. The P values were obtained using the student t-test, and results with lower values (< 0.05) were regarded as significant.

Results and discussion

Antibacterial activity

The ethyl acetate fraction (400 µg/disc) of *P. niruri* exhibited strong antimicrobial activity in the disc diffusion method. This fractionate produced broad-spectrum activity by inhibiting all thirteen tested gram-positive and gram-negative bacterial growths. Except for *Vibrio mimicus* and *Vibrio parahemolyticus*, the minimum zone of inhibition (ZoI) value of the ethyl acetate soluble fraction was 18 mm against *Sarcina lutea* and *Shigella boydii*, whereas the zone of inhibition was 10 mm for both *Vibrio mimicus* and *Vibrio parahemolyticus* species (Table 1). This fraction showed the highest activity against *Staphylococcus paratyphi* (25 mm ZoI value), followed by *Shigella dysenteriae* (22 mm ZoI value). The zone of inhibition (ZoI) value against the other remaining bacterial species was between 18 and 22 mm. The zone of inhibition (ZoI) of the standard Ciprofloxacin was between 37 and 41 mm. The chloroform soluble fraction (400 µg/disc) also revealed strong antibacterial activity, whose highest zone of inhibition (ZoI) value was 25 mm against *Staphylococcus paratyphi*. This fraction showed antibacterial activities against all tested bacterial species except *Vibrio parahemolyticus*, which also indicates broad-spectrum activity of the chloroform-soluble fractionate (CF) of *P. niruri*.

The obtained zone of inhibition values of the test fractions were lower than the standard Ciprofloxacin. This might be

Table I. Antibacterial activity of different fractions of *P. niruri*

Test Organisms	Diameter of zone of inhibition (mm)				
	Methanolic extract 400 µg/disc	Hexane fraction 400 µg/disc	Chloroform fraction 400 µg/disc	Ethyl acetate fraction 400 µg/disc	Ciprofloxacin 5 µg/disc
	Gram Positive Bacteria				
Bacillus cereus	8	11	10	20	39
Bacillus megaterium	12	10	15	19	37
Bacillus subtilis	8	8	12	20	39
Staphylococcus aureus	8	14	10	20	40
Sarcina lutea	14	11	15	18	40
	Gram Negative Bacteria				
Escherichia coli	15	10	10	20	40
Pseudomonas aureus	12	8	15	20	39
Staphylococcus paratyphi	10	12	25	25	40
Salmonella typhi	12	-	14	20	40
Shigella boydii	8	-	17	18	38
Shigella dysenteriae	11	16	20	22	40
Vibrio mimicus	13	-	15	10	40
Vibrio parahemolyticus	12	-	-	10	41

due to the utilization of crude forms in the assay. There is a possibility of obtaining greater microbial growth inhibition if isolated compounds of the plant are used. The outcome indicates the presence of bioactive compounds with broad-spectrum antimicrobial potency, notably in the ethyl acetate soluble fraction and the chloroform soluble fraction of *P. niruri*. Alkaloids, terpenoids, saponins, tannins, phenols, and flavonoids were qualitatively and quantitatively analyzed in previous work on the plant (Bagalkotkar *et al.* 2010), and these substances were reported to produce antimicrobial effects (Ramandeep *et al.* 2017). Other antimicrobial substances, including lignans (like phyllanthin and hypophyllanthin), astragalin, and glycosides (geraniin, quercitrin etc.), were reported to be produced in the plant as well (Somanabandhu *et al.* 1993; Yeap, 1995). The antimicrobial molecules of the plant have been shown to affect the bacterial cell wall, and these compounds are also responsible for increasing membrane permeability by being locked on the bacterial surface (Hyldgaard *et al.* 2012). Due to the disruption effects on the bacterial cell wall, the extract of *P. niruri* was suggested to be used topically (Ibrahim *et al.* 2013). Thus, this plant can be claimed to be a potential reservoir of antibacterial compounds.

Antioxidant activity

The chloroform soluble fraction of *P. niruri* exhibited potential antioxidant activity in the DPPH scavenging activity assay with an IC_{50} value of 22.01 μg/mL, and that value of the standard was 6.39 μg/mL (Table II). The methanol extract showed mild antioxidant potential (89.69 μg/mL IC_{50} value) compared to the standard. The IC_{50} values of the hexane and ethyl acetate fractions were 138.73 μg/mL and 180.91 μg/mL, respectively.

