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Investigation for phytochemical composition, antioxidant activities and antibacterial properties of *Achyranthes aspera* L.

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Abstract

Plants and plant-based medicines form the foundation of many modern pharmaceuticals used to treat various ailments. Thus, this study aimed to investigate the phytochemical, antioxidant, and antibacterial properties of *A. aspera* leaves. Phytochemical screening revealed the presence of alkaloids, flavonoids, terpenoids, glycosides, carbohydrates, coumarins, and steroids in both ethanol and methanol extracts. However, cardiac glycosides and phenols were exclusively found in the ethanol extract. Methanol leaf extracts exhibited the highest levels of flavonoids (41.16 mg QE/gm) and phenols (9.99 mg GAE/gm), respectively. Significant antioxidant activity was observed with IC50 values of 237.83 µg/ml and 279.14 µg/ml for methanol and ethanol leaf extracts, respectively. The antibacterial efficacy of *A. aspera* leaf extracts was tested against *Bacillus* sp., *E. coli*, and *Enterobacter* sp. Methanolic extracts showed stronger antibacterial effects compared to ethanolic extracts, with the highest zone of inhibition (20.67 mm) against *Bacillus* sp. and the lowest MIC (75 mg/ml) and MBC (150 mg/ml) values. These findings suggest that *A. aspera* leaf extract could serve as a source of novel antioxidant and antibacterial compounds.

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Introduction

Therapeutic chemicals derived from natural sources have been used for thousands of years. Across the globe, people have traditionally employed a wide variety of medicinal plants to treat a range of ailments. Many of the chemicals found in these plants can be used to develop new drugs. These include secondary metabolites or phytochemicals such as tannins, terpenoids, alkaloids and flavonoids, which are abundant in plants. These compounds exhibit diverse bioactivities including antimutagenic, anticarcinogenic, antioxidant, antibacterial and anti-inflammatory properties (Yen *et al.*, 1993). The three main categories of plant secondary metabolites are terpenoids, alkaloids and polyphenols (phenolics). The antimicrobial activity of plants is largely attributed to these secondary metabolites (Keita *et al.*, 2022).

The use of medicinal plants particularly their phenolic constituents such as anthocyanins, flavonoids, tannins, stilbenes and phenolic acids as safe, effective and sustainable sources of natural antioxidants and free radical scavengers has long been recognized.

The primary natural dietary antioxidants include carotenoids, phenolic compounds (especially flavonoids) and vitamins C and E. According to (Aguoru *et al.,* 2015), nearly 80% of the global population now relies on plants for primary medical care.

Medicinal plants provide a valuable source of lead compounds for the development of new drugs with fewer side effects. However, in the latter part of the 19th century, the pharmaceutical industry began to grow rapidly, prompting researchers to begin developing and characterizing various drugs derived from plant sources (Ovais *et al.*, 2021).

Achyranthes aspera L., a member of the Amaranthaceae family, is currently being evaluated for its medicinal properties. In northern Bangladesh this widespread plant is considered both a weed and an invasive alien species (Akter and Zuberi, 2009). According to (Talreja and Tiwari, 2023), *A. aspera* commonly known as "Prickly Chaff Flower," holds significant ethno-pharmacological value. It is commonly found as a roadside weed.

A. aspera is a well-known medicinal plant traditionally used to treat a variety of ailments. The roots are employed as an astringent for treating stomachaches and wounds while leaf extracts have demonstrated antiperoxidative and thyroid-stimulating properties.

The plant exhibits a wide range of pharmacological effects including hepatoprotective, anti-allergic, cardiovascular, hypoglycemic, antidiabetic, analgesic, antiperiodic, antipyretic, purgative and laxative activities, making it effective in managing gastrointestinal issues and general body pain.

According to (Hai-Anh *et al.,* 2024), it contains high concentrations of essential phytochemicals such as phenolics, saponins, tannins, alkaloids, flavonoids, cardiac glycosides, steroids and phlobatannins.

Achyranthes aspera is traditionally used to treat a variety of conditions including fever, wound healing, toothache, arthritis, gynecological disorders, urinary issues, insect bites, snake bites, abdominal tumors and stomach pain (Ribeiro, 2021).

Given its rich phytochemical composition and wide range of medicinal uses, the present study aimed to investigate the phytochemical profile, antioxidant capacity, and antibacterial activity of *A. aspera* leaves. Medicinal plants with such potent phyto-constituents serve as valuable sources for the development of effective therapeutic agents.

Materials and methods

Samples

Fresh plant material was gathered from the Rajshahi University region to guarantee that the plants were disease-free and healthy. The University of Rajshahi, Rajshahi, Bangladesh's Plant Taxonomy Laboratory identified the plant.

Chemicals

Various tests were conducted using the following analytical-grade chemicals and reagents such as nitric acid (HNO_3), potassium nitrate, picric acid (2.4.6-trinitro phenol), mercuric chloride, potassium iodide (KI), iodine, tannic acid, ferric chloride, lead acetate, copper sulfate ($CuSO_4$), sodium hydroxide (NaOH), hydrochloric acid (HCl), sulphuric acid (H_2SO_4), alcoholic ferric chloride, glacial acetic acid, ninhydrin reagent, Molisch's reagent, gallic acid, Folin-ciocalteu reagent (FCR), sodium carbonate (Na_2CO_3), aluminium chloride ($AlCl_3$), methanol, ethanol, quercetin, potassium acetate ($C_2H_3KO_2$), DPPH (2,2-diphenyl-1- picrylhydrazyl) reagent.

Preparation of crude extracts

After collection of the leaves were thoroughly washed 3-4 times through running tap water and dried under shade with room temperature (26 ± 2 °C). The dried leaves were made into fine powder by using grinder machine and stored in airtight glass container. Then, leaf powder was mixed with methanol and ethanol solvents in a ratio of 1:10 (w/v) and shaken for 48 h by a shaker machine. After that the solution was filtered via cotton cloth and Whatman No. 1 filter paper. The solvent was evaporated at 35 ± 2 °C in a water bath. Finally, semisolid crude extract was produced within 5 days and transferred into airtight screw cap vials and stored at 4°C for further analysis.

Determination of phytochemicals

With few modifications, the conventional methods of (Balamurugan *et al.,* 2019) were used to perform a qualitative phytochemical analysis of *A. aspera* extracts.

Qualitative phytochemical analysis

Test for alkaloids

Each extract was separately dissolved in diluted hydrochloric acid and then filtered.

Wagner's test

Take 1 ml of extract and add 3-5 drops of Wagner's reagent and observe for the formation of reddish-brown precipitate indicate positive result.

Meyer's test

2 ml of extract and added 1 ml Mayer reagent, alkaloids give cream color precipitate with this reagent.

Dragendroff's test

2 ml of extract and added 1 ml Dragendroff reagent, alkaloids give reddish brown precipitate with this reagent.

Hager's test

2 ml of extract and added 1 ml Hager reagent, alkaloids give yellow color precipitate with this reagent.

Tannic acid test

2 ml extract and added 1 ml tannic acid, alkaloids give buff color precipitate with tannic acid.

Test for Flavonoids (alkaline reagent test)

2 ml of extract was treated with 3-5 drops of 1 ml 10% NaOH solution. Observe for the formation of intense yellow color, which becomes colorless on addition of 0.5 ml diluted HCl indicates the presence of flavonoids.

Test for Saponins (Foam test)

2 ml of extract was added 2 ml distilled water and shake 1 minute vigorously and incubated 5 minutes. Observe the formation of persistence foam that confirms the presence of saponins.

Test for Terpenoids (Salkowski's test)

2 ml of extract was mixed in 1 ml of chloroform and then 1.5 ml concentrated H_2SO_4 carefully added to form layer. The reddish-brown ring of the mixture was formed show positive result for the presence of terpenoids.

Test for Tannins (Braymen's test)

1 ml of extract was treated it with 1 ml of 10% alcoholic ferric chloride solution and observed for the formation of dark green or blue-black color.

Test for cardiac glycosides (keller-killani test)

1 ml extract was treated it with 1 ml of glacial acetic acid and 2-3 drops of 5% ferric chloride solution.

After that, add 1 ml of conc. H_2SO_4. Observe for a brown ring at the interface shows the presence of glycosides.

Test for Phenol (Lead acetate test)

2 ml extract was added 1 ml 10% lead acetate solution and observe for the bulky white precipitate.

Test for Coumarins

2 ml extract was added 3ml 10% NaOH and observed for the formation of yellow color for positive result.

Test for Xanthoprotein

2 ml of extract was added 2 ml nitric acid. This mixture needed Sometimes heat for formation of light-yellow color. Then added 1 ml 40% NaOH and observed for the formation of Dark yellow or orange color.

Test for Steroids

2 ml of extract was mixed in 1 ml of chloroform and concentrated 1ml H_2SO_4 was carefully added to form red and yellowish green color for confirming positive result.

Test for Proteins

1 ml extract was added 1 ml 40% NaOH. Then added few drops of 1% $CuSO_4$ for formation of violet or pink color that showed positive result for this test.

Test for Amino acids (Ninhydrin reagent)

2 ml of extract was mixed with 2 ml ninhydrin reagent and boil for few minutes. Then observed for the formation of purple/blue color for ensuring positive result.

Test for carbohydrates (Molisch's test)

1 ml extract was taken and added 3-5 drops of Molisch's reagent. In addition, added 1 ml H_2SO_4 in this mixture and to strand for 2-3 min for red or dull violet color that ensuring the positive result.

Test for Glycosides

At first taken 2 ml extract and carefully added 1 ml conc. H_2SO_4. Then observed red color for ensuring the positive result.

Quantitative phytochemical analysis

Estimation of Total Phenolic Content in the Plant Extracts

The total phenolic content (TPC) of different extracts was determined by UV-vis spectrophotometry using Folin-Ciocalteu (FC) assay (Alhakmani *et al.*, 2013) with few modifications. The calibration curve was constructed with different concentrations (25 µg/ml, 50 µg/ml, 75 µg/ml, 100 µg/ml and 150 µg/ml) of gallic acid as the standard. For this test 1 ml (1000µg/ml) of plant extracts (Methanolic and ethanolic) were taken into test tubes separately. Then 3 ml of distilled water was added in each test tubes and after that mixed thoroughly with 1 ml of Folin-Ciocalteu reagent (1:10 v/v diluted with distilled water). The solutions were left to rest for 5 min before being added with 2 ml of 7.5% sodium carbonate (Na_2CO_3) and stand 30 min of incubation at room temperature in the dark place. The absorbance of the samples was measured at 765 nm UV-vis spectrophotometer.

Estimation of Total Flavonoid Content in the Plant Extracts

The total flavonoid content (TFC) was estimated by aluminum chloride colorimetric method (Aruna *et al.*, 2015) with slight modification. Different concentrations of quercetin (12.5-200 µg/ml) were used as a standard to created calibration curve. For this test 1 ml (1000 µg/ml) of plant extracts (ethanolic and methanolic) were taken into test tubes separately. Then 100µl of 10% aluminum chloride ($AlCl_3$) solution, 100µl of 1M potassium acetate ($C_2H_3KO_2$) solution and 2.8 ml of double distilled water added in each test tubes and mixed thoroughly. After 30 min of incubation at room temperature, the absorbance of the sample was measured at 415 nm with a UV-vis spectrophotometer.

Determination of antioxidant activity

DPPH (2,2-diphenyl-1-picrylhydrazyl) Radical Scavenging Assay

Using a modified approach, the diphenyl-picrylhydrazyl (DPPH) radical degradation was used to assess the antioxidant activity of methanolic,

ethanolic and aqueous extracts (Alvarez-Jubete *et al.*, 2010) as a standard ascorbic acid was utilized. For this assay 2 ml of methanol and ethanol solution of plant extract or standard at different concentration (25 µg/ml, 50 µg/ml, 100 µg/ml, 200 µg/ml, 400 µg/ml) was taken in test tubes separately. 3 ml of methanol and ethanol solution of DPPH was added in to the test tubes. The test tubes were incubated at room temperature for 30 minutes in dark place to complete the reaction. Then the absorbance of the solution was measured at 519 nm using a spectrophotometer against control. A typical control solution contains all reagents except plant extract or standard solution. The percentage (%) scavenging activity was calculated from the following equation:

$\% SCV = \{(A_0-A_1)/A_0\}*100$

Where,

A_0 is the absorbance of the blank, and

A_1 is the absorbance of the extract/standard.

Determination of antibacterial activity

Plant materials and bacterial species

In the present investigation, ethanolic and methanolic crude extract of *A. aspera* leaves were used for antibacterial activity by disc diffusion assay. Three bacterial species were used to study to determine the antibacterial activity. The bacterial species were collected from Microbiology Laboratory, Department of Botany, University of Rajshahi. The concentrations (200 mg/ml, 400 mg/ml, 600 mg/ml, 800 mg/ml) of *A. aspera* were made from the mother extracts by using standard formula for respective solvents.

Culture preparation of bacterium

The studied species maintained in laboratory by 33% glycerol stock culture in eppendorf tube. During this investigation, all the bacterial species were cultured on nutrient agar medium. This step was repeated to go a single colony. Single colony was then transferred into nutrient broth medium and incubated at 30 ± 2 °C for 24 hours. After 24 hours, broth culture of bacteria was used as inoculums.

Inoculation, discs placing and incubation

In vitro antibacterial activities of the extracts were measured by employing standard agar disc diffusion method (Bauer *et al.*, 1966; Barry, 1980). In the disc diffusion method, the disc was placed aseptically over the bacterial culture on Mueller Hinton Agar Medium plates and incubated at 30 ± 2 °C for about 24 hours. After incubation for about 24 hours the antibacterial activities were determined by measuring the diameter of the inhibition zone in mm. The experiment was replicated three times to confirm the reproducible results. Sterile, blank paper discs were impregnated with sterile solvents (Methanol and Ethanol leaf extract) were used and tetracycline contained paper disc were used as positive control each time.

Determination of the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The minimum inhibitory concentrations (MIC) of the extracts were determined for each of the test organisms in triplicate in test tubes. According to (Doughari *et al.*, 2007) with some modification that 0.5 ml of varying concentrations of the extracts in test tubes nutrient broth was added and then a loopful of the test organism, previously diluted to 0.5 Mc Farland turbidity standard was introduced. The procedure was repeated on the test organisms using the standard antibiotics tetracycline. A tube containing nutrient broth only was seeded with the test organisms as described above to serve as controls. The culture tubes were then incubated at 30 ± 2 °C for 24 h. After incubation the tubes were then examined for microbial growth by observing for turbidity. To determinate the MBC for each set of test tubes in the MIC determination, a loopful of broth was collected from those that did not show any growth and inoculated onto sterile Nutrient agar by streaking. Nutrient agar plates only were also streaked with the respective test organisms to serve as controls. All the plates were then incubated at 37°C for 24 h. After incubation the concentration at which no visible growth was seen was noted as the Minimum Bactericidal Concentration (MBC).

Results

Qualitative phytochemical analysis

The findings of the qualitative analysis of the phytochemical screening of *A. aspera* leaf extracts in methanol and ethanol were shown in Table 1. and included 14 components. Out of the two solvents the ethanolic leaf extract included nine different compounds: (alkaloids, flavonoid, terpenoids, glycosides, cardiac glycosides, phenol, coumarins, xanthoproteins and steroids). In contrast, methanolic leaf extracts had a maximum of seven different compounds: (alkaloids, flavonoids, terpenoids, glycosides, coumarins, steroids and carbohydrates).

In contrast, methanol leaf extracts did not contain cardiac glycosides, xanthoprotein and phenol, while ethanol leaf extracts did not contain carbohydrates. Methanolic and ethanolic leaf extracts did not include saponins, tannins, proteins and amino acids. Due to its ability to identify the top nine phytochemicals, ethanol solvents outperformed methanol solvents.

Table 1. Phytochemical screening of methanol leaf extract and ethanol leaf extract of *A. aspera*.

Name of the phytochemicals	Name of specific test	Methanol	Ethanol
Alkaloids	Dragendroff's test	-	-
	Hager's test	-	-
	Wagner's test	+	+
	Mayer's test	-	-
	Tannic acid test	+	+
Flavonoids	Alkaline test	+	+
Saponins	Foam test	-	-
Terpenoids	Salkowski's test	+	+
Tannins	Braymen's test	-	-
Glycosides	NaOH test	+	+
Cardiac Glycosides	Killer-killiani test	-	+
Phenol	Lead acetate test	-	+
Coumarins	10%NaOH test	+	+
Xanthoprotein	HNO3 test	-	+
Steroids	Salkowski test	+	+
Proteins	FeCl3 test	-	-
Amino acids	Ninhydrin reagent	-	-
Carbohydrates	Molich's test	+	+

Total phenol content

The findings of the total phenol content of *A. aspera* in methanol and ethanol leaf extracts are shown in Table 2. The maximum concentration of phenolic components was found in the methanolic leaf extract of *A. aspera*, which measured 9.99 ± 0.43 mg GAE/g, which was statistically significant when compared to ethanolic leaf extracts (2.44 ± 0.46 mg GAE/g) at the $p<0.05\%$ level.

Total flavonoid content

A. aspera leaf extracts in methanol and ethanol are tested for total flavonoid content (TFC) and the findings are shown in Table 2. Quercetin equivalents per gram of dry weight were used to express the combined flavonoid concentration of the two extracts.

Comparative analysis showed that the methanolic leaf extract from *A. aspera* had the highest flavonoid content (41.16 ± 0.93 mg QE/g), while the ethanol leaf extract had the lowest amounts (16.07 ± 0.28 mg QE/g), which is not statistically significant.

Antioxidant activity by DPPH method

The IC_{50} values of the methanolic and ethanolic leaf extracts of *A. aspera* as well as the percentage of scavenging activity of the leaf extracts are shown in Figure 1. and Figure 2.

Table 2. Total phenol and flavonoid content of methanol and ethanol leaf extracts of *A. aspera*.

Plant Name	Solvents	Equivalents per gm dry weight of extract	
		Total Phenol Content (mg GAE/gm)	Total Flavonoid Content (mg QE/gm)
A. aspera	Methanol	9.99 ± 0.43[a]	41.16 ± 0.93[a]
	Ethanol	2.44 ± 0.46[b]	16.07 ± 0.275[b]

Note: Values are mean ± Standard error (n = 3). Catalog 'a, b' reveals statistically significant differences between the column of TPC and TFC calculated by t-test ($p < 0.05$). The identical designation (e.g., 'a') do not differ significantly.

They calculated their decreasing power using the 50% inhibition (IC_{50}) values. Ascorbic acid has an IC_{50} of 46.24 ± 1.52 µg/ml. As evidenced by their lower IC_{50} value, plants have superior antioxidant activity. Strong antioxidant activity was indicated by the scavenging activity range of *A. aspera* methanolic extracts (39.44 ± 0.45% to 54.96 ± 0.09%), as well as the lowest IC_{50} values (237.83 ± 1.70 µg/ml) and ethanolic extracts (37.64 ± 0.55% to 56.77 ± 0.16%) and the highest IC_{50} values (279.14 ± 2.51 µg/ml). The methanolic extracts were non-significant with methanolic extracts at the $p < 0.05$ level.

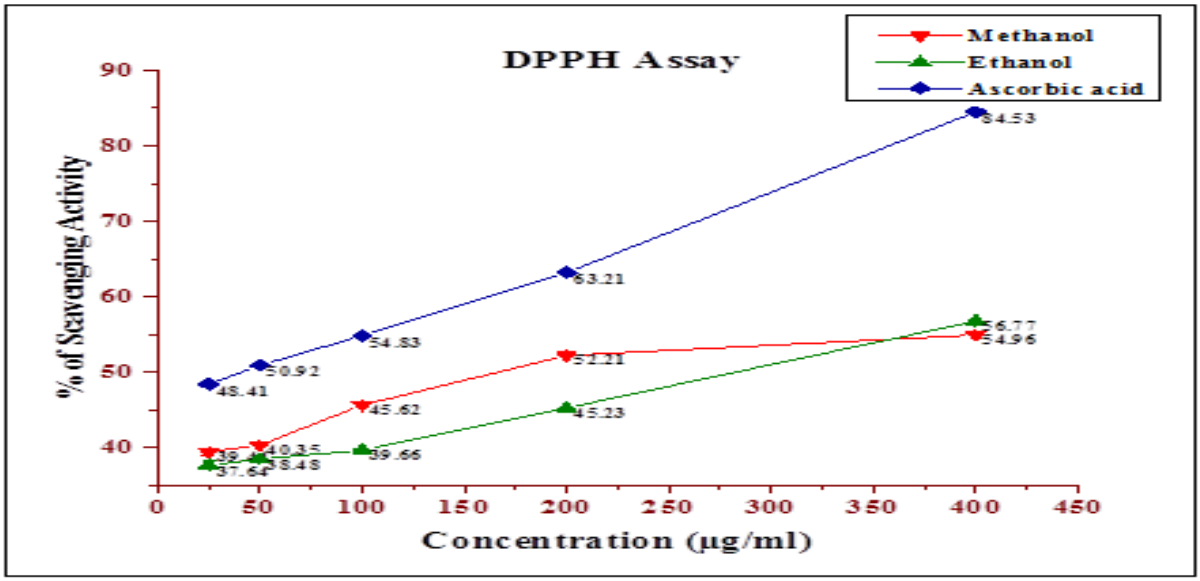

Fig. 1. Scavenging activity of standard, methanolic and ethanolic leaf extract of *A. aspera*.

The antibacterial activity of methanol leaf extract of A. aspera

The antibacterial activity of *A. aspera* methanol leaf extract against three bacterial strains is shown Figure 3. Against *Bacillus* sp., the methanolic leaf extract showed the highest inhibitions zone 20.67 ± 0.32 mm in 800 mg/ml concentration and *Enterobacter* sp. was showed lowest inhibition zone 7.25± 0.27 mm in 600 mg/ml concentration respectively. Positive control exhibited zone of inhibition against all the organisms tested. The range of inhibition zone was 31.20 ± 0.13 - 34.09 ± 0.19 mm.

Antibacterial activity of ethanol leaf extract of A. aspera

The antibacterial activity of *A. aspera* methanol leaf extract against three bacterial strains is shown Figure 4. In ethanol leaf extract, against *Bacillus* sp showed the highest inhibition zone (17.76 ± 0.47mm) in 800 mg/ml concentration and lowest inhibition zone 7.98 ± 0.22 mm was found in 800 mg/ml concentration against *Enterobacter* sp. respectively. Positive control exhibited zone of inhibition against all the organisms tested. The range of inhibition zone was 30.21 ± 0.30 - 33.30 ± 0.16 mm.

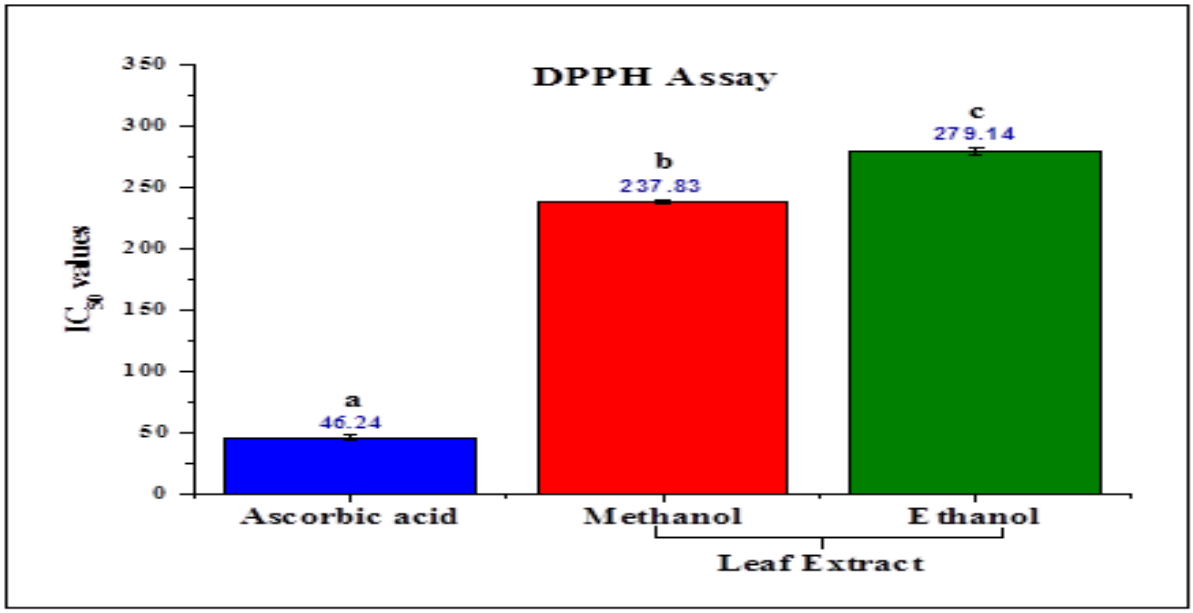

Fig. 2. IC_{50} values of *A. aspera* methanolic and ethanolic leaf extract. Catalog a-c reveals statistically significant differences between the bars calculated by DMRT test ($p < 0.05$).

Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC)

MIC is important in diagnostic laboratory to confirm resistance of microorganisms to an antimicrobial agent and also to monitor the activity of new antimicrobial agents. MBC is the lowest concentration of antibiotic required to kill an organism (Mathews, 2002). Results of Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of three different extract are shown in Figure 5. The result of methanol extract showed in the range from 75-500 mg/ml. The lowest MIC value 75 mg/ml was recorded against *Bacillus* sp. and the highest MIC value was 500 mg/ml was recorded against *Enterobacter* sp. The other tested bacteria *E. coli* had medium MIC value (100 mg/ml).

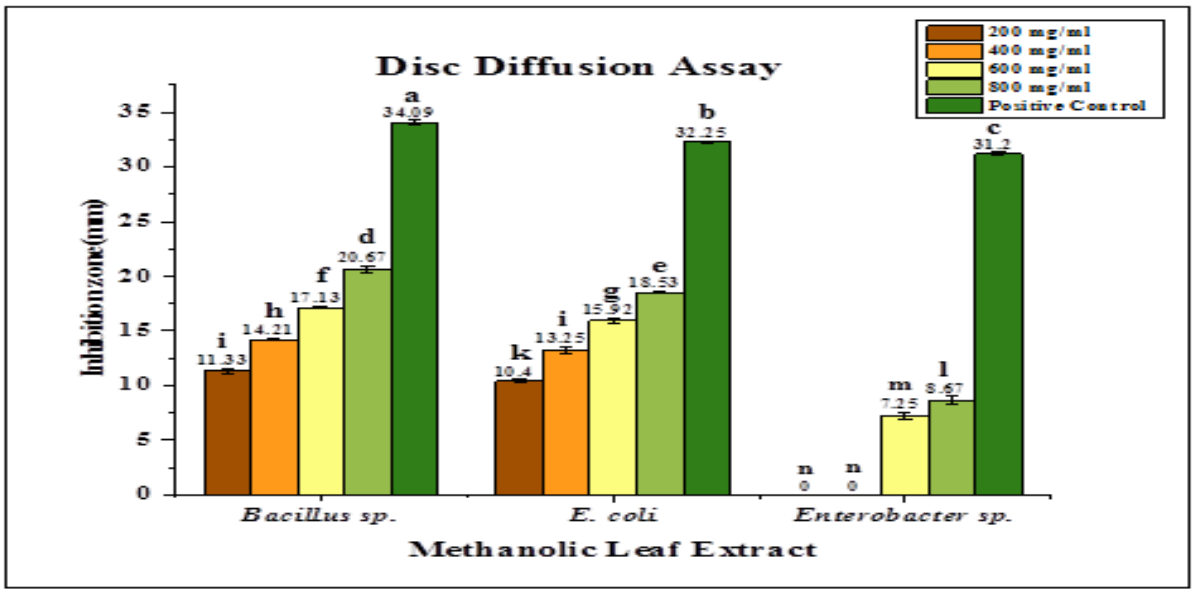

Fig. 3. Antibacterial activity of methanol leaf extract of *A. aspera* against three human pathogenic bacteria.

The lowest MBC value 150 mg/ml was recorded against *Bacillus* sp. and highest MBC value 650 mg/ml was recorded against *Enterobacter* sp. The MIC value of ethanol extract was range from 100

mg/ml to 700 mg/ml. The lowest MIC value 100 mg/ml was recorded against *Bacillus* sp. and highest MIC value 700 mg/ml was recorded against *Enterobacter* sp. The other tested bacteria *E. coli* had medium MIC value 125 mg/ml. The lowest MBC value 175 mg/ml was recorded against *Bacillus* sp. and highest MBC value 850 mg/ml was recorded against *Enterobacter* sp.

Discussion

Medicinal plants are an important source of basic healthcare services for rural populations. Originally herbal remedies were made up of crude pharmaceuticals including tinctures, teas, powders, poultices and other herbal preparations (Balick and Cox, 1997).

The secondary metabolites of medicinal plants particularly terpenoids, phenolic compounds and alkaloids are primarily responsible for their therapeutic effects. It can be utilized to look for bioactive substances that might be synthesized and prepared into very beneficial medications (Harborne, 1998).

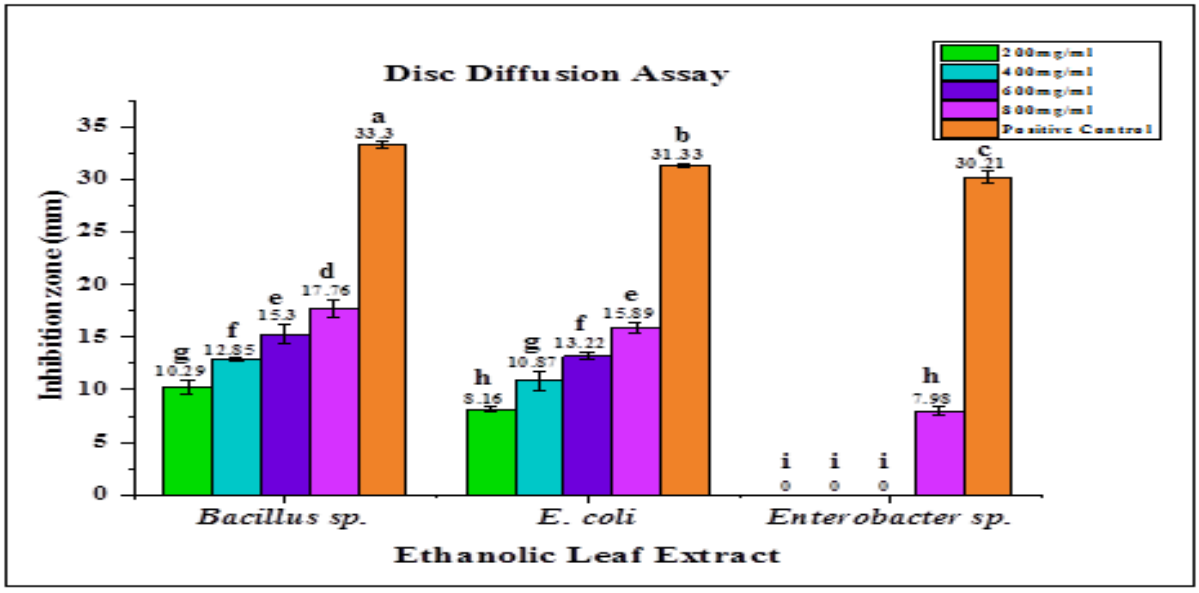

Fig. 4. Antibacterial activity of ethanol leaf extract of *A. aspera* against three human pathogenic bacteria.

In the present work *A. aspera* leaf extract showed the presence of alkaloids, flavonoids, terpenoids, glycosides, cardiac glycosides, phenol, coumarins, xanthoproteins and steroids. The earlier reports confirmed the presence of similar phytochemicals in *A. aspera* (Manandhar *et al.*, 2021; Thapa *et al.*, 2023). On the other hand, some research confirmed the presence of saponins, tannin, protein and amino acid (Thapa *et al.*, 2023) while our studies giving negative results. The presence of phytochemical constituents such as flavonoids, triterpenoids, polyphenolic compounds and steroids that have antibacterial and antioxidant properties. Alkaloids are essential for the invention of strong analgesic, cytotoxic, antispasmodic and antibacterial effects in both conventional and current medicine (Faruq *et al.*, 2024).

The presence of significant amounts of phenol and flavonoids are potent antioxidants, free radical scavengers and metal chelators as well as inhibit lipid peroxidation and exhibit various physiological activities, such as anti-bacterial, anti-viral, anti-cancer, anti-inflammatory, anti-allergic etc. (Nayan *et al.*, 2021) reported that *A. aspera* leaf contained flavonoids at 17.59 µg QE/mg and 297.20 mg QE/100g (Imtiaz *et al.*, 2020), which is approximately closer to our result. Phenolic compounds are widely distributed in plants and have shown to possess antimicrobial properties. Some previous studies reported that phenolic content 22.282 gm/100 gm (Kodidhela, 2023), 209.007 µg GA/mg (Manandhar *et al.*, 2021), 461.09 mg GA/100gm (Imtiaz *et al.*, 2020) was found in leaf

extract of *A. aspera*. The high phenolic content suggests significant antioxidant potential contributing to the therapeutic properties of *A. aspera* making it a valuable resource in traditional medicine for various disorders.

Antioxidant-based medication formulations are used to treat and prevent complex illnesses like Alzheimer's disease, diabetes, stroke, cancer and atherosclerosis. The DPPH free radical scavenging assay is the most straightforward and trustworthy methods for determining antioxidant activity (Manandhar *et al.*, 2021; Priyamvada *et al.*, 2021; Ahmed *et al.*, 2022). In the present investigation, the antioxidant activities of methanol and ethanol leaf extract of *A. aspera* showed significant antioxidant activity. (Priyamvada *et al.*, 2021) showed that, the methanolic extract was higher than petroleum ether. This result approximately similar with our investigation.

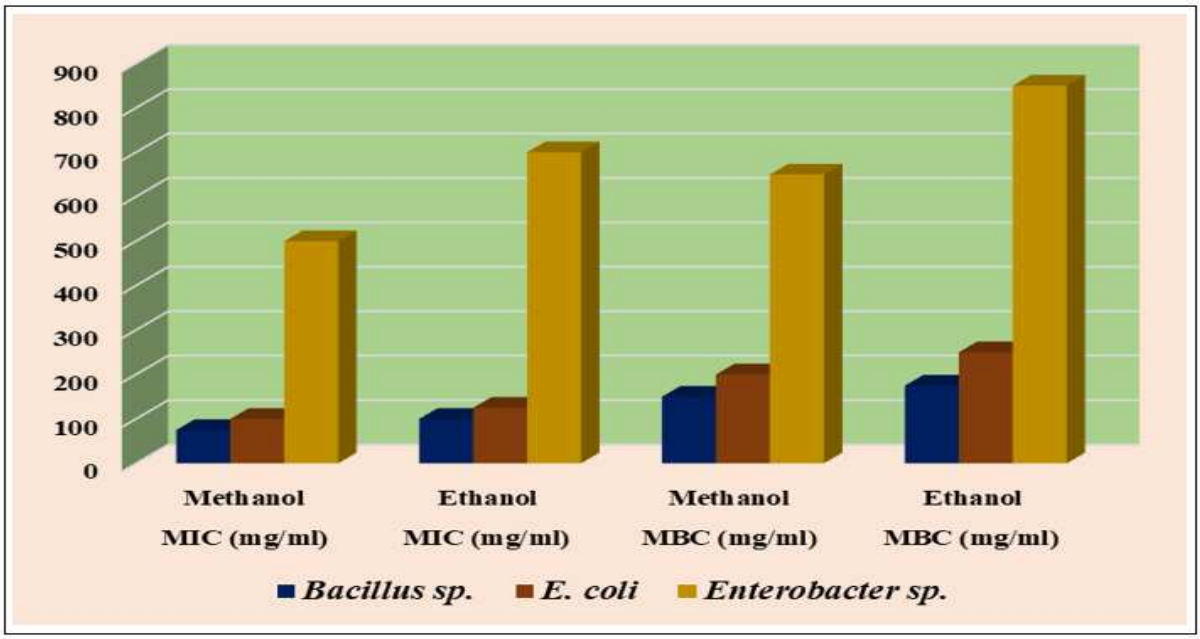

Fig. 5. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) for the methanol and ethanol Leaf extract of *A. aspera*.

In the present investigation, the methanolic extract of *A. aspera* was produced the highest zone of inhibition was 20.67 mm against *Bacillus* sp. Another researcher found methanol extracts was to be higher than that of aqueous, chloroform and hexane extracts for antimicrobial activity (Aniel *et al.*, 2014) and the highest activity was observed against *Bacillus subtillis* (Mishra, 2018). On the other hand, the ethanolic extract of *A. aspera* produced the highest zone of inhibition was showed in 800mg/ml in value of 17.76 mm against *Bacillus* sp. In this study Gram positive bacteria is more prominent to gram negative bacteria. Other researcher found, ethanol extracts of *A. aspera* was shown to be higher antibacterial activity than that of aqueous, chloroform and hexane extracts (Aniel *et al.*, 2014) and similar our research result.

However, if any differences were observed between antibacterial activities of studied extracts. These differences could be due to the differences in the phytochemical constituents its quantity, quality, age difference of plant etc.

Conclusion

In conclusion, the study investigating the phytochemical, antioxidant and antibacterial analysis of *Achyranthes aspera*. underscores its potential as a valuable natural resource for medicinal purposes. Phytochemical screening revealed the presence of various bioactive compounds including alkaloids, flavonoids, terpenoids, glycosides, coumarins and steroids in both ethanol and methanol extracts.

Additionally, quantitative analysis highlighted the high levels of flavonoids and phenols in methanol leaf extracts suggesting their potential health benefits.

Moreover, the antioxidant activity of *A. aspera* extracts assessed through the DPPH free radical scavenging assay, demonstrated significant antioxidant potential with noteworthy IC_{50} values in both methanol and ethanol extracts. This suggests the plant's ability to combat oxidative stress, thereby potentially preventing various diseases associated with oxidative damage.

Furthermore, the antibacterial activity evaluation against *Bacillus* sp., *E. coli*, and *Enterobacter* sp. revealed promising results with methanol leaf extracts exhibiting superior antibacterial effects compared to ethanol extracts. The observed inhibition of bacterial growth suggests *A. aspera*'s potential as a natural antibacterial agent which could be valuable in combating bacterial infections.

Overall, the findings suggest that *A. aspera* holds promise as a source of bioactive compounds with antioxidant and antibacterial properties warranting further investigation for its potential therapeutic applications in medicine and healthcare. However, further studies, including in vivo experiments and clinical trials are necessary to validate its efficacy and safety for human use.

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