

***In-vivo* Analgesic, Diuretic, Anti-diarrheal and *in-vitro* Anti-microbial activity study of the ethanolic extract of *Phyllanthus reticulatus* fruits**

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Abstract

Background

Phyllanthus reticulatus commonly known as Pancoli. It is used as a traditional medicinal with properties such as antioxidant, antibacterial and anti-HIV-1.

Methods

Analgesic activity was determined by the acetic acid writhing test and the diuretic activity was assessed in albino mice by measuring urine volume and electrolyte excretion over 24 hours following oral administration. The anti-diarrheal effect was evaluated using the castor oil-induced model, and antimicrobial activity was tested through the disc diffusion method.

Results

In the analgesic assay, the PRFEE at a dose of 300 mg/kg showed the highest inhibition (89%, 1.50 ± 0.50 writhes, $P < 0.001$). The diuretic activity assessment indicated a substantial increase in urine output with a dose of 500 mg/kg producing 2.50 ± 0.289 ml of urine ($P < 0.001$). Additionally, this dose significantly enhanced sodium (133.16 ± 8.186 mmol/L, $P < 0.001$), Potassium (87.25 ± 3.792 mmol/L, $P < 0.001$), and chloride (106.79 ± 4.49 mmol/L, $P < 0.001$) excretion. In the anti-diarrheal study, PRFEE 500 mg/kg achieved 81.82% inhibition reducing stool count to 1 ± 0.408 ($P < 0.001$), demonstrating strong efficacy. The antimicrobial activity showed a Potent inhibitory effect with a dose of 750 mg/mL, exhibiting inhibition zones of 17 mm (*Escherichia coli*), 15 mm (*Pseudomonas aeruginosa*) and 19 mm (*Staphylococcus aureus*), comparable to those of kanamycin (16–19 mm).

Conclusion

The PRFEE exhibited significant Pharmacological effects across all evaluated Parameters.

INTRODUCTION

Medicinal plants have long played a significant role in healthcare systems across various regions of the world. These plants contain a wide range of bioactive compounds with therapeutic properties that are used in the treatment of numerous health conditions in both humans and animals [1, 2]. Among these, *Phyllanthus reticulatus* (commonly known as Pancoli) is a shrub or small tree belonging to the Euphorbiaceae family. It has been traditionally used throughout Asia and Africa to treat liver disorders, gastrointestinal issues, and various infections. The plant typically grows to a height of 1 to 5 meters [3] with its flowering, and fruiting season extending from July to March [4]. Phytochemical analyses show that flavonoids, alkaloids, saponins, phenols, cardiac glycosides, tannins, anthocyanins, and terpenoid

compounds are found in plant [5]. These constituents are believed to contribute to the plant's wide range of pharmacological effects [6]. Previous studies have documented the traditional and pharmacological uses of *P. reticulatus*. This plant has demonstrated antioxidant, antibacterial, anti-HIV-1, antidiabetic, antifungal, antiplasmodial and antidiarrheal effects [7–10]. Additionally, an herbal hair dye formulation has been developed using an aqueous leaf extract of *P. reticulatus* in combination with other natural ingredients [11]. Methanolic extracts of the stem bark have shown antimicrobial activity [12]. Its fruits have been used traditionally for their anti-inflammatory effects [13]. Furthermore, traditional practices include the preparation of a pill made from leaf juice mixed with camphor and cubebs, which is dissolved in the mouth to treat bleeding gums, while the roots are used as a natural red dye [14]. *Phyllanthus* species is known for its diuretic effects, contributing to fluid and electrolyte balance [15]. Methanolic and ethyl acetate extracts from the plant have shown promising antimicrobial activity against both bacterial and fungal pathogens [16, 17]. Pain can be measured using tests like the acetic acid-induced writhing and hot-plate methods, which show how drugs work in both the brain and the nerves. These drugs also help with diarrhea by controlling how the intestines move and produce liquid, which stops too much water from being lost, as seen in tests using castor oil-induced diarrhea [18, 19]. Additionally, these drugs have a diuretic effect, meaning they increase urine and salt loss, showing their effect on kidney function and the body's water balance [20]. Both Gram-positive and Gram-negative bacteria are believed to be a big issue for public health [21]. While extensive research has confirmed the pharmacological potential of the plant's leaves, bark, and stem, the fruit of *P. reticulatus* remains significantly underexplored. Despite its traditional medicinal use and potential as a source of novel bioactive compounds, there is a lack of comprehensive phytochemical and pharmacological data specifically focused on the fruit. The chemical constituents, biological properties, and therapeutic potential of the fruit have not yet been systematically investigated. Given this knowledge gap, further scientific investigation is essential. The fruit of *P. reticulatus* may contain unique active compounds that could offer therapeutic benefits in the treatment of pain, gastrointestinal disorders, metabolic diseases and microbial infections. A thorough study of the fruit could contribute to the development of affordable, accessible, and safe natural therapeutics, particularly important for populations in developing countries that rely heavily on herbal medicine. Therefore, the present study was undertaken to investigate the phytochemical composition and pharmacological properties of the ethanolic extract of *Phyllanthus reticulatus* fruits (PRFEE) with a specific focus on in vivo analgesic, diuretic, antidiarrheal, and in vitro antimicrobial activities. This research aims to bridge traditional knowledge and modern pharmacological validation, potentially identifying new bioactive compounds of therapeutic value.

MATERIALS AND METHODS

Plant Materials and Extraction Process

The fruits of *P. reticulatus* were gathered from Savar, Dhaka, Bangladesh between October to November 2023. They were washed carefully to remove any unwanted stuff. The plant was confirmed by the Bangladesh National Herbarium in Mirpur, Dhaka, Bangladesh with the accession number DACB 87899.

After washing, the fruits were dried in the shade for seven days and then dried in an oven at 40°C for 24 hours. The dried fruits were then turned into a rough powder using a disc mill grinder machine. Before storing, the powder was cleaned again and dried properly. The powder was kept in a sealed container in a dark and dry area. 341 g of dry powder of *P. reticulatus* fruits was taken by the extractor apparatus, and 2.5 liters of 98% ethanol were used in the Soxhlet apparatus, model-509.303.

Chemicals

Aspirin (Albion Laboratories Limited), Furosemide (Square Pharmaceutical Limited), and Loperamide (ACME Laboratories Limited) were purchased from the market. The bacterial strain organisms were collected from the Microbiology Research Laboratory, Gono Bishwabidyala. All the chemicals are sourced from the Department of Pharmacy Laboratory, Gono Bishwabidyalay, Savar, Dhaka.

Experimental Animals

Swiss albino mice both male and female aged between 6 and 7 weeks and weighing 20 to 30 grams were acquired from the Animal Research Laboratory at the Department of Pharmacy, Jahangirnagar University located in Savar, Dhaka. They were maintained under standard laboratory conditions including a temperature of $25 \pm 1^\circ\text{C}$, a humidity level of 55–65% and a 12-hour light-dark cycle. The mice had free access to food and water and were allowed to acclimate to the laboratory environment for one week before the start of the experiments. The mice were divided into five groups for analgesic and anti-diarrheal test, and four groups for diuretics test with each group containing five mice. All experiments conducted on animals were approved by the Research Ethical Committee under the Center for Multidisciplinary Research of Gono Bishwabidyalay, Dhaka 1344, Bangladesh (Permission number: CMR/EC/023).

Phytochemical activitys

The PRFEE was checked for plant-based chemical components through several simple tests. Different chemicals were used according to standard methods, and the results showed clear color changes that showed the presence of carbohydrates, saponins, tannins, alkaloids, flavonoids, steroids, glycosides, and phenolic compounds, and the absence of steroid compounds [22].

Antioxidant activitys

Reducing Power Capacity Assay (RPC)

Reducing power capacity of the fruit extract was evaluated using the ferric–ferricyanide reduction method [23]. 1.0 ml of Plant extract or standard of different concentration mixed with 2.5ml of phosphate buffer (pH 6.6) & 2.5 ml potassium ferricyanide (1%). The reaction mixture is incubated for 20

min at 50⁰ C to complete the reaction. 2.5ml of trichloro acetic acid (10%) solution is added into the test tube and the total mixture was centrifuged at 3000 rpm for 10 min. 2.5ml of supernatant solution is withdrawn from the mixture and mixed with 2.5 ml of distilled water. 0.5ml of ferric chloride (0.1%) solution was added to the diluted reaction mixture. Then the absorbance of the solution is measured at 700 nm using a spectrophotometer against blank. An increase in absorbance indicated enhanced reducing power.

DPPH Free Radical Scavenging Assay

The in vitro antioxidant activity of the fruit extract was assessed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay, which measures the ability of antioxidants to quench DPPH radicals through hydrogen atom or electron donation [24, 25], which measures the ability of antioxidants to quench DPPH radicals through hydrogen atom or electron donation. A 0.004% (w/v) DPPH solution was freshly prepared by dissolving 0.004 g DPPH in 100 mL ethanol. Stock solutions of the fruit extract and ascorbic acid (5 mg/mL) were prepared in ethanol, and serial dilutions yielded concentrations of 6.25 to 800 µg/mL. Aliquots (1 mL) of each concentration were mixed with 2 mL of DPPH solution and incubated in the dark at room temperature for 30 min. Absorbance was measured at 517 nm using a UV-Vis spectrophotometer. All tests were conducted in triplicate. The percentage of DPPH radical inhibition was calculated as:

$$\% \text{ Inhibition} = \left(1 - \frac{\text{Absorbance of sample}}{\text{Absorbance of control}} \right) \times 100$$

The IC₅₀ value (µg/mL) was determined from a plot of % inhibition versus concentration by linear regression.

Acute Toxicity Study

An acute oral toxicity study was conducted on Swiss albino mice in accordance with OECD Guideline 423 [26]. Twelve mice were randomly allocated into four groups (n = 3) and administered a single oral dose of PRFEE at 250, 500, 1000 and 2000 mg/kg, respectively. The animals were monitored for 14 days for signs of toxicity, behavioral alterations, and mortality.

In-vivo Evaluation of Analgesic Activity.

Acetic acid induced writhing test

The abdominal writhing test was done using acetic acid following the method by Saha et al. [27] with some changes. The animals were randomly divided into five groups. The control group got normal saline at a dose of 10 ml/kg of body weight. The standard group was given aspirin at 75 mg/kg, and the test groups were given PRFEE at doses of 100, 200 and 300 mg/kg, all by mouth. Thirty minutes after drug administration, all animals were injected intraperitoneal with 0.6% acetic acid to induce pain. After a

latency period of 5 minutes, the number of abdominal writhes was counted over the next 30 minutes. The pain-relieving effect was shown as the percentage of writhing that was prevented.

$\% \text{ Inhibition} = \frac{\text{Number of writhes in control group} - \text{Number of writhes in treated group}}{\text{Number of writhes in control group}} \times 100$

In vivo Evaluation of Diuretic Activity.

Diuretic activity was assessed using the method of Geleta et al. [28] with slight modifications. Swiss albino mice (20–30 g) were ensured to have similar body weights. All the mice were not allowed to eat for the whole night but could drink water freely, and were acclimatized in individual metabolic cages for 24 hours before the experiment. The control group received 0.9% NaCl, the standard group was administered furosemide at a dose of 10 mg/kg and the test groups received PRFEE at 250 and 500 mg/kg by mouth. After dosing, urine was collected over 24 hours in individual metabolic cages. Collected urine was filtered and stored at -20°C for analysis of sodium, potassium, and chloride levels. The following parameters were calculated.

$\text{Diuretic action (\%)} = \frac{\text{Total urinary output}}{\text{Total liquid administered}} \times 100 \text{ (1)}$

$\text{Urinary excretion} = \frac{\text{Urinary excretion of test group}}{\text{Urinary excretion of control group}} \text{ (2)}$

$\text{Diuretic activity} = \frac{\text{Diuretic action of the test group}}{\text{Diuretic action of standard drug}} \text{ (3)}$

Analytical Procedure:

The levels of sodium, potassium, and chloride in both the urine samples and the plant extract were checked. These measurements were done with an ion-selective electrode analyzer called the 9180 Electrolyte Analyzer Reagent, which was made by Roche in Germany.

Evaluation of the Antidiarrheal Activity

Castor Oil-Induced Diarrheal Test

The diarrhea model caused by castor oil as described by Afroz et al. [29] was used with slight modifications. Mice were not given food for 18 hours. All mice were given 1.0 ml of castor oil by mouth. After 30 minutes, the control group was given normal saline at a dose of 10 ml/kg of body weight. The standard group received loperamide at 2 mg/kg. The test groups were given PRFEE at doses of 150, 300, and 500 mg/kg. Each mice were placed in a separate cage with paper lining. The paper was changed every hour for 4 hours. The number and weight of feces, along with stool consistency were recorded. The results were shown as a percentage of how much diarrhea was prevented [30].

$\% \text{ Inhibition of defecation} = \frac{\text{Mean of defecation control group} - \text{Mean of defecation treated group}}{\text{Mean of defecation control group}} \times 100$

In vitro Evaluation of Antimicrobial Activity

Media Preparation:

Mueller-Hinton agar and broth were prepared and sterilized according to the manufacturer's instructions and standard protocols [31]. About 20–25 ml of agar was added to sterile petri dishes and let dry at room temperature. The dishes were kept in a refrigerator at 4°C until they were needed [32]. The bacteria used in this study were *Staphylococcus aureus*, which is Gram-positive and *Escherichia coli*, *Salmonella enterica* and *Pseudomonas aeruginosa*, which are all Gram-negative from the Microbiology Research Laboratory, Gono Bishwabidyalay, Savar, Dhaka.

Disc diffusion method:

Antimicrobial activity of PRFEE was tested using a modified disc diffusion (Hole-Plate) method [33, 34]. Bacterial suspensions were placed on nutrient agar plates and small wells, each about 8 millimeters in size were created using a clean cork borer. The extract was dissolved in a 1:1 mixture of DMSO and water for injection and applied at concentrations of 250, 500 and 750 µg/ml (100 µl Per well). Plates were kept at a temperature of 37°C for 24 hours. The areas where bacteria did not grow were measured in millimeters to assess the effectiveness of the substance in inhibiting bacterial growth. Water for injection and the solvent mixture served as negative controls, while Kanamycin-K (30 µg/disc) was used as the positive standard. All tests were performed in duplicate for accuracy.

Statistical analysis

Statistical analysis was done using SPSS version 30.0 for Windows [35]. The results are shown as S.E.M. A one-way ANOVA was used along with Dunnett's post hoc test to compare multiple groups. Differences were considered statistically significant when the P value was below $P < 0.05$ - $P < 0.001$.

RESULTS

Screening Phytochemical Components

The PRFEE was found to contain carbohydrates, tannins, saponins and phenolic constituents upon phytochemical analysis. However, steroids were absent.

Antioxidant activity

Determination of Reducing Power capacity (RPC)

The reducing properties are generally associated with the presence of reluctance which have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom). Absorbance Concentration (µg/ml) reducing power (Table 1). In the present study ferric reducing power

activity of the extract of fruits of *P. reticulatus* plants are for $(118.39 \pm 4.30 \text{ mg/g})$ as Gallic acid (Table 2). The experimental results indicate lower ferric ($y = 0.0029x + 0.039$, $R^2 = 0.9842$) (Fig. 1).

Table 1
Absorbance found with different concentration of Gallic Acid.

Concentration ($\mu\text{g/ml}$)	Absorbance
6.25	0.032
12.5	0.147
25	0.223
50	0.269
100	0.484
200	0.788

Table 2
Determination of Reducing Power capacity of the ethanolic extract of fruits of *P. reticulatus*.

Sample Name	Wt. of plant extract ($\mu\text{g/ml}$)	Abs.	GAE conc.(C) ($\mu\text{g/ml}$)	GAE conc.(C) (mg/ml)	V (ml)	C $\times$ V (mg)	TPC as GAE, A= (c $\times$ V)/m (mg/gm)	Mean value $\pm$ SD
<i>P.</i>	200	0.107	23.445	0.023	1	0.023	117.241	118.39 $\pm$ 4.30 mg/g
<i>reticulatus</i>	200	0.105	22.756	0.022	1	0.022	113.793	
	200	0.111	24.828	0.024	1	0.024	124.128	

DPPH free radical scavenging assay

When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized, which can be quantitatively measured from the changes in absorbance. DPPH free radical scavenging study of the extract of fruits of *P. reticulatus* determine as % of inhibition and IC_{50} (inhibition concentration 50%) value. Lower IC_{50} values indicates good scavenging capacity and antioxidant property. The results of the present study showed that ethanolic extract have IC_{50} value $639.033 \mu\text{g/mL}$ (Table 4) (Fig. 3), exhibited poor antioxidant property compared to standard $3.725 \mu\text{g/ml}$ (Table 3) (Fig. 2).

Table 3
The percentage (%) scavenging Activity of Ascorbic Acid.

Concentration (µg/ml)	Control	Absorbance sample	% Inhibition	IC ₅₀ value
6.25	1.748	1.347	22.940	3.725
12.5	1.748	1.089	37.700	
25	1.748	0.957	45.251	
50	1.748	0.729	58.295	
100	1.748	0.641	63.329	
200	1.748	0.578	66.933	
400	1.748	0.489	72.025	
800	1.748	0.317	81.864	

Table 4
The results and IC₅₀ ethanol extract of fruits of *P. reticulatus* is presented in the Table.
The percentage (%) scavenging Activity of *P. reticulatus* fruits extract.

Concentration (µg/ml)	Control	Absorbance sample	% Inhibition	IC ₅₀ value
6.25	1.748	1.514	13.39%	639.033
12.5	1.748	1.467	16.08%	
25	1.748	1.268	27.46%	
50	1.748	1.025	41.36%	
100	1.748	0.991	43.31%	
200	1.748	0.953	45.48%	
400	1.748	0.679	61.16%	
800	1.748	0.575	67.11%	

Acute Toxicity

No mortality was observed in any of the groups. The body weight remained normal and no significant toxic symptoms were observed.

Analgesic activity

PRFEE showed a strong pain-relieving effect that increases with higher doses in the acetic acid-induced writhing test. Aspirin at 75 mg/kg produced 80% inhibition ($P < 0.001$). PRFEE at 100, 200 and 300 mg/kg

showed 20%, 71% and 89% inhibition, respectively. Significant reductions in writhing were observed at 200 mg/kg ($P < 0.01$) and 300 mg/kg ($P < 0.001$), while 100 mg/kg was not statistically significant (Table 5) (Fig. 4).

Table 5
Effect of Aspirin and PRFEE on acetic acid-induced writhing in mice.

Group	Dose	Mean of Writhing $\pm$ S.E.M	% Inhibition
Control	10 ml/kg	13.75 $\pm$ 0.48	-
Aspirin	75 mg/kg	2.75 $\pm$ 0.48 ***	80%
PRFEE	100 mg/kg	11.00 $\pm$ 1.00	20%
	200 mg/kg	4.00 $\pm$ 0.50 **	71%
	300 mg/kg	1.50 $\pm$ 0.50 ***	89%

Values are represented as S.E.M, (n = 5); $P < 0.05$ – $P < 0.001$.

Diuretic activity

Effect of PRFEE on 24-hour urine output and diuretic indices in mice. The control group produced 1.00 ± 0.00 ml of urine, while furosemide raised output to 2.75 ± 0.25 ml ($P < 0.001$). PRFEE at 250 and 500 mg/kg increased urine volume to 2.00 ± 0.001 ml ($P < 0.01$) and 2.50 ± 0.289 ml ($P < 0.001$), respectively. The diuretic actions were 2.00 and 2.50 with corresponding diuretic activities of 0.72 and 0.91, indicating that the 500 mg/kg dose achieved the standard drug effect (Table 6) (Fig. 5).

Table 6
Diuretic effect of PRFEE by increasing urine volume.

Group	Dose	Total urine volume (ml) $\pm$ S.E.M	Diuretic action (%)	Diuretics activity
Control	25 ml/kg	1 $\pm$ 0.00	-	
Furosemide	10 mg/kg	2.75 $\pm$ 0.250 ***	2.75	-
PRFEE	250 mg/kg	2 $\pm$ 0.001 **	2	0.72
	500 mg/kg	2.50 $\pm$ 0.289 ***	2.50	0.91

Values are represented as S.E.M, (n = 5); $P < 0.05$ - $P < 0.001$

Electrolyte Excretion

PRFEE significantly increased urinary excretion of Na^+ , K^+ and Cl^- levels. In the control group urine levels were 87.85 ± 2.87 (Na^+), 55.58 ± 4.73 (K^+) and 61.84 ± 2.20 (Cl^-). Furosemide at 10 mg/kg markedly increased electrolyte excretion Na^+ to 125.16 ± 4.54 , K^+ to 80.45 ± 1.82 and Cl^- to 95.89 ± 3.10 mmol/l ($P < 0.01$ - $P < 0.001$). PRFEE at 250 mg/kg significantly raised Na^+ , K^+ and Cl^- to 106.28, 76.32 and 82.51

mmol/l, respectively ($P < 0.05$ - $P < 0.001$). At 500 mg/kg excretion levels further increased to 133.16 (Na^+), 87.25 (K^+) and 106.79 mmol/l (Cl^-), all highly significant ($P < 0.001$), exceeding the effect of furosemide (Table 7) (Fig. 6).

Table 7
Effect of PRFEE on urinary electrolyte excretion.

Group	Dose	Total Urinary electrolyte excretion		
		Na^+ mmol/l $\pm$ mean	K^+ mmol/l $\pm$ mean	Cl^- mmol/l $\pm$ mean
Control	25 ml/kg	87.85 ± 2.866	55.58 ± 4.729	61.84 ± 2.197
Furosemide	10 mg/kg	125.16 ± 4.535 ***	80.45 ± 1.821 **	95.89 ± 3.095 ***
PRFEE	250 mg/kg	106.28 ± 3.361 *	76.32 ± 5.434 *	82.51 ± 2.437 ***
	500 mg/kg	133.16 ± 8.186 ***	87.25 ± 3.792 ***	106.79 ± 4.490 ***
Values are represented as S.E.M, (n = 5); $P < 0.05$ - $P < 0.001$				

Antidiarrheal Activity

PRFEE has dose-dependent antidiarrheal effects in the control group had the mean of 5.50 ± 0.65 watery stools were observed, while loperamide at 2 mg/kg significantly reduced this to 2.25 ± 0.63 ($P < 0.01$) showing 60% inhibition. PRFEE at 150 mg/kg caused a mild reduction of 3.75 ± 0.48 (27% inhibition) while 300 mg/kg showed significant activity ($P < 0.01$) with 2.75 ± 0.48 stools (50% inhibition). The 500 mg/kg dose produced a highly significant reduction to 1.00 ± 0.41 stools ($P < 0.001$), achieving 81.82% inhibition exceeding that of Loperamide as shown (Table 8) and (Fig. 7).

Table 8
Showing the percentage reduction in volume of small intestinal content.

Group	Dose	Mean number of watery stools $\pm$ S.E.M	Inhibition of diarrhea (%)
Control	10 ml/kg	-	-
Loperamide HCl	2 mg/kg	2.25 ± 0.629 **	60%
PRFEE	150 mg/kg	3.75 ± 0.479	27%
	300 mg/kg	2.75 ± 0.479 **	50%
	500 mg/kg	1 ± 0.408 ***	81.82%

Values are represented as S.E.M, (n = 5); $P < 0.05$ - $P < 0.001$

Antimicrobial Activity

The PRFEE exhibits the capability to combat both Gram-positive and Gram-negative bacteria. No inhibition was observed at 250 µg/ml. The dose at 500 µg/ml showed moderate zones of inhibition were seen against *Pseudomonas aeruginosa* (13 mm) and *Staphylococcus aureus* (17 mm), while *Escherichia coli* showed no response. At 750 µg/ml, PRFEE showed strong antibacterial activity with inhibition zones of 17 mm (*E. coli*), 15 mm (*P. aeruginosa*) and 19 mm (*S. aureus*), comparable to the standard antibiotic kanamycin (30 µg). These results are summarized and visually represented in (Table 9) (Fig. 8).

Table 9
Inhibitory effect of PRFEE on (1) *Escherichia coli* (2) *Pseudomonas aeruginosa* (3) *Staphylococcus aureus* (4) Negative control (5) Kanamycin.

Group	Zone of inhibition (mm)		
	<i>Escherichia coli</i>	<i>Pseudomonas aeruginosa</i>	<i>Staphylococcus aureus</i>
Control	0	0	0
Kanamycin	19 mm	16 mm	19 mm
PRFEE 250 µg/ml	0	0 mm	0
PRFEE 500 µg/ml	0	13 mm	17 mm
PRFEE 750 µg/ml	17 mm	15 mm	19 mm

DISCUSSION

The Present study evaluated the dose-dependent pharmacological activities. This research is the first to report on the pharmacological potential of the fruits clearly demonstrates that the PRFEE exhibits significant pharmacological properties including analgesic, diuretic, antidiarrheal and antimicrobial activities. These effects can be attributed to its rich content of bioactive phytochemicals compounds, which are well-known for their diverse therapeutic effects. The results indicate that the fruits of *P. reticulatus* extract exhibited low DPPH radical scavenging activity and limited reducing power capacity, indicating weak chemical antioxidant potential. However, these assays mainly assess in vitro electron-donating ability and do not necessarily correlate with pharmacological effects. The observed biological activities may be attributed to the presence of alkaloids, flavonoids and phenolic compounds, which exert analgesic, diuretic and antidiarrheal effects through mechanism-specific pathways independent of antioxidant capacity [36–38]. PRFEE showed a strong analgesic effect in the acetic acid-induced writhing test with 300 mg/kg producing 89% inhibition, which was significantly greater than aspirin (75 mg/kg, 80%). Acetic acid-induced pain is mediated by the release of endogenous substances such as prostaglandins particularly PGE₂ and PGF₂α, which stimulate nociceptors [39]. Flavonoids and alkaloids reported are known to inhibit cyclooxygenase and lipoxygenase enzymes thereby suppressing prostaglandin synthesis and producing analgesia [40, 41]. Further supporting the role of polyphenolic compounds in analgesic activity [42]. PRFEE significantly increased urine output with the 500 mg/kg dose (2.50 ml) achieving a response comparable to furosemide (2.75 ml). The extract also markedly enhanced urinary electrolyte excretion (Na⁺, K⁺, Cl⁻) with the higher dose surpassing the standard drug.

Flavonoids and saponins have been reported to exert diuretic effects by altering renal tubular ion transport and increasing renal blood flow [43]. The observed saluretic effect (Na^+ and Cl^- excretion) and kaliuretic activity (K^+ excretion) suggest that PRFEE may act through a mechanism similar to loop diuretics possibly mediated by prostaglandins [44]. These findings indicate that PRFEE could be a promising natural diuretic.

In the castor oil-induced diarrheal model, PRFEE exhibited protection with the 500 mg/kg dose producing 81.82% inhibition surpassing loperamide (60%). Castor oil causes diarrhea by releasing ricinoleic acid, which stimulates intestinal peristalsis and secretion of electrolytes and water via prostaglandin pathways [45]. Tannins, flavonoids and steroids are known to reduce intestinal motility and secretion by inhibiting prostaglandin synthesis and relaxing intestinal smooth muscles [46]. The significant effect of PRFEE can therefore be linked to its tannin and flavonoid content consistent with earlier findings in other *Phyllanthus* species [47]. The fruit extract also demonstrated broad-spectrum antibacterial activity with strong inhibition zones against *Staphylococcus aureus* (19 mm), *Pseudomonas aeruginosa* (15 mm) and *Escherichia coli* (17 mm) at 750 $\mu\text{g}/\text{ml}$ comparable to the standard antibiotic kanamycin. Phenolic, flavonoids and alkaloids compounds are well known for their ability to disrupt bacterial cell walls inhibit nucleic acid synthesis and interfere with microbial enzymes, thereby exerting antimicrobial effects [48, 49]. These findings justify the ethnomedicinal use of *P. reticulatus* in infectious diseases.

CONCLUSION

The PRFEE exhibits potent analgesic, diuretic, antidiarrheal and antimicrobial activities. The highest doses of PRFEE often surpassed standard drugs in efficacy, indicating the fruit as a Promising source of bioactive compounds. The study highlights the relevance of plant part selection in pharmacological research, as the fruit may contain higher levels of active constituents than the leaves and bark. These results support the traditional medicinal use of *P. reticulatus* and suggest that PRFEE could serve as a foundation for developing natural therapeutic agents. Further studies focusing on molecular docking, isolation of bioactive compounds, mechanistic evaluation, toxicity profiling and clinical trials are necessary to establish its safety and therapeutic applicability.

Abbreviations

PRFEE, *Phyllanthus reticulatus* Fruit Ethanolic Extract; S.E.M., Standard Error of Mean.

Declarations

Author contributions

PR assisted in doing the project (experiments) for her thesis and analyzed the data and contributed to the writing of the paper. KZ supervised the project. He was involved in all parts of the experiments. JN analyzed the data and contributed to the writing of the paper. SM advised on the project and performed

some experiments. TR and AM advised on the project. All authors read and approved the final manuscript.

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animal rights

Animals were used in the studies that are the basis of this research.

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Animal Ethics approval and consent to participate

Applicable.

Consent for publication

All authors have read and agreed to the published version of the manuscript. Competing interests The authors declare no competing interests.

Human Clinical trial number

Not applicable.

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Figures

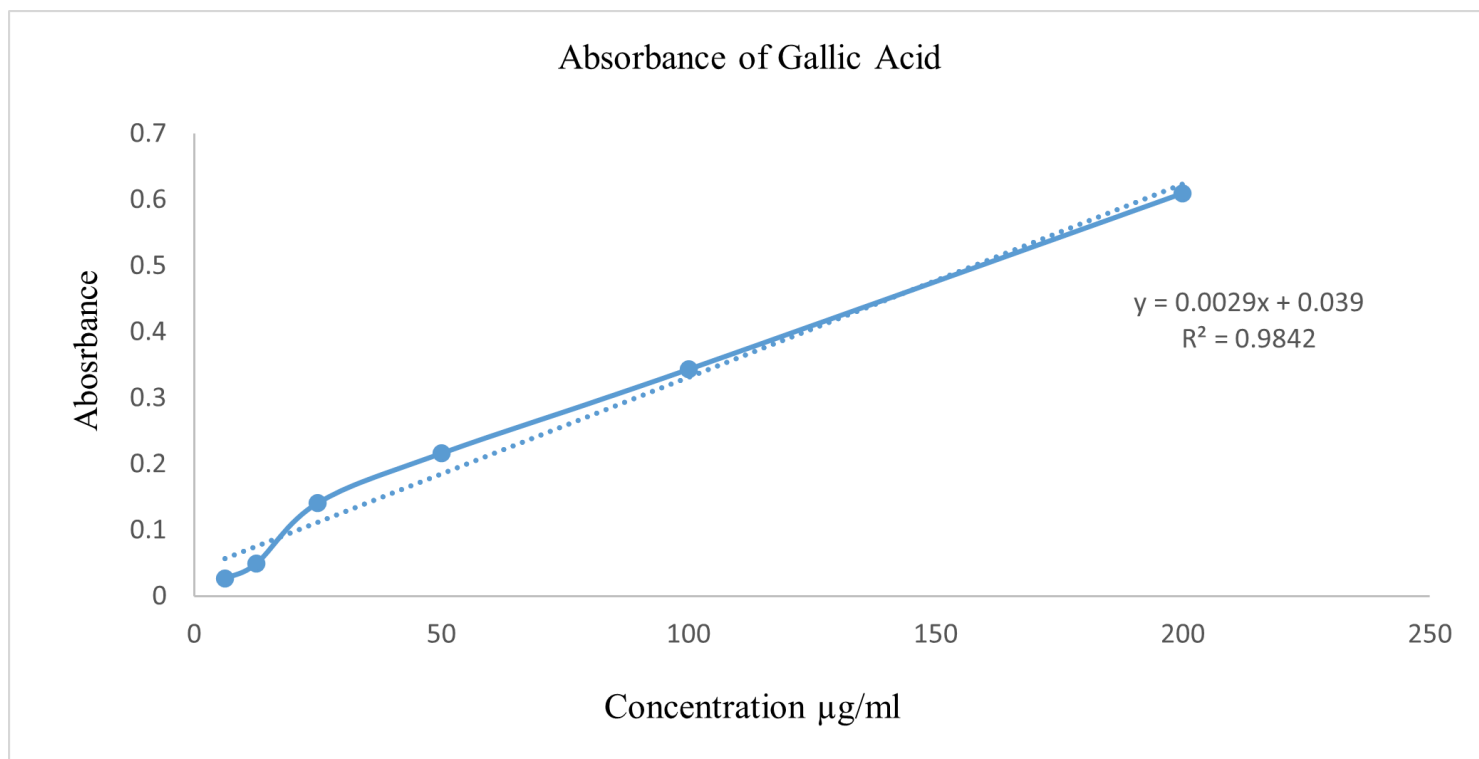


Figure 1

Calibration curve of Gallic Acid of reducing power capacity.

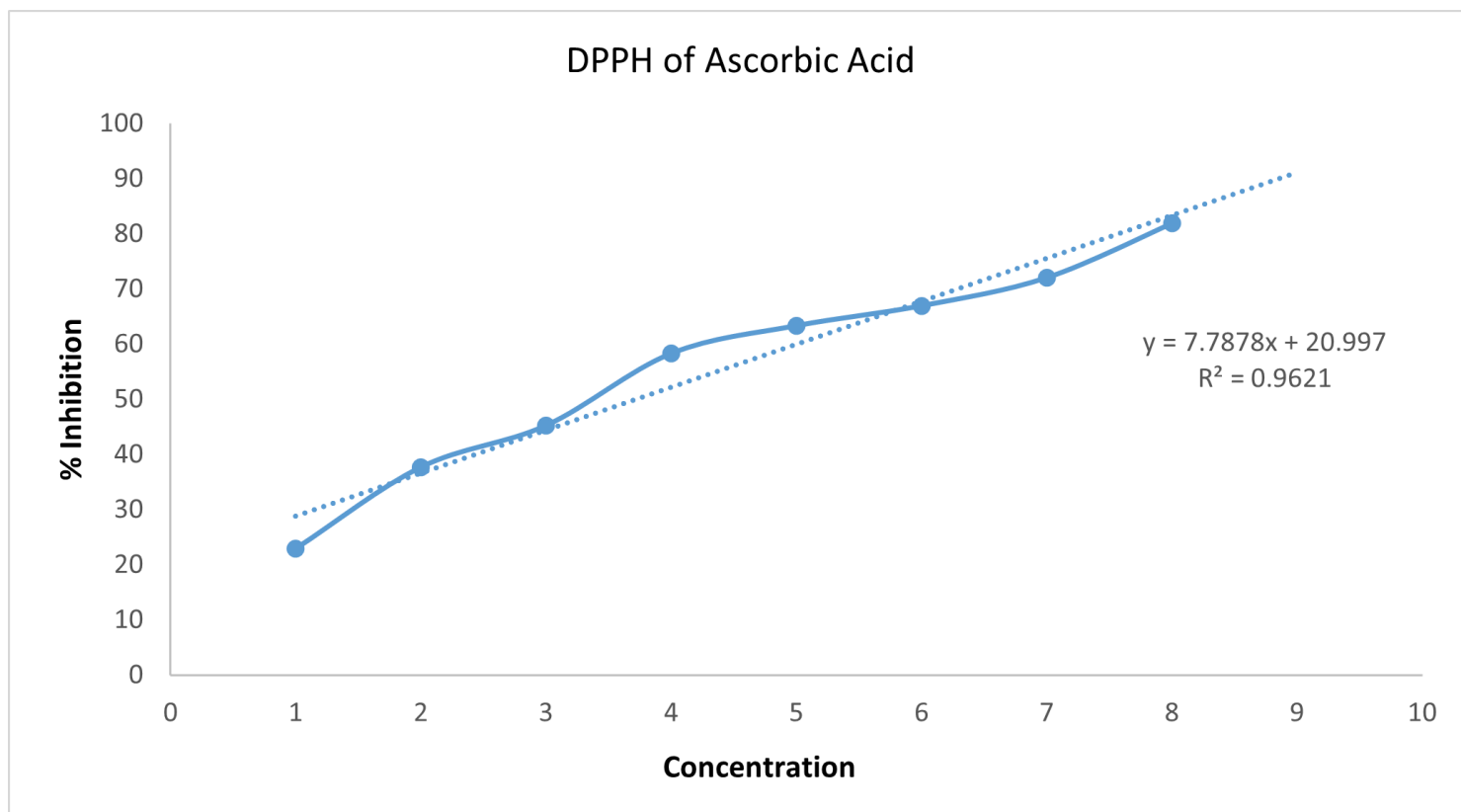


Figure 2

Calibration curve of DPPH of Ascorbic Acid.

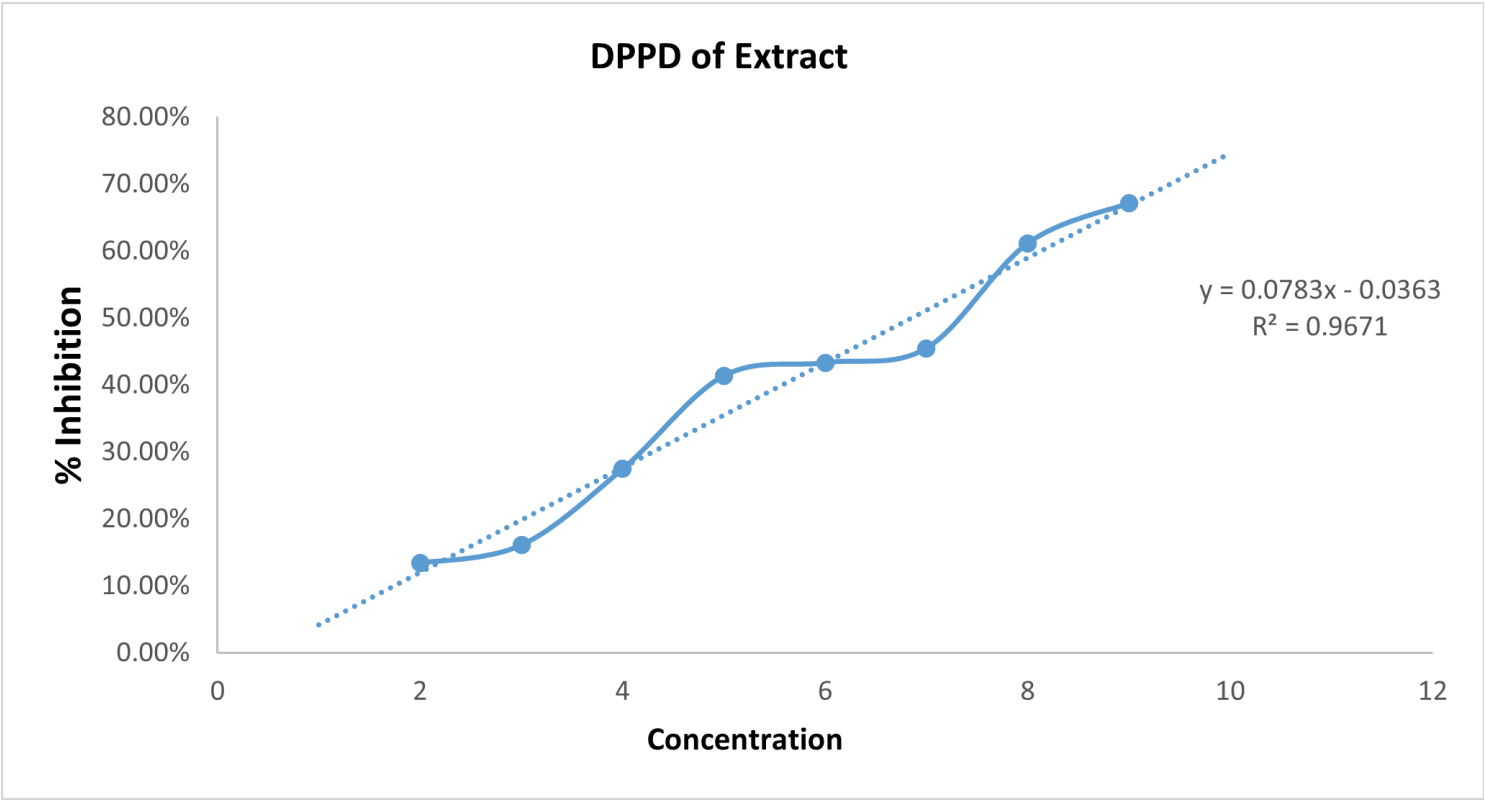


Figure 3

Calibration curve of DPPH of Extract.

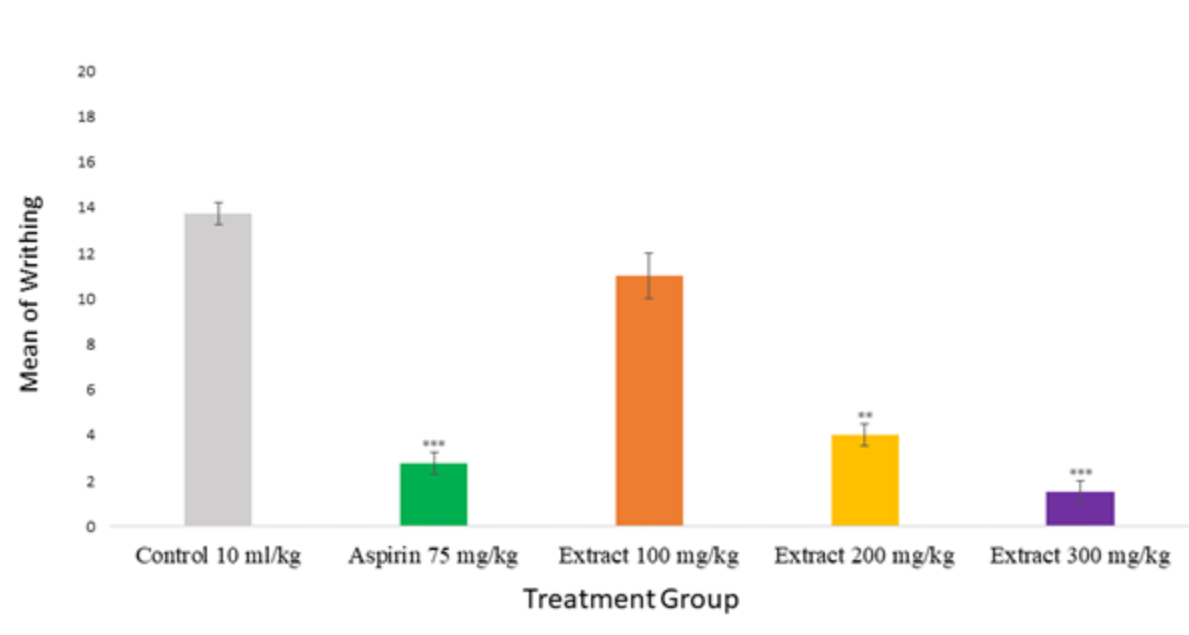


Figure 4

Acetic acid-induced writhing test of PRFEE.

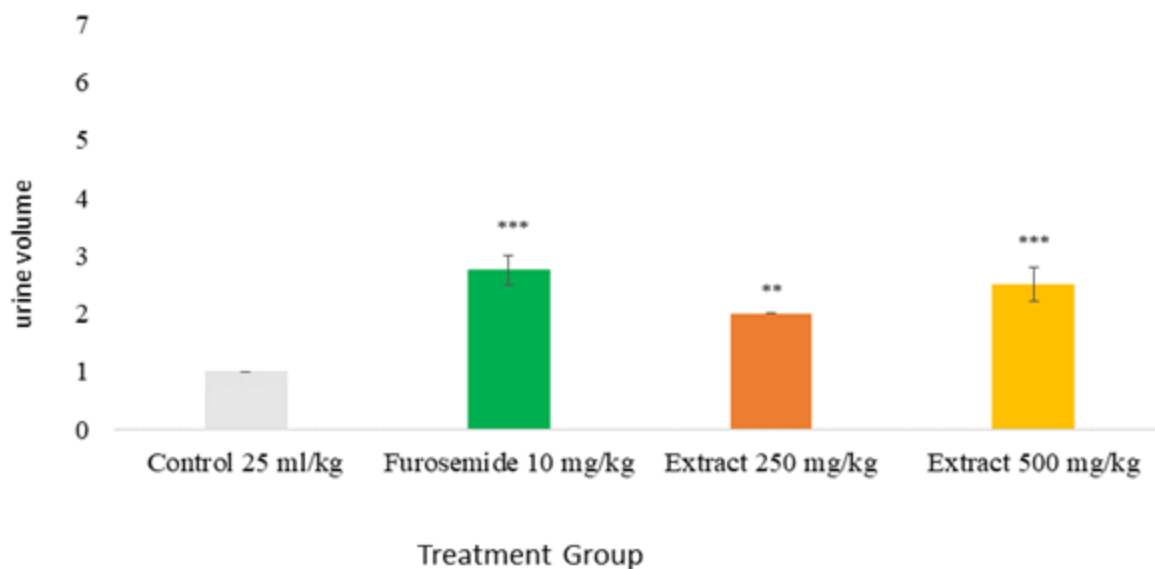


Figure 5

Effect of furosemide and PRFEE on urine volume in albino mice.

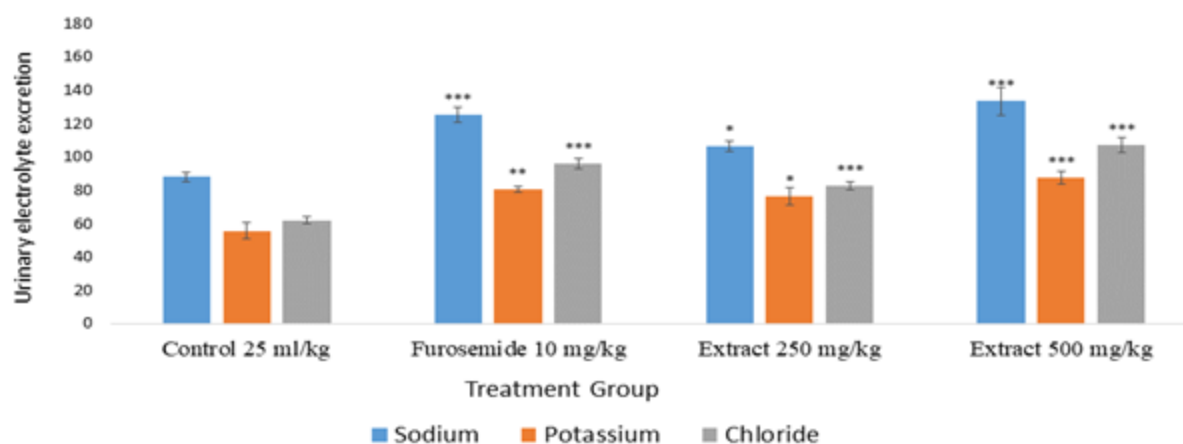


Figure 6

Effect of PRFEE on urinary sodium, potassium and chloride (mmol/L) ions concentration in albino mice.

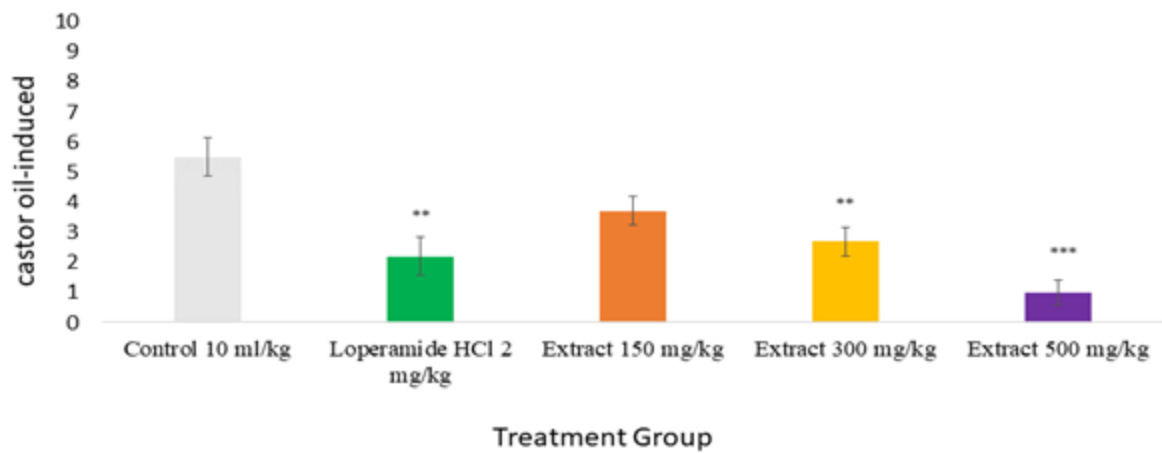


Figure 7

Antidiarrheal activity of the PRFEE.



Figure 8

Anti-microbial activity in (Hole-Plate) disk diffusion