Table II. Antioxidant activity of different fractions of *P. niruri*

Test Samples	IC_{50} values (μg/mL)
Standard (Ascorbic acid)	6.39
Methanolic extract	89.69
Hexane fraction	138.73
Chloroform fraction	22.01
Ethyl acetate fraction	180.91

According to the assay's findings, antioxidant compounds are present in *P. niruri*, primarily in the chloroform fraction. Many bioactive substances, such as polyphenols, coumarins, alkaloids, and flavonoids, are reported to possess antioxidant properties and to be synthesized as secondary metabolites in the plant (Giribabu *et al.* 2014). A little relation between the phenolic contents and antioxidant capability was found in *P. niruri*, which helped to conclude that, together with phenolic chemicals, the plant may also contain non-phenolic molecules that contribute to its antioxidant properties (Harish and Shivanandappa, 2006). In addition to these phenolic and non-phenolic substances, a unique structural protein of 35 kDa was isolated from *P. niruri* that possessed antioxidant properties. The protein molecule was predicted to be accountable for *P. niruri*'s antioxidant action to some extent (Sarkar *et al.* 2009). The presence of antioxidant protein molecules created an opportunity to search for more such types of agents in *P. niruri*. The work was conducted on the whole plant, so more investigation is needed to reveal the definite distribution of the potential antioxidant substances. As natural antioxidants provide notable benefits over synthetic antioxidants (Shebis *et al.* 2013), the plant may contribute substantially to limiting the adverse effects of oxidative damage.

Anti-inflammatory activity

Among the test samples, the highest anti-inflammatory activity was exhibited by the ethyl acetate fraction of *P. niruri*, with 30.97% ($p < 0.01$), 36.55% ($p < 0.01$), 44.78% ($p < 0.001$), and 53.45% ($p < 0.001$) edema inhibition after 1 hour, 2 hours, 3 hours, and 4 hours, respectively (Table III). At the same time interval, 41.59% ($p < 0.001$), 51.68% ($p < 0.001$), 60.45% ($p < 0.001$), and 67.93% ($p < 0.001$) edema inhibitory findings were observed in the experimental animals treated with the standard. The n-hexane and the chloroform fraction showed relatively lower anti-inflammatory activity after 3 hours and 4 hours. The methanolic extract of *P. niruri* also produced significant anti-inflammatory properties after 3 and 4 hours with 31.72% ($p < 0.01$) and 40.34% ($p < 0.001$) edema inhibition, respectively.

Being a body defense mechanism, inflammation responds to various injurious stimuli of pathological processes. Inflammation is thus involved in controlling homeostasis in the body. But when the inflammation continues for a longer duration, it plays a major role in developing mental and physical health problems as well as organ damage (Chen *et al.* 2018). Common diseases, including diabetes mellitus, ischemic heart disease, neurodegenerative condi-

Table III. Anti-inflammatory activity of different fractions of *P. niruri*

Test Samples	Dose	Mean paw edema (mL) ±SD (% Edema inhibition)			
		1 hr	2 hr	3 hr	4 hr
Control (1% Tween 80 in saline solution)	-	0.452±0.05	0.476±0.06	0.536±0.07	0.580±0.05
Standard (diclofenac sodium)	50 mg/kg	0.264±0.08 (41.59%)***	0.230±0.05 (51.68%)***	0.212±0.09 (60.45%)***	0.186±0.08 (67.93%)***
Methanolic extract	400 mg/kg	0.402±0.06 (11.06%)	0.390±0.03 (18.07%)	0.366±0.03 (31.72%)**	0.346±0.10 (40.34%)***
Hexane fraction	400 mg/kg	0.434±0.05 (03.98%)	0.422±0.09 (11.34%)	0.410±0.07 (23.51%)*	0.402±0.06 (30.69%)**
Chloroform fraction	400 mg/kg	0.428±0.05 (05.31%)	0.416±0.05 (12.61%)	0.408±0.04 (23.88%)*	0.420±0.07 (27.59%)**
Ethyl acetate fraction	400 mg/kg	0.312±0.08 (30.97%)**	0.302±0.10 (36.55%)**	0.296±0.04 (44.78%)***	0.270±0.04 (53.45%)***

Data are presented as mean ± SD values, n=5. *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$

tions, stroke, cancer, and so on, are closely linked to chronic inflammation; over 50% of all deaths globally are caused by these clinical disorders (Furman *et al.* 2019). To manage inflammatory diseases, non-steroidal anti-inflammatory drugs (NSAIDs) are widely used. These medications have well-established adverse effects on the tract and the kidney (Mahesh *et al.* 2021). Furthermore, corticosteroids are among the best anti-inflammatory drugs for a variety of chronic inflammatory conditions, but they are not without side effects. Common side effects of these agents include weight gain, hypertension, mood swings, and gastrointestinal problems like ulcers and bleeding (Alorfi, 2023). Monoclonal antibodies like infliximab, natalizumab, adalimumab, etc. have been used to treat inflammatory diseases in recent times, but they are more costly than conventional drugs, making them less accessible to the general population (Makurvet, 2021). Consequently, researchers and industry are continuously trying to develop side-effect-free anti-inflammatory drugs. *P. niruri's* ethyl acetate fraction showed potent anti-inflammatory effects in the study. Research on this plant has shown that it contains lignans, tannins, coumarins, terpenes, flavonoids, and other compounds that have anti-inflammatory properties (Bagalkotkar *et al.* 2010). From the results, it can be hypothesized that the ethyl acetate fraction contains such anti-inflammatory compounds. Given the significance of developing novel, effective medications to control inflammation, the ethyl acetate fraction may be a useful resource for prospective lead finding.

Anti-diarrheal activity

The ethyl acetate fraction showed statistically significant anti-diarrheal activity that inhibited 40.67% ($p < 0.01$) of defection compared to the control group, whereas the standard produced 61.02% ($p < 0.01$) fecal inhibitory results (Table IV). The chloroform fraction showed moderate anti-diarrheal property with 28.81% ($p < 0.05$) defecation inhibition, induced by castor oil. The n-hexane fraction and the methanolic extract were not able to produce significant anti-diarrheal activity in the experimental animals.

Table IV. Anti-diarrheal activity of different fractions of *P. niruri*

Test Samples	Dose	Mean number of fecal pellets ± SD	% Inhibition of defecation
Control (1% Tween 80 in saline solution)	-	11.8 ± 2.59	-
Standard (loperamide)	50 mg/kg	4.6 ± 1.95	61.02***
Methanolic extract	400 mg/kg	9.4± 2.87	20.34
Hexane fraction	400 mg/kg	10.2 ± 2.77	13.56
Chloroform fraction	400 mg/kg	8.4 ± 2.19	28.81*
Ethyl acetate fraction	400 mg/kg	7.0 ± 2.24	40.68**

Data are presented as mean ± SD values, n=5. *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$

Thousands of people get diarrhea every year, and a significant portion of them pass away from the condition. Children, especially those under 5 years old, are vulnerable to this disease. Most of the affected populations belong to low- and middle-income countries (Hartman *et al.* 2023). In acute diarrheal cases, antibiotics such as tetracycline, ciprofloxacin, erythromycin, metronidazole, ampicillin, amoxycyline, and so on are common therapies. But the use of these antibiotics causes a reduction in the beneficial bacterial count of the GI tract and also shows an allergic reaction sometimes. Besides, many microorganisms are becoming resistant, making antibiotics ineffective therapies, which leads to a more dangerous condition termed antibiotic-associated diarrhea (Rawat *et al.* 2017). Antisecretory agents (loperamide, diphenoxylate) and anticholinergics (atropine, propantheline) are other available pharmacological options for diarrhea treatment. Many of these agents are insufficient to treat diarrhea sometimes. Additionally, these drugs also produce negative effects on the GI tract, kidneys, and other organs (Schiller, 2017). The therapeutic resistance and drug-related negative effects imply the importance of the development of new, effective anti-diarrheal drugs. As a significant portion of diarrheal disease sufferers are children, drugs with a less toxic profile are indispensable to treat their condition. It has long been known that plants can be used to treat diarrhea and related symptoms. The ethyl acetate fraction of *P. niruri* produced significant antidiarrheal efficacy in the study. Different phytocompounds are reported to have the ability to reduce diarrheal disease burden by various physiological mechanisms: alkaloids reduce peristaltic movement, tannins inhibit fluid secretion, histamine release is restricted by sponins, terpenoids interfere with the production of prostaglandins, and so on (Megersa *et al.* 2023). Findings from the current assay indicate that these bioactive compounds with antidiarrheal properties are present in the plant's ethyl acetate fraction. The isolation of potent anti-diarrheal compounds from *P. niruri* and the exploration of their mechanisms are evocative of the study.

Conclusion

Pharmacological activity screening of the whole plant, *P. niruri*, revealed its potent antibacterial, antioxidant, anti-diarrheal, and anti-inflammatory properties. The chloroform-soluble fractionate possessed both antimicrobial and antioxidant activities. Strong antibacterial potency was revealed by the ethyl acetate fraction. Significant anti-inflammatory and anti-diarrheal effects were also produced by the ethyl acetate fraction. The presence of phytocompounds with potent biological activities in *P. niruri* was indicated by the study, mostly in the ethyl acetate and n-hexane fractions. To obtain these active compounds, consecutive investigation is required.

Acknowledgement

The antibacterial assay of the work was conducted in the Biomedical Research Center of the University of Dhaka, and *in vivo* experiments were conducted in the animal house of the Institute of Nutrition and Food Science (INFS), University of Dhaka. The authors are grateful to the research center and the institution for the support.

References

Ahmed SK, Hussein S, Qurbani K, Ibrahim RH, Fareeq A, Mahmood KA and Mohamed MG (2024), Antimicrobial resistance: Impacts, challenges, and future prospects, *J Med Surg and Public Health.* **2**: 100081. DOI: org/10.1016/j.glmedi.2024.100081

Alorfi NM (2023), Pharmacological Methods of Pain Management: Narrative Review of Medication Used, *Int J Gen Med.* **16**: 3247-3256. DOI: org/10.2147/IJGM.S419239

Bagalkotkar G, Sagineedu SR, Saad MS and Stanslas J (2010), Phytochemicals from *Phyllanthus niruri* Linn. and their pharmacological properties: a review, *J Pharm Pharmacol.* **58**: 1559-1570. DOI: org/10.1211/jpp.58.12.0001

Bauer AW, Kirby WM, Sherris JC and Turck M (1966), Antibiotic susceptibility testing by a standardized single disk method, *Am J Clin Pathol.* **45**: 493-496.

Brand-Williams W, Cuvelier ME and Berset C (1995), Use of a free radical method to evaluate antioxidant activity, *LWT-Food Sci Technol.* **28**: 25-30. DOI: org/10.1016/S0023-6438(95)80008-5

Chen L, Deng H, Cui H, Fang J, Zuo Z, Deng J, Li Y, Wang X and Zhao L (2018), Inflammatory responses and inflammation-associated diseases in organs, *Oncotarget*. **9**: 7204-7218. DOI: org/10.18632/oncotarget.23208

Debono M, Barnhart M, Carrell CB, Hoffmann JA, Occolowitz JL, Abbott BJ, Fukuda DS, Hamill RL, Biemann K and Herlihy WC (1987), A21978C, a complex of new acidic peptide antibiotics. Isolation, chemistry, and mass spectral structure elucidation, *J Antibiot.* **40**: 761-777. DOI: org/10.7164/antibiotics.40.761

Furman D, Campisi J, Verdin E, Carrera-Bastos P, Targ S, Franceschi C, Ferrucci L, Gilroy DW, Fasano A, Miller GW, Miller AH, Mantovani A, Weyand CM, Barzilai N, Goronzy JJ, Rando TA, Effros RB, Lucia A, Kleinstreuer N and Slavich GM (2019), Chronic inflammation in the etiology of disease across the life span, *Nat Med.* **25**: 1822-1832. DOI: org/10.1038/s41591-019-0675-0

Giribabu N, Rao PV, Kumar KP, Muniandy S, Swapna Rekha S and Salleh N (2014), Aqueous extract of *Phyllanthus niruri* leaves displays *in vitro* antioxidant activity and prevents the elevation of oxidative stress in the kidney of streptozotocin-induced diabetic male rats, *J Evid Based Complementary Altern Med*. 1-10. DOI: org/10.1155/2014/834815

Haque SS, Rashid MM, Prodhan MA, Noor S and Das A (2014), In vitro evaluation of antimicrobial, cytotoxic and antioxidant activities of crude methanolic extract and other fractions of *Sterculia villosa* barks, *J Appl Pharm Sci.* DOI: org/10.7324/JAPS.2014.40308

Harish R and Shivanandappa T (2006), Antioxidant activity and hepatoprotective potential of *Phyllanthus niruri*, *Food Chem.* **95**: 180-185. DOI: org/10.1016/j.foodchem.2004.11.049

Hartman RM, Cohen AL, Antoni S, Mwenda J, Weldegebriel G, Biey J, Shaba K, de Oliveira L, Rey G, Ortiz C, Tereza M, Fahmy K, Ghoniem A, Ashmony H, Videbaek D, Singh S, Tondo E, Sharifuzzaman M, Liyanage J and Nakamura T (2023), Risk factors for mortality among children younger than age 5 years with severe diarrhea in low- and middle-income countries: findings from the World Health Organization-coordinated global Rotavirus and pediatric diarrhea surveillance networks, *Clin Infect Dis.* **76**: e1047-e1053. DOI: org/10.1093/cid/ciac561

Huang S and Eze UA (2023), Awareness and knowledge of antimicrobial resistance, antimicrobial stewardship and barriers to implementing antimicrobial susceptibility testing among medical laboratory scientists in Nigeria: A Cross-Sectional Study, *Antibiotics*. **12**: 815. DOI: org/10.3390/antibiotics12050815

Hyldgaard M, Mygind T and Meyer RL (2012), Essential oils in food preservation: mode of action, synergies, and interactions with food matrix components, *Front microbiol.* **3**: 12. DOI: org/10.3389/ fmicb.2012.00012

Ibrahim D, Hong LS and Kuppan N (2013), Antimicrobial activity of crude methanolic extract from *Phyllanthus niruri*, *Nat Prod Commun.* **8**: 493-496.

Kamatnur N and Chawan A (2013), PA03.02. The effect of bhumyamalaki (*Phyllanthus Niruri*) in balatisara with special reference to viral diarrhea, *Anc Sci Life.* **32**: 71. DOI: org/10.4103/0257-7941.123894

Kupchan SM, Tsou G and Sigel CW (1973), Datiscacin, a novel cytotoxic cucurbitacin 20-acetate from *Datisca glomerata*, *J Org Chem.* **38**: 1420-1421. DOI: org/10.1021/jo00947a041

Lee NYS, Khoo WKS, Adnan MA, Mahalingam TP, Fernandez AR and Jeevaratnam K (2016), The pharmacological potential of *Phyllanthus niruri*, *J Pharm Pharmacol.* **68**: 953-969. DOI: org/10.1111/-jphp.12565

Mahesh G, Anil KK and Reddanna P (2021), Overview on the discovery and development of anti-inflammatory drugs: should the focus be on synthesis or degradation of PGE2? *J Inflamm Res.* **14**: 253-263. DOI: org/10.2147/JIR.S278514

Makurvet FD (2021), Biologics vs. small molecules: drug costs and patient access, *Med Drug Discov.* **9**: 100075. DOI: org/10.1016/j.medidd.2020.100075

Mao X, Wu LF, Guo HL, Chen WJ, Cui YP, Qi Q, Li S, Liang WY, Yang GH, Shao YY, Zhu D, She GM, You Y and Zhang LZ (2016), The Genus *Phyllanthus*: An ethnopharmacological, phytochemical, and pharmacological review, *J Evid Based Complementary Altern Med*. **1**: 1-36. DOI: org/10.1155/ 2016/ 7584952

Megersa A, Dereje B, Adugna M, Ayalew GK and Birru E (2023), Evaluation of anti-diarrheal activities of the 80% methanol extract and solvent fractions of *Maesa lanceolata* Forssk (Myrsinaceae) leaves in mice, *J Exp Pharmacol.* **15**: 391-405. DOI: org/10.2147/-JEP.S429403

Nisar M, He J, Ahmed A, Yang Y, Li M and Wan C (2018), Chemical components and biological activities of the genus Phyllanthus: A review of the recent literature, *Molecules*. **23**: 2567. DOI: org/10.3390/molecules23102567

Ramandeep K, Nahid A, Neelabh C and Navneet K (2017), Phytochemical screening of *Phyllanthus niruri* collected from Kerala region and its antioxidant and antimicrobial potentials, *J Pharm Sci Res.* **9**: 1312.

Rawat P, Singh PK and Kumar V (2017), Evidence-based traditional anti-diarrheal medicinal plants and their phytocompounds, *Biomed Pharmacother*. **96**: 1453-1464. DOI: org/10.1016/j.biopha.2017.11.147

Saha P, Rahman FI, Hussain F, Rahman SMA and Rahman MM (2022), Antimicrobial Diterpenes: Recent Development From Natural Sources. *Front pharmacol*. **12:** 820312. DOI: org/10.3389/fphar.2021.820312

Samali A (2012), Evaluation of chemical constituents of *Phyllanthus Niruri*, *Afr J Pharm Pharmacol.* **6**: 125-128. DOI: org/10.5897/AJPP10.363

Sarkar MK, Kinter M, Mazumder B and Sil PC (2009), Purification and characterization of a novel antioxidant protein molecule from *Phyllanthus niruri*, *Food Chem.* **114**: 1405-1412. DOI: org/10.1016/j.foodchem.2008.11.022

Schiller LR (2017), Antidiarrheal drug therapy, *Curr Gastroenterol Rep.* **19**: 1-12. DOI: org/10.1007/s11894-017-0557-x

Sharifi-Rad M, Anil Kumar NV, Zucca P, Varoni EM, Dini L, Panzarini E, Rajkovic J, Tsouh Fokou PV, Azzini E, Peluso I, Prakash MA, Nigam M, El Rayess Y, Beyrouthy ME, Polito L, Iriti M, Martins N, Martorell M, Docea AO and Sharifi-Rad J (2020), Lifestyle, oxidative stress, and antioxidants: Back and forth in the pathophysiology of chronic diseases, *Front Physiol.* **11**: 694. DOI: org/10.3389/fphys.2020.00694

Shebis Y, Iluz D, Kinel YY, Dubinsky Z and Yehoshua Y (2013), Natural antioxidants: Function and sources, *Food Nutr Sci.* **4**: 643-649. DOI: org/10.4236/fns.2013.46083

Smith R and Coast J (2013), The true cost of antimicrobial resistance, *BMJ*, *346*(mar11 3), f1493-f1493. DOI: org/10.1136/bmj.f1493

Somanabandhu A, Nitayangkura S, Mahidol C, Ruchirawat S, Likhitwitayawuid K, Shieh H, Chai H, Pezzuto JM and Cordell GA (1993), ^{1}H- and ^{13}C-Nmr assignments of Phyllanthin and Hypophyllanthin: Lignans that enhance cytotoxic responses with cultured multidrug-resistant cells, *J Nat Pro.* **56**: 233-239. DOI: org/10.1021/np50092a008

Taniyama Y and Griendling KK (2003), Reactive oxygen species in the vasculature, *Hypertension*. **42**: 1075-1081. DOI: org/10.1161/01. HYP.00001 00443.09293.4F

Terefe L, Nardos A, Debella A, Dereje B, Arega M, Abebe A, Gemechu W and Woldekidan S (2023), Antidiarrheal activities of the methanol leaf extracts of *Olinia rochetiana* (Oliniaceae) against castor oil-induced diarrhea in mice, *J Exp Pharmacol.* **15**: 485-495. DOI: org/10.2147/JEP.S441555

Vaou N, Stavropoulou E, Voidarou C, Tsigalou C and Bezirtzoglou E (2021), Towards advances in medicinal plant antimicrobial activity: A review study on challenges and future perspectives, *Microorganisms*. **9**: 2041. DOI: org/10.3390/microorganisms9102041

Venkateswaran PS, Millman I and Blumberg BS (1987), Effects of an extract from *Phyllanthus niruri* on hepatitis B and woodchuck hepatitis viruses: *in vitro* and *in vivo* studies, *Proc Natl Acad Sci.* **84**: 274-278. DOI: org/10.1073/pnas.84.1.274

Winter CA, Risley EA and Nuss GW (1962), Carrageenin-induced edema in hind paw of the rat as an assay for antiinflammatory drugs, *Exp Biol Med.* **111**: 544-547. DOI: org/10.3181/00379727-111-27849

Yeap FL (1995), Amariinic acid and related ellagitannins from *Phyllanthus amarus*, *Phytochemistry*. **39**: 217-224. DOI: org/10.1016/0031-9422(94)00836-I

Zimmermann M (1983), Ethical guidelines for investigations of experimental pain in conscious animals, *Pain*. **16**: 109-110. DOI: org/10.1016/ 0304-3959 (83)90201-4