ORIGINAL ARTICLE

In-vitro anti-bacterial activity of medicinal plants against Urinary Tract Infection (UTI) causing bacteria along with their synergistic effects with commercially available antibiotics

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Abstract

Background: Plants contain a variety of bioactive compounds that provide them antimicrobial properties, which can be used to develop novel antibiotics. The current research evaluated the antibacterial activity of 6 medicinal plants *Sphagneticola calendulacea* (Chinese wedelia), *Enydra fluctuans* (Buffalo spinach), *Chenopodium album* (Goosefoot), *Mentha arvensis* (Wild mint), *Mimosa diplotricha* (Nila grass), *and Averrhoa bilimbi* (Cucumber tree) against Urinary Tract Infection (UTI)- causing pathogens (*Staphylococcus spp*., *Proteus spp*., *Pseudmonas spp*., *Escherichia coli* and *Enterobacter spp*.).

Methods: The bacterial contamination of these plants was evaluated by using their surface-washed water. The combined effects of commercially available antibiotics along with these medicinal plants were also tested. We used the solvent extraction method, conventional cell culture technique, minimum inhibitory concentration (MIC) assay, and disc diffusion method for our analysis.

Results: The surface-washed water was contaminated with variable bacteria. The plants displayed notable antibacterial activity against most of the tested bacteria. Ethanol and hot water extract of plants exhibited minimum inhibitory effects, while the methanol extract of plants showed very potent antibacterial activity against most of the bacteria with inhibitory zone diameter up to 14 mm. In the case of combined effects, the zone diameter increased up to 26 mm, which is a significant improvement compared to the individual plant extracts.

Interpretation: This data suggested that the combination of two antibacterial agents, one natural and the other synthetic, would be more efficient in the treatment of multidrug-resistant bacteria than a single monotherapy of either of the antibacterial agents.

Keywords: Medicinal Plants, Antibacterial activity, UTI causing-pathogens, Synergistic effects
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1. Introduction

Antibiotic resistance is currently a major global health concern. Multidrug-resistant bacteria are emerging and spreading rapidly, threatening our ability to treat common infectious diseases. Indiscriminate use of commercial antibiotics is considered to be one of the main reasons for drug-resistance. According to the World Health Organization (WHO), drug-resistant diseases could cause 10 million deaths each year by 2050 and damage the global economy significantly [1]. Thus, it is very crucial to identify products with antimicrobial properties that could be utilized to develop novel and efficient antibiotics.

Plants have been used since ancient times to treat diseases and the practice is continued till today. It is estimated that about 80% of the population from developing countries uses medicines derived from plant species [2]. As plant-derived medicines are safer than synthetic alternatives [3], their usage will continue to grow in the future. It is expected that by the year 2050, the value of international trade of medicinal plants and their products would reach USD 5 trillion [4]. Plants produce a variety of compounds that are not used in their primary metabolism but increase their ability to survive environmental

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challenges. These secondary metabolites (e.g., alkaloids, phenols, terpenes, etc) are bioactive and provide defense against herbivores and microbes [5,6]. Different parts of plants such as the leaf, root, flower, fruit, peel, bark, seed, stem, rhizome, etc can be used for therapeutic purposes as they contain a high amount of bioactive compounds with therapeutic potential [7].

Bangladesh is a tropical country with a rich variety of medicinal plants. It is estimated that there are about 5000 plant species, of which around 1500 species are expected to have therapeutic benefits [8]. Over the last decade, numerous studies have been conducted in Bangladesh to analyze the antibacterial properties of eclectic plants like *Azadirachta indica* (Neem) [9–11], *Rosa kordesii* [12], *Hibiscus rosa-sinensis* (China rose) [13,14], *Curcuma longa* (Turmeric) [14,15], *Zingiber officinale* (Ginger) [15,16], *Allium sativum* (Garlic) [15], *Piper betle* (Betel leaf) [17,18], *Launaea sarmentosa* [19], *Ficus racemosa* (Fig) [20], *Nigella sativa* (Black cumin) [18], *Cinnamomum cassia* (Chinese cinnamon) [21], *Ocimum tenuiflorum* (Tulsi) [10], *Melastoma malabathricum* [22], *Polygonum hydropiper* L. (Water pepper) [23], *Ipomoea mauritiana* (Morning glory) [24], *Eclipta prostrata* [11,22], *Tagetes minuta* (Marigold) [9,14] and so on. A more diverse range of medicinal plants needs to be analyzed extensively to enhance our knowledge further. Thus, we designed this study to investigate the antimicrobial traits of plants that are not widely studied in Bangladesh. We chose *Sphagneticola calendulacea* (Chinese Wedelia), *Enydra fluctuans* (Buffalo Spinach), *Chenopodium album* (Goosefoot), *Mentha arvensis* (Wild mint), *Mimosa diplotricha* (Nila grass), *and Averrhoa bilimbi* (Cucumber tree). These plants were collected from different areas in Bangladesh and their microbial loadings and antibacterial activity were evaluated. We tested these plants, individually and in combination with commercially available antibiotics, against pathogens responsible for Urinary Tract Infection (UTI), which is a very common infectious disease in Bangladesh.

2. Materials and methods

2.1. Collection of plant samples

A total of 6 medicinal plant samples, commonly known as Chinese Wedelia, Buffalo Spinach, Goosefoot, Wild mint, Nila grass, and Cucumber tree, were collected from different areas of Bangladesh.

