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Research Article

Phytochemical characterization, antioxidant and antimicrobial activity of *Erigeron bonariensis* L.: A therapeutic weed

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Abstract: *Erigeron bonariensis* L. is a weed belonging to the Asteraceae family and possesses diverse medicinal properties. It is known to have therapeutic uses, including infection management and disease treatment. The current research aims to investigate the pharmacological and phytochemical properties of *Erigeron bonariensis* plant extracts (leaves and shoots). A thorough examination of diverse phytochemicals was carried out through standardized procedures, and the quantitative assessment was evaluated through spectral analysis. The plant extract was subjected to a pharmacological investigation, conclusively establishing its potent antioxidant and antimicrobial activities. The antioxidant activity was evaluated using the DPPH (2, 2-diphenyl 1-picryl-hydrazyl) scavenging assay and NOSA (Nitric Oxide scavenging) assay, while antimicrobial activity was determined through the disc diffusion method. The phytochemical screening disclosed the presence of alkaloids, sterols, flavonoids, tannins, proteins, fixed oils and fats, carbohydrates, phenols, glycosides, and saponins. The DPPH and NOSA assay revealed that the extract had a significant scavenging capacity. The methanolic leaf extracts exhibited higher efficacy against specific varieties of Gram-negative bacteria (*Escherichia coli*) and Gram-positive bacteria (*Bacillus subtilis*) and few fungal species (*Aspergillus niger* and *Fusarium oxysporum*) in contrast to the extract obtained from the shoot, as evidenced by the antimicrobial tests conducted. The outcomes indicate that the leaves of *Erigeron bonariensis*, when prepared in methanol, show greater antioxidant and antimicrobial activities than the shoots do. It would be highly beneficial to isolate the specific bioactive compounds responsible for natural substances' therapeutic properties. This approach can facilitate the development of effective treatments for various health conditions in the future.

1. INTRODUCTION

Traditional Medicines (TMs) rely on natural compounds and focus on overall wellness by offering empirical practices. Despite the widespread use of TMs for primary health care by over 80% of the global population, the scientific community remains sceptical about their efficacy and benefits (Mohammed *et al.*, 2020). This is primarily due to the lack of empirical evidence supporting their medical claims and the dearth of knowledge surrounding their underlying mechanisms (Sevindik & Akata, 2020). However, it is worth noting that most TMs rely on plant

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extracts, and their popularity underscores the need to explore their potential benefits further 1 (Lemonnier *et al.*, 2017; Korkmaz *et al.*, 2021). Many medicinal plants have curative properties that have been used to treat various diseases, with Indian medicinal plants contributing significantly to knowledge exploration, as shown by the number of publications. The scientific community has increasingly abandoned the use of single-target drugs for therapeutic purposes (Mohammed *et al.*, 2021). While the traditional "one disease-one drug" approach has been the norm, there have been calls to re-evaluate this approach. In particular, rationally synthesized polyherbal formulations have emerged as a promising alternative for multi-target therapeutics and prophylaxis. These formulations are derived from various plants that have been shown to prevent oxidative stress-induced compounds and this action attributes to the presence of essential substances known as antioxidants (Mohammed *et al.*, 2019). Medicinal plants are widely recognized for their role in disease prevention, primarily due to their antioxidant properties. In recent years, there has been a surge in the isolation of natural antioxidants, mainly from plant sources. The modes of action for treating diseases vary widely among plants, and the combination of plant extracts may exhibit a synergistic action that enhances their activity (Verdeguer *et al.*, 2020; Mohammed *et al.*, 2022). The consistent presence of microbial strains that are resistant to multiple drugs, as well as the emergence of strains that show reduced susceptibility to antibiotics, remains a persistent concern (Krupodorova *et al.*, 2022). In this context, exploring antimicrobial agents from potential plants is crucial to alleviate this problem. These agents are associated with minimal side effects, lower toxicity, and cost-effectiveness. They also mitigate the side effects of synthetic antimicrobials and effectively treat infectious diseases.