2.2. Determination of microbial contamination of the medicinal plants

The external areas of all the plant samples were washed with double distilled water (DDW) to be able to determine the types of bacteria that the plant samples could be contaminated with. The surface-washed water was inoculated onto different selective and non-selective media to evaluate specific microbes. For the enumeration of total viable bacteria (TVB) and total fungal load, 0.1 ml of each sample was spread into nutrient agar and sabouraud dextrose agar (SDA) media by spread plate technique. The plates were incubated at 37°C for 24 hours for a total viable bacterial count and at 25° C for 48 hours for a total fungal count [25]. For the detection of *Klebsiella* spp., 0.1 ml of each sample was spread onto MacConkey agar and then incubated at 37°C for 24 hours. To confirm the survival of *Escherichia coli*, 0.1 ml of each sample was spread over the eosin-methylene Blue (EMB) agar and incubated at 37°C for 24 hours. The presence of *E. coli* was confirmed by the appearance of bluish-black colonies with a green metallic sheen on the EMB agar [25]. For the detection of *Staphylococcus* spp., *Pseudomonas* spp., and *Bacillus* spp., 0.1 ml of each sample was spread onto mannitol salt agar (MSA), *Pseudomonas* agar (PA), and starch agar (SA) and incubated at 37°C for 24 hours. MSA, PA, and SA are selective media to identify *Staphylococcus* spp., *Pseudomonas* spp., *and Bacillus* spp. consecutively.

2.3. Pathogenic bacterial species used in this study

Previously isolated clinical pathogenic strains from a urinary tract infected (UTI) patient were collected from the department of Microbiology Culture lab of Brahmanbaria Medical College, Bangladesh [26]. Five bacterial isolates, including *Staphylococcus* spp., *Proteus* spp., *Pseudomonas* spp., *Escherichia coli*, and *Enterobacter* spp. were collected from the previous stock culture and sub-cultured on the selective media for the corresponding bacteria [25]. We followed routine precautions while dealing with pathogenic bacteria. For instance, decontamination of work surfaces, washing hands with soap/detergent after handling the samples, wearing lab coats only in the work area, keeping biohazardous waste in a covered container, etc [27].

2.4. Preparation of bacterial suspension

Loop full culture of each isolate from the stock culture was suspended into the LB broth and incubated at 37°C for 16-18 hours. After incubation, the bacterial growth was observed by measuring the optical density through the spectrophotometer, which was adjusted at 0.5 OD_{600} nm [28,29].

2.5. Processing of medicinal plant extracts

The whole part of the washed plants was used to make the extracts for this study. The samples were dried thoroughly and pestled to form smooth powder by a grinder. Afterward, all the powder samples were stored in airtight containers. The ethanolic, methanolic, and hot water extracts of all seven medicinal plants were prepared by dissolving 10 g of fine powder of each

TABLE 1. Microbial count of surface-washed water of medicinal plants (CFU/ml)

Sample	TVB	Fungi	*E.coli.*	*Klebsiella* spp.	*Staphylococcus* spp.	*Bacillus* spp.	*Pseudomonas* spp.
Sphagneticola calendulacea	2.64×10^5	3.2×10^4	0	2.0×10^4	1.8×10^5	1.5×10^4	1.7×10^4
Enhydra fluctuans	3.0×10^5	3.8×10^4	1.5×10^4	3.3×10^4	1.81×10^5	1.4×10^4	1.9×10^4
Chenopodium album	2.84×10^5	4.2×10^4	0	1.8×10^4	1.84×10^5	2.1×10^4	1.9×10^4
Averrhoa bilimbi	2.08×10^5	2.5×10^4	0	0	1.58×10^5	1.1×10^4	1.4×10^4
Mentha arvensis	2.7×10^5	2.9×10^4	1.0×10^4	2.6×10^4	1.75×10^5	1.6×10^4	1.4×10^4
Mimosa diplotricha	2.36×10^5	3.0×10^4	0	0	1.76×10^5	1.2×10^4	1.8×10^4

The experiments were conducted three times independently, and the results were found to be reproducible. TVB is Total viable bacteria.

plant in 50 ml of ethanol, methanol, and hot water (distilled water) respectively. The contents were kept in a rotary shaker for 48 h. Then the extracts were filtered and dried in a hot air oven at 40°C. Then the extracts were refrigerated at 4°C for further studies [12].

2.6. Assay of antimicrobial activity using agar well diffusion method

20 ml of sterilized Muller Hinton Agar was poured into sterile Petri dishes. After solidification, the fresh culture of microorganisms such as *E.coli, Klebsiella* spp., *Staphylococcus* spp., *Bacillus* spp., and *Pseudomonas* spp. were swabbed on the respective plates. Wells were punched on the agar plates by using a sterile borer. About 500 µl of each plant extract (ethanol, methanol, and hot water) was added to the wells. Gentamicin was used as a positive control. The plates were incubated for 24 hours at 37°C. After incubation, the diameter of inhibitory zones (in mm) formed around each well was measured [12].

2.7. Determination of Minimal Inhibitory Concentration (MIC)

To verify the antibacterial activity of the medicinal plants, we implemented the two-fold broth micro-dilution assay to determine the potent minimal concentration of the medicinal plants that can inhibit microbial growth [30]. An aliquot of 100 µl of the overnight (~12 hours) culture of each of the tested bacteria was inoculated into the appropriately labeled sterile tubes containing Mueller-Hinton (MH) broth (Oxoid Ltd, England). The turbidity was adjusted with 0.5 McFarland standard and the different concentrations (32 µL, 64 µL, 128 µL, 256 µL, 512 µL, 1024 µL, and 2048 µL) of medicinal extracts were introduced afterward. All the tubes were incubated at 37°C for 24 hours. The least concentration of sample which could retard the multiplication of the tested bacteria was recorded as the MIC.

2.8. Determination of the efficacy of antibiotics against the bacteria

The potency of the commercially available synthetic drugs was examined against the same clinically isolated pathogens. Five broad-spectrum antibiotics (commonly prescribed by physicians in Bangladesh) such as Streptomycin(30 µg), Gentamicin (30 µg), Methicillin (10µg), and Erythromycin (15 µg) were selected to use in this experiment by following the amount used in our previous work [31]. The loop-full inoculum from the stock culture was evenly smeared onto Mueller-Hinton agar (Difco, Detroit, MI). Then the antibiotic discs were employed onto the surface of the bacterial lawn through the disc-diffusion method (Kirby Bauer technique) [32]. The plates were then inverted and incubated at 37°C for 24 hours. After incubation, the zone of inhibition was measured and compared with the standard chart to ensure the drug sensitivity or resistance array [14].

2.9. Combined effects of antibiotics and medicinal plant extracts

We tried to evaluate the combined antibacterial effects of both synthetic and natural sources against the same bacterial strains. All five pathogens were cultured separately on Muller-Hinton agar plates. 500 µL of each of the medicinal plant extracts (methanolic) was added to five antibiotics discs such as Streptomycin (Str), Gentamicin (Gen), Methicillin (Meth), Azithromycin (Azi), and Erythromycin (Ery) separately. The plates were incubated at 37°C for 12-16 hours and the inhibitory zone diameters were determined afterward [33].

2.10. Statistical analysis

All the data were statistically analyzed by determining the p-value through the t-test and the significant level was considered as <0.1. The GraphPad Prism (San Diego, CA) was customized to calculate the one-way analysis of variance (one-way ANOVA)

3. Results

3.1. Microbial loading in surface-washed water of medicinal plants

All 6 plants were contaminated with variable microorganisms, as verified from the surface-washed water samples. While the

bacteria count was in the range of 10^5-10^4 CFU/ml, the fungi loading was on average around 10^4 CFU/ml (Table 1). We identified *Staphylococcus* spp., *Bacillus* spp., and *Pseudomonas* spp. in all the cases. In contrast, *Klebsiella* spp. was present in 4 out of 6 plants and *E. coli* was present in 2 out of 6 plants. This shows the importance of decontaminating the plants thoroughly before using them for any medicinal purpose.

3.2. Antibacterial activity of medicinal plant extracts against the UTI causing bacteria

The antimicrobial activity of methanol, ethanol, and hot water extracts of the plants was investigated using Agar well diffusion method. All the plant extracts showed a varying degree of antimicrobial activity against the selected pathogens with an average inhibitory zone diameter in the range of 4-14 mm [(Figs. 1–6), (Supplemental information)].

The three extracts of *Sphagneticola calendulacea* (Chinese Wedelia) showed antibacterial activity against all the tested pathogens, with methanol extract displaying the best activity (Fig. 1). The methanol extract of Chinese Wedelia showed the highest average zone diameters against *Staphylococcus* spp. (13 mm) and *Pseudomonas* spp. (14 mm). Similar to Chinese Wedelia, all three extracts of *Mentha arvensis* (Wild mint) displayed full activity, with methanol extract resulting in the highest average zone diameter against *Staphylococcus* spp. (14 mm) (Fig. 4).

The ethanol and water extracts of *Enydra fluctuans* (Buffalo Spinach) and *Mimosa diplotricha* (Nila grass) were active against all the pathogens except *Enterobacter* spp. (Figs. 2 and 5). Only the methanol extract of Nila grass worked against *Enterobacter* spp. (Fig. 5). While for *E. coli* both methanol and ethanol extracts of Buffalo Spinach showed similar zones of diameter, methanol extract excelled over the other extracts against *Proteus* spp., *Staphylococcus* spp., and *Pseudomonas* spp. (Fig. 2). The methanol extract of Nila grass showed the highest zone diameter (13 mm) against *Pseudomonas* spp. (Fig. 5).

For *Chenopodium album* (Goosefoot) only the methanol extract showed antibacterial efficacy against all the pathogens (Fig. 3). The best activities were noted against *Staphylococcus* spp., *Pseudomonas* spp., and *Enterobacter* spp. with an average zone diameter around 12-13 mm (Fig. 3). Lastly, for *Averrhoa bilimbi* (Cucumber tree), the ethanol extract worked against all the pathogens while the methanol extract showed the best activity against *Proteus* spp. and *Pseudomonas* spp. (Fig. 6).

Overall, the methanol extracts of all 6 plants have the efficacy to fight *E. coli*, *Proteus* spp., and *Pseudomonas* spp. The

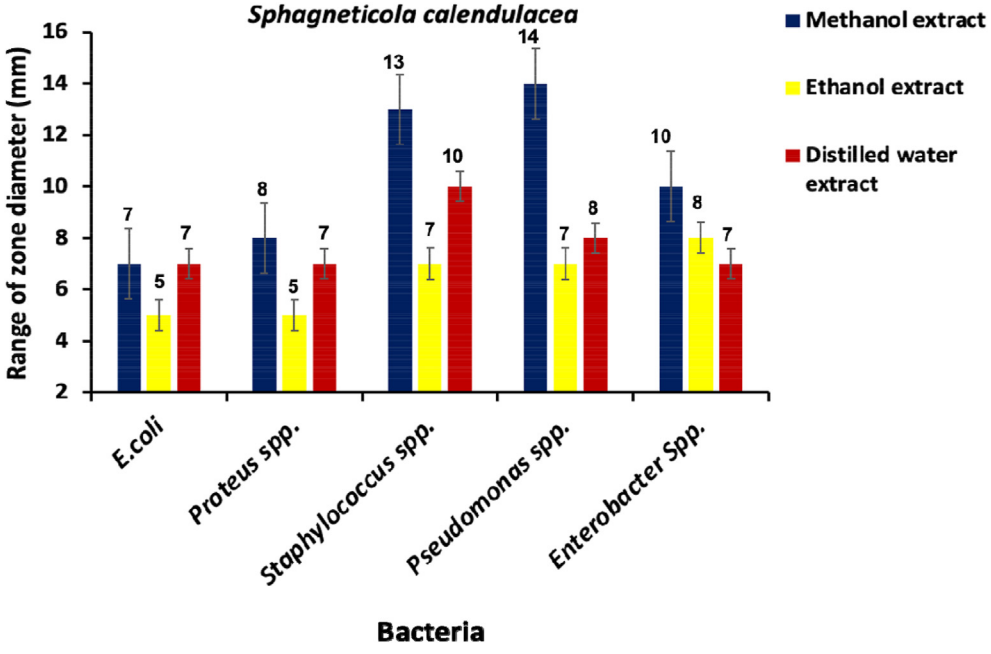

FIG. 1. Antibacterial activity of *Sphagneticola calendulacea* (Chinese wedelia) against UTI-causing pathogens: the figure indicates the bacterial growth retardation ability of the plant extracts (methanol, ethanol and distilled water), the blue bar presenting the effectivity of methanol extract, the yellow bar showing the activity of ethanol extract and the red bar displaying the activity of distilled water extract. According to the the graph the maximum antibacterial activity was recorted for methanol extract especially the uppermost zone diameter was estimated against the growth of *Staphylococcus* spp. and *Pseudomnas* spp. The ethanol extract showed relatively lower activity than methanol and distilled water extarcts. As a positive control gentamycin was used. All the experiments were performed three times.

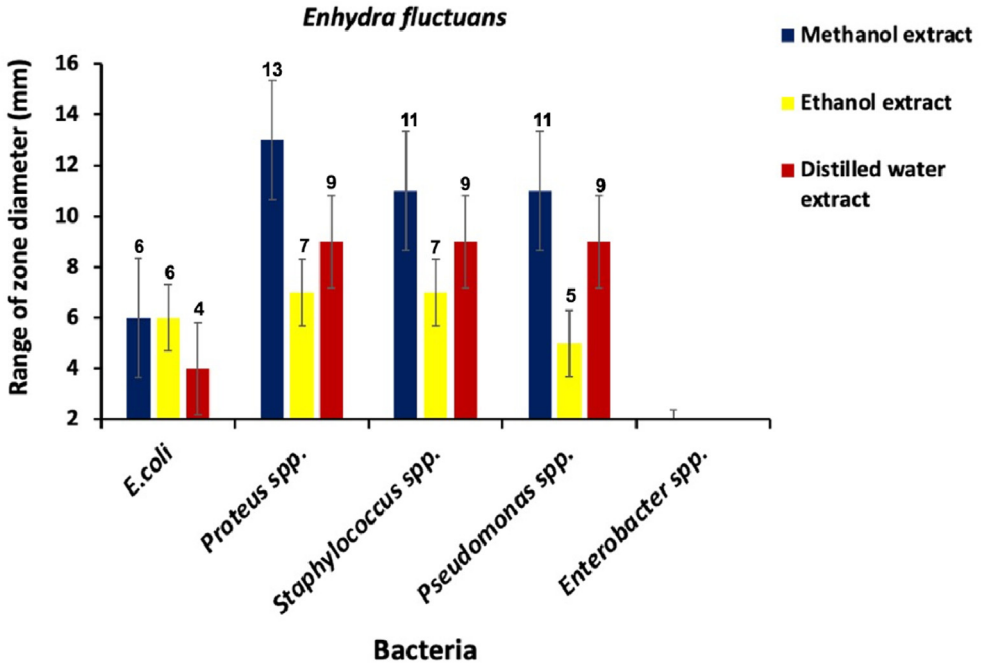

FIG. 2. Antibacterial activity of *Enydra fluctuans* (Buffalo spinach) against UTI-causing pathogens: the figure indicates the bacterial growth retardation ability of the plant extracts (methanol, ethanol and distilled water), the blue bar presenting the effectivity of methanol extract, the yellow bar showing the activity of ethanol extract and the red bar displaying the activity of distilled water extract. According to the the graph the maximum zone diameter was recorted for methanol extract especially against the *Proteus* spp. *Staphylococcus* spp. and *Pseudomnas* spp. while in case of distilled water extract the zone diameter was almost same against the growth of *Proteus* spp. *Staphylococcus* spp. and *Pseudomnas* spp. Against the Enterobacter growth, no activity was found by the extracts of *Enydra fluctuans*. As a positive control gentamycin was used. All the experiments were performed three times.

better efficacy of the methanol extracts of the plants could be caused by the type of bioactive compounds that is present as well as their extent. While studying the rhizomes of the plant Heliconia rostrate, Moonmun and colleagues [34] determined that all phytoconstituents detected in the methanol extract were found in the ethanol extract, except for the glycoside content which was found only in the methanol extract. Total phenolic and flavonoid content was higher in the ethanol extract compared to the methanol extract, whereas total tannin content was higher in the methanol extract [34].

It is worth mentioning that the antibacterial activity of the plants comes from the bioactive compounds, released as a result of the extraction of the plants in specific solvents. The plant surface itself does not have any antibacterial activity, so it is possible for the surface to be contaminated with bacteria.

In this study, Gentamicin was used as a positive control with a zone diameter of 12mm, 13mm, 10mm, 11mm & 14mm against *E. coli*, *Proteus* spp., *Pseudomonas* spp., *Enterobacter* spp., and *Staphylococcus* spp. consecutively. However, the data was not included with the findings of methanol, ethanol, and hot water extraction.

3.3. Minimal Inhibitory Concentration (MIC) of medicinal plants

The antibacterial activity of the medicinal plants was further assessed by evaluating the value of Minimal Inhibitory Concentration (MIC), which is the lowest concentration of a drug/chemical required to prevent the visible growth of bacteria. The MIC values for the medicinal plants were recorded within the range of 1-30 mg/ml (Table 2). The lowest MIC values (1-6 mg/ml) were determined with *Chenopodium album* (Goosefoot) for all the pathogens. In contrast, *Mentha arvensis* (Wild mint) displayed the highest MIC values (18-30 mg/ml) for all the pathogens. Thus, in terms of efficiency, Goosefoot seems to be a better antimicrobial agent compared to the rest of the plants.

Interestingly, although *Chenopodium album* (Goosefoot) and *Sphagneticola calendulacea* (Chinese Wedelia) had comparable antibacterial activity as indicated by the agar well diffusion method, the *Chenopodium album* had a lower MIC value than *Sphagneticola calendulacea* (Table 2). While the agar well diffusion method confirms the presence of antibacterial activity, it might not provide the most precise extent of antibacterial activity as the evaluation method is qualitative [35]. In

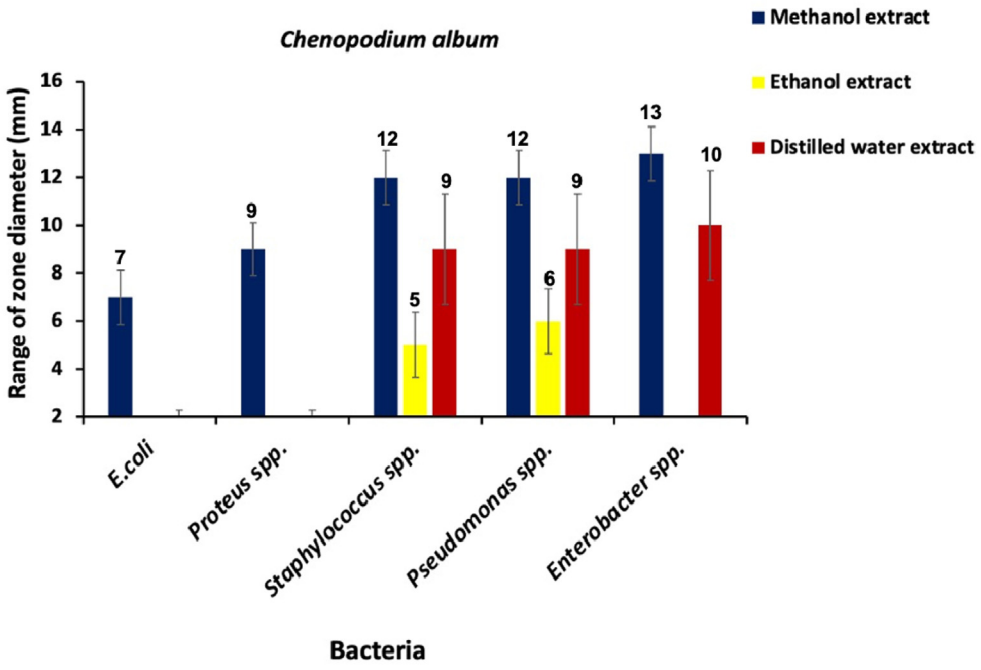

FIG. 3. Antibacterial activity of *Chenopodium album* (Goosefoot) against UTI-causing pathogens: the figure indicates the bacterial growth retardation ability of the plant extracts (methanol, ethanol and distilled water), the blue bar presenting the effectivity of methanol extract, the yellow bar showing the activity of ethanol extract and the red bar displaying the activity of distilled water extract. According to the the graph the methanol extract was able to show the antibacterial activity against all the bacteria while the distilled water extract exhibited the bacteria inhibiting ability against *Staphylococcus* spp. *Pseudomnas* spp. and *Enterobacter* spp. The ethanol extract showed antibacterial activity against *Staphylococcus* spp. and *Pseudomnas* spp. As a positive control gentamycin was used. All the experiments were performed three times.

comparison, as the determination of MIC is a quantitative method, it provides a more precise estimation of the extent of the antibacterial activity in different components.

3.4. Individual activity of antibiotics and their synergistic effect with medicinal plants

5 of the commercially available antibiotics were tested against the pathogens. The inhibitory zone diameters varied between 0-15 mm (Table 3). Streptomycin (30 µg), Gentamicin (30 µg), and Azithromycin (30 µg) were effective against all the pathogens. Methicillin (10 µg) and Erythromycin (15 µg) were completely ineffective against *E. coli*, *Proteus* Spp. and *Pseudomonas* spp., but were able to prevent the growth of *Staphylococcus* spp, exhibiting zone diameters of 15 mm (Table 3). The inefficacy of Methicilin and Erythromycin against *E. coli*, *Proteus* spp. and *Pseudomonas* spp. could be a result of these bacteria developing drug-resistance against these antibiotics. These bacteria could be producing hydrolyzing enzymes to cleave these antibiotics [35].

Later we combined each of the antibiotics with all 6 medicinal plants. In all combinations, the zone diameters increased and were recorded in the range of 20-26 mm (Fig. 7), indicating a significant improvement in antibacterial activity. When Methicillin (10 µg) and Erythromycin (15 µg) were combined with the plants, they became highly active against *E. coli*, *Proteus* spp. and *Pseudomonas* spp., which is a remarkable achievement.

4. Discussion

The emergence of different pathogenic microorganisms is currently a severe threat all over the world along with the existing hazard of drug-resistant microorganisms, which are resistant to traditional antibiotics. Despite the rapid succession of modern science, the spread of drug-resistant microorganisms has been expanding day by day due to the infirm medication policy and insufficient development of novel therapeutic agents. To overcome this situation, experts from different fields such as physician, pharmacist, microbiologist, molecular biologist, and phytochemists are working together to sort out potential candidates with high antibacterial activity from natural resources that could be effective against the pathogenic microorganisms.

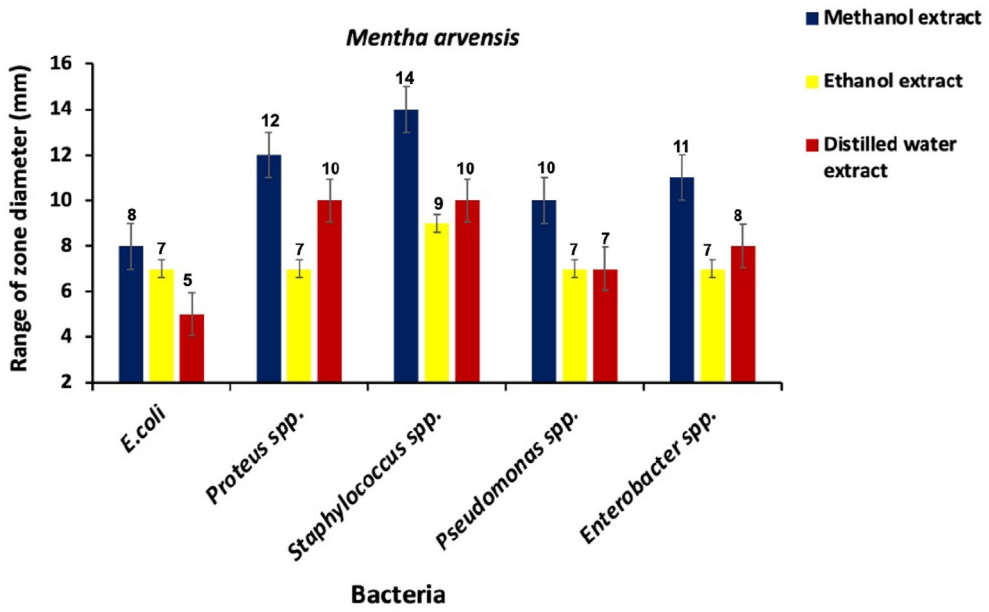

FIG. 4. Antibacterial activity of *Mentha arvensis* (Wild mint) against UTI-causing pathogens: the figure indicates the bacterial growth retardation ability of the plant extracts (methanol, ethanol and distilled water), the blue bar presenting the effectivity of methanol extract, the yellow bar showing the activity of ethanol extract and the red bar displaying the activity of distilled water extract. According to the the graph, the antibacterial activity was found for methanol, ethanol and distilled water extract of plant against all the UTI causing bacteria. Among the three extraction, the maximum antibacterial activity was recorted for methanol extract especially the uppermost zone diameter was estimated against the growth of *Staphylococcus* spp. As a positive control gentamycin was used. All the experiments were performed three times.

The findings from our study unveiled that some commonly available, but not widely studied, medicinal plants in Bangladesh are highly effective against the pathogenic bacteria isolated from the patients suffering from Urinary Tract Infection (UTI), which is one of the most common diseases not only in Bangladesh but also in other developing and developed countries. We studied the effect of three different plant extracts for all the plants we studied: *Sphagneticola calendulacea* (Chinese Wedelia), *Enydra fluctuans* (Buffalo Spinach), *Chenopodium album* (Goosefoot), *Mentha arvensis* (Wild mint), *Mimosa diplotricha* (Nila grass), *and Averrhoa bilimbi* (Cucumber tree). While the ethanol and hot water extracts of these plants showed minimum antibacterial activity, the methanol extracts in general showed the highest antibacterial activity against the UTI-causing pathogens. This suggests that the specific bioactive compounds present in the methanol extracts have strong efficacy against the UTI- causing pathogens. *Chenopodium album* (Goosefoot), displayed the lowest MIC (in the range of 1-6 mg/ml) against *E. coli, Proteus* spp., *Pseudomonas* spp., *Staphylococcus* spp., and *Enterobacter* spp., demonstrating high potential to be developed into a novel antibiotic.

The most important aspect of this study was to evaluate the combined effect of medicinal plants and commercial antibiotics on UTI-causing pathogens. The combined drug action displayed a 2-3 times larger zone diameter, as recorded in the range of 20-26 mm in comparison to the individual drug effects. After combining previously ineffective antibiotics Methicillin and Erythromycin with the plant extracts, the antibacterial activity against *E. coli*, *Proteus* spp., and *Pseudomonas* spp., was significantly increased. This synergistic effect is complementary to the work done by Hossan and colleagues [21], which showed that combining *Cinnamomum cassia* with vancomycin decreases its resistance to Methicillin-resistant *Staphylococcus aureus* (MRSA). Therefore, combining medicinal plants with commercially available antibiotics is a great way to address the global issue of drug-resistant bacteria.

Due to limited research funding, we were unable to purify and identify the specific bioactive compounds in the methanol extracts of the plants. Utilization of high-pressure liquid chromatography (HPLC) along with nuclear magnetic resonance (NMR) would be highly beneficial in this regard. However, the application of these advanced methods would be suggestive for the others researchers for the development of novel antibiotics.

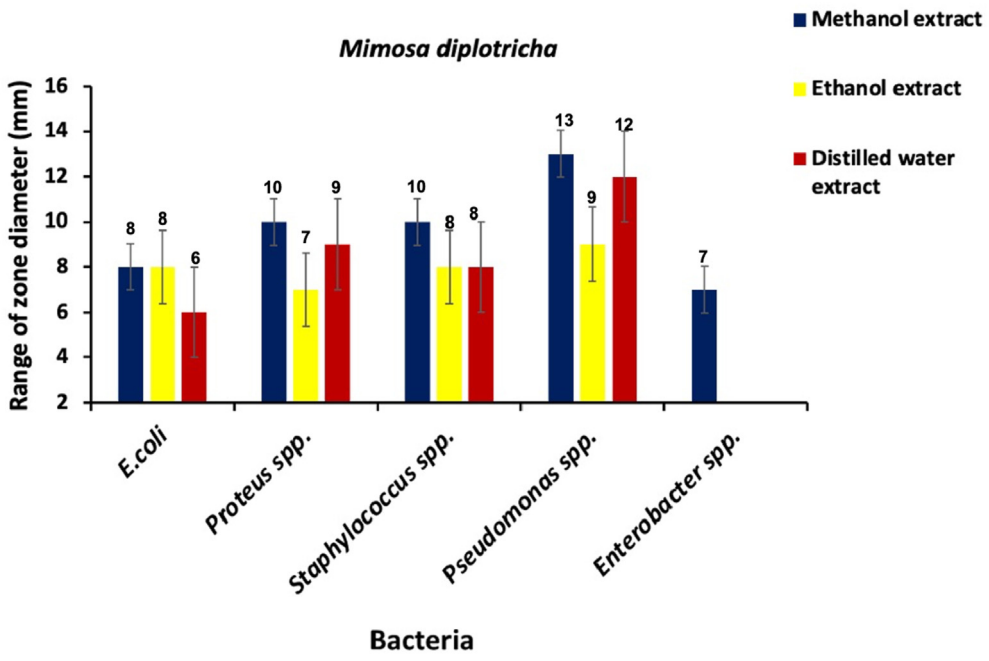

FIG. 5. Antibacterial activity of *Mimosa diplotricha* (Nila grass) against UTI-causing pathogens: the figure indicates the bacterial growth retardation ability of the plant extracts (methanol, ethanol and distilled water), the blue bar presenting the effectivity of methanol extract, the yellow bar showing the activity of ethanol extract and the red bar displaying the activity of distilled water extract. According to the the graph, the ethanol and distilled water extract couldn't inhibit the growth of *Enterobacter* spp. In this case the methanol extract was the most potent extract against the growth of all bacteria. As a positive control gentamycin was used. All the experiments were performed three times.

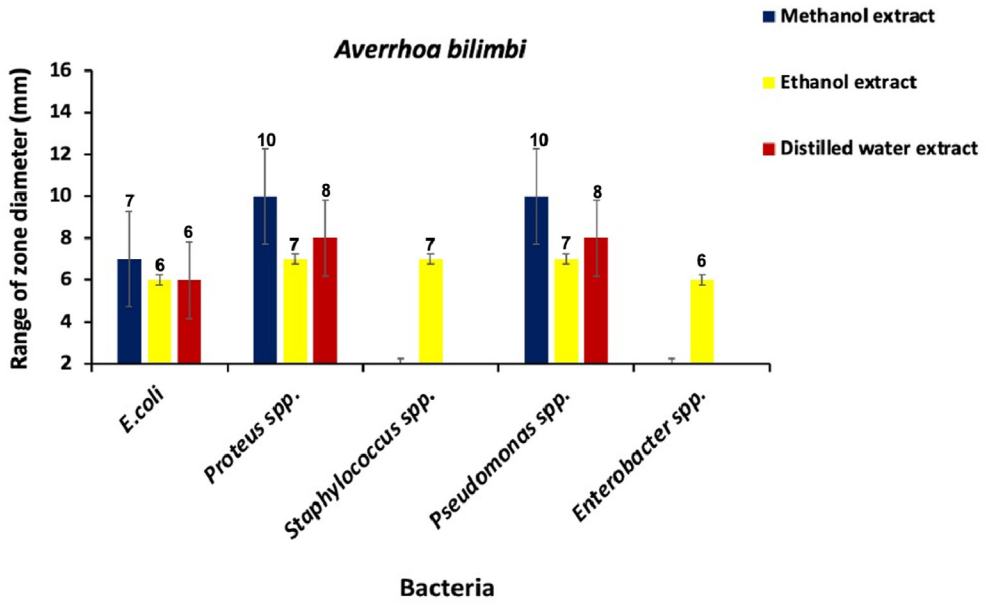

FIG. 6. Antibacterial activity of *Averrhoa bilimbi* (Cucumber tree) against UTI-causing pathogens: the figure indicates the bacterial growth retardation ability of the plant extracts (methanol, ethanol and distilled water), the blue bar presenting the effectivity of methanol extract, the yellow bar showing the activity of ethanol extract and the red bar displaying the activity of distilled water extract. In this case, the ethanol extract of the plant showed antibacterial activity against all the bacteria wherease the methanol and distilled water extract couldn't produce any zone against *Staphylococcus* spp. and *Enterobacter* spp. As a positive control gentamycin was used. All the experiments were performed three times.

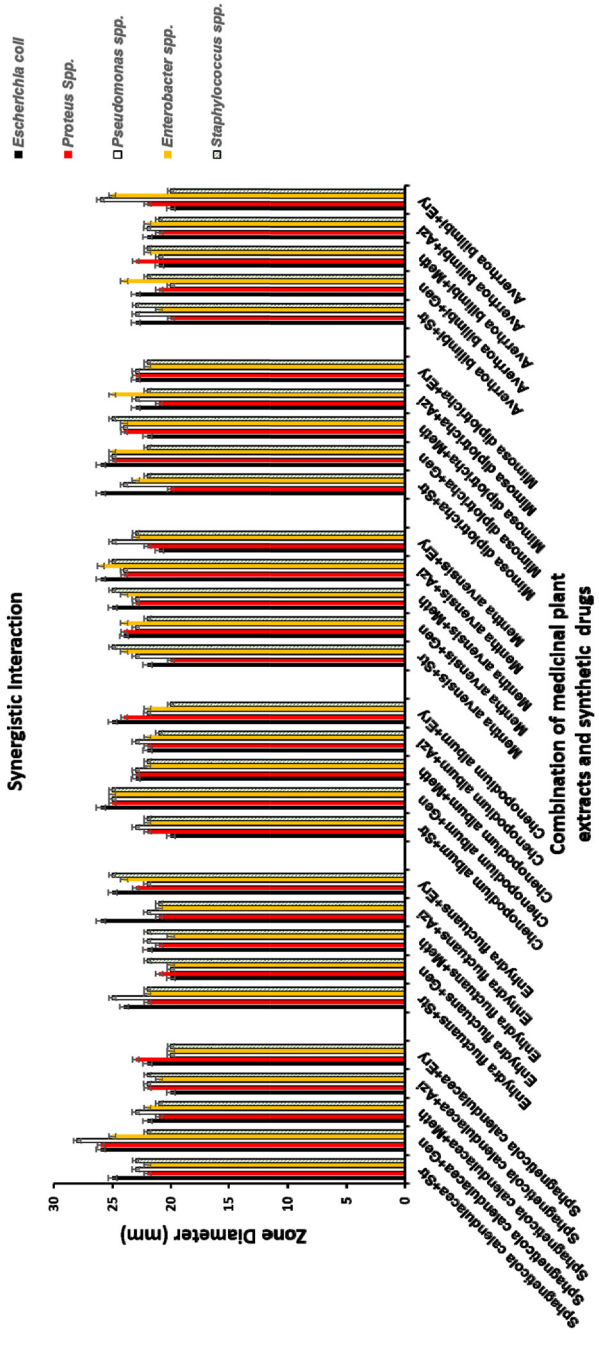

FIG. 7. Synergestic effects of medicinal plant extracts and synthetic drugs against UTI-causing pathogens: in the figure the black bar indicates the *E. coli*, red bar for *Proteus* spp., white bar for *Pseudomonas* spp., yellow bar for *Enterobacter* spp. and *geometric* pattern represents *Staphylococcus* spp. The combination of Six medicinal plants extracts and five antibitics showed significant range of zone diameter against the five bacteria isolated from UTI patients. The estimated zone diameter was 2-3 times higher thant the individual effects of drugs and plants extracts. All the experiments were performed three times.

TABLE 2. **Minimum Inhibitory Concentration (MIC) of the plant samples against UTI-causing pathogens**

	Minimum Inhibitory Concentration (MIC) (mg/ml)				
Sample	***E. coli***	***Proteus* spp.**	***Pseudomonas* spp.**	***Staphylococcus* spp.**	***Enterobacter* spp.**
Sphagneticola calendulacea	20	20	25	20	20
Enhydra fluctuans	7	3	7	7	7
Chenopodium album	3	1	6	3	3
Mentha arvensis	5	7	7	7	7
Mimosa diplotricha	18	30	30	18	30
Averrhoa bilimbi	10	10	10	10	10

The experiments were conducted three times independently, and the results were found to be reproducible.

TABLE 3. **Antibiotic susceptibilities of the UTI-causing pathogens**

	Zone of inhibition (mm)				
Name of Antibiotics	***E. coli***	***Proteus* spp.**	***Pseudomonas* spp.**	***Enterobacter* spp.**	***Staphylococcus* spp.**
Streptomycin (30μg)	11	13	13	14	14
Gentamycin (30μg)	12	13	10	11	14
Methicillin (10μg)	0	0	0	0	15
Azithromycin (15μg)	14	14	13	14	15
Erythromycin (15μg)	0	0	0	14	15

The experiments were conducted three times independently, and the results were found to be reproducible.

5. Conclusions

This study is an important contribution to addressing the global crisis of antibiotic or drug-resistance. We tested the antibacterial activity of 6 medicinal plants that are available in Bangladesh against Urinary Tract Infection (UTI) causing bacteria. These plants are commonly known as Chinese Wedelia, Buffalo Spinach, Goosefoot, Wild mint, Nila grass, and Cucumber tree. All the plants showed activity against the pathogens. The methanol extracts of the plants were more effective than the ethanol and water extracts. When these medicinal plants were combined with commercially available antibiotics, their efficiency improved significantly. This provides an immediate solution to improve the currently used antibiotics with the help of naturally available antibacterial agents.

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Declaration of competing interest

Authors have no conflict of interest.

CRediT authorship contribution statement

Mrityunjoy Acharjee: Conceptualization, Methodology, performed experiment, Software, Writing – original draft, Supervision. **Nagma Zerin:** Data curation, Writing – review & editing. **Touhida Ishma:** Data curation. **Md. Rayhan Mahmud:** Writing – review & editing.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.nmni.2022.101076.

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