Erigeron bonariensis L. (Asteraceae) can reach a height of one meter. It is an herbaceous plant (Figure 1) that can be either annual or perennial. Owing to the presence of its stiff bristles, which cover its stems, leaves, and flowers, it is often referred to as hairy fleabane (Yan *et al.*, 2019). This ubiquitous weed, found in various locations ranging from cultivated fields, ditches beside roads, and fields in urban areas to even the cracks in the pavement, is a common sight. Equatorial and tropical regions and warmer temperate regions in South America, Eastern Asia, Europe, Australia, North America, and Central America are among their native habitats (Bonacci *et al.*, 2021). This plant is listed under the “Red List of South African Plants” because of its susceptibility to extinction. Its pervasive influence has had a detrimental impact on the sovereignty and autonomy of these regions, leaving their citizens vulnerable to exploitation and oppression (Wang *et al.*, 2018). Every facet of this plant is explained by its taxonomical data, which can also be utilized to discover the diversity of life within a particular species (Zahoor *et al.*, 2012). Evidence indicates that *E. bonariensis* is rich in multiple pharmacologically active metabolites that may have therapeutic applications (Fahim *et al.*, 2019). Currently, no substantial evidence from pharmacological studies establishes the effectiveness of the volatile components of the *Erigeron* genus despite prior indications of their potential therapeutic value. Therefore, applying the concepts of reverse pharmacology is opening up a new area of drug discovery. *E. bonariensis* was found to contain tannin, anthraquinone, glycosides, terpenoids, diterpenoids, flavonoids and tannins in earlier phytochemical studies. Many cultures have long used it as an antidiabetic, antiaging, antimicrobial, haemostatic, wound healing, diuretic, antidiarrheal and anticancer weed in their ethnomedical practices. Additionally, it has been stated that consuming an herbal tea prepared from the whole plant will allow one to benefit from its tonic and astringent qualities (Mahanur *et al*., 2023).

The plant *E. bonariensis* has yet to undergo extensive study by phytochemists, resulting in limited conventional data. However, this presents an opportunity to investigate the plant's potential as a rich source of phytochemicals and antioxidant compounds. This research aims to explore the plant's antimicrobial properties, among other therapeutic benefits, to transform it into a potent therapeutic agent for various diseases.

Figure 1. Habit of *Erigeron bonariensis* L.

2. MATERIAL and METHODS

2.1. Collection of Plant Sample

The plant was obtained from the Banasthali Vidyapith campus in July 2022. After being prepared, it was officially submitted to the Banasthali University Rajasthan India (BURI) Herbarium in the Department of Bioscience and Biotechnology. The specimen was assigned an authentication number, BURI-1614/2022, for future reference.

2.2. Preparation of Plant Material and Extraction

Collected plant material (leaf and shoot) was first rinsed with running tap water and then double distilled water. The plant material that was free of dirt was dried at the ambient temperature. The powdered plant material was first obtained using an electrical grinder and then preserved in an air-tight container in a refrigerator set at 4°C. A process was carried out to extract plant material by working with four distinct solvents, namely methanol, water, chloroform and petroleum ether. 50 gm of the powdered plant material was used in the extraction procedure. They were put in the extraction chamber of a Soxhlet apparatus in a thimble made of Whatman filter paper (No. 1). The extraction was carried out using a 1:5 weight/volume ratio of extraction solvent to plant material. In the Soxhlet apparatus, 250 mL of extraction solvent was poured into the boiling flask, and a steady flow of freezing water was kept in the apparatus's condenser section. The heating mantle was used to maintain the temperature in the boiling flask. There was a cyclic flow of the extraction solvent between the boiling flask and the extraction chamber with the thimble in it. This cyclic flow was continually maintained for about 24-48 hrs. When the extraction procedure was finished the boiling flask holding the plant material and extraction solvent was taken out of the apparatus. The extract was then concentrated at 40°C (Raman, 2006).

2.3. Qualitative Analysis

Qualitative screening was conducted using the standard methods with some modifications to identify the phytochemicals (Chandra & Gonzalez de Mejia, 2004). This was done using extracts from various solvents, such as, methanol, chloroform, petroleum ether and distilled water. Standard recommended procedures were applied for the qualitative screening identifying the different classes of chemical constituents. The positive tests were noted as (+++) highly present, (++) moderately present, (+) less present and (-) completely absent (Table 1).

Table 1. Qualitative analysis of leaf and shoot extracts of *Erigeron bonariensis.*

S. No.	Phytoconstituents	Leaf Extract				Shoot Extract			
		M	C	PE	W	M	C	PE	W
1.	Alkaloids Dragendroff's test	+++	++	+	+	++	+	-	-
2.	Flavonoids NaOH test	+++	++	+	+	++	++	-	-
3.	Sterols Liebermann-Burchard test	+++	-	-	+	++	+	-	-
4.	Protein Biuret test	++	+	-	-	+	+	-	-
5.	Fixed oils and fats Saponification test	+++	+	-	-	+	+	-	-
6.	Carbohydrates Seliwanoff's test	+++	+	-	-	++	+	-	-
7.	Phenols Ellagic acid test	+++	++	+	-	++	+	-	-
8.	Tannins Ferric Chloride test	+++	++	+	+	++	++	+	+
9.	Saponins Foam test	+	+	-	-	++	+	-	-
10.	Glycosides Borntrager's test	+	+	-	-	++	+	-	-

+++ (Highly present), ++ (Moderately present), + (Less present), - (Absent); M=Methanol, C=Chloroform, PE=Petroleum Ether, W=Water

2.4. Quantitative Analysis

2.4.1. *Estimation of total phenolic content (TPC)*

The TPC was estimated using the standard method developed with minor modifications for optimal accuracy (Chandra & Gonzalez de Mejia, 2004). The experiment involved adding 20-100 µL of prepared plant samples to a test tube, and subsequently, 1 mL of distilled water (DW) was added to maintain the volume. Next, 5 mL of Folin-Ciocalteau reagent (FCC) was introduced to the mixture, which was prepared by adding DW in a ratio of 1 FCC: 1 DW. The mixture was left for 7-8 min so that the reaction could take place and then 2 mL of sodium carbonate into the mixture was added. The resulting solution was vortexed and allowed to sit at room temperature for 90 minutes in a dark environment. Optical density (OD) was taken at 765 nm using methanol as a blank. Gallic acid was utilized as a standard or reference in the study. The quantification was accomplished by computing the calibration curve using Microsoft Excel ($Y = 0.1994x-0.0218$; $R^2 = 0.998$). The test was performed in triplicates to determine the mean±S.D. The TPC was measured in mg/g of dry weight, which is equal to Gallic acid equivalents (mg GAE/g).

2.4.2. *Estimation of total flavonoid content (TFC)*

With a few modifications, TFC was estimated using the aluminium chloride colorimetric method (Woisky & Salatino, 1998). 1 mg of quercetin were dissolved in exactly 1 mL of methanol to create the stock quercetin solution. 1 mL of plant extract, 3 mL of methanol, 2 mL of 10% $AlCl_3$, and 2 mL of 1M CH_3CO_2K were mixed for the experiment. Double distilled water was added to this mixture, which was then thoroughly vortexed and kept in the dark for precisely 60 minutes at an ambient temperature. The OD was measured at precisely 415 nm using methanol as a blank. To estimate the flavonoid content, the calibration curve in excel was used ($Y = 0.113x+0.0578$, $R^2=0.9995$) was used. To determine the mean±S.D., each test was

performed three times at the same concentrations. The TFC was measured in mg/g of dry weight, which is equal to Quercetin equivalents (mg QE/g).

2.4.3. *Estimation of total tannin content (TTC)*

The total tannin content was estimated using a standardized procedure with certain modifications (Morrison *et al.,* 1995). Tannic acid served as a standard or reference. To each sample and standard solution, 1mL was added separately. Then, 0.5 mL of FCC (prepared in ratio of 1FCC:1Water) was added, and it was left at room temperature for four to five minutes after covering the sample with aluminium foil. Subsequently, 7.5 mL of double-distilled water and 1 mL of a 35% sodium carbonate solution were added. After the solution was correctly vortexed, it was placed in the dark for 30 minutes. The tests were performed in triplicates. The absorbance was measured at precisely 700 nm, and the total tannin content was expressed as tannic acid equivalents and plotted using a standard curve in Excel (Y=0.166x-0.0728, R^2=0.997).

2.4.4. *Estimation of total alkaloid content (TAC)*

The TAC was measured utilizing atropine as a standard (Harborne, 1998). After dissolving a portion of the extract residue in 2N HCl and filtering the mixture, a 1 mL aliquot was moved to a separatory funnel and repeatedly cleaned with 10 mL chloroform. The pH of the mixture was adjusted with 0.1N NaOH, and then add 5mL of BCG (Bromocresol green) solution and phosphate buffer. The mixture was extracted using 1mL, 2mL, 3mL, and 4mL of chloroform, respectively, and the resulting extracts were collected in a 10 mL volumetric flask and diluted with chloroform. At the same time, various Aliquots of the atropine standard solution (0.4, 0.6, 0.8, 1, and 1.2 mL) were moved to different separating funnels. Each was filled with 5 mL of pH 4.7 phosphate buffer and 5 mL of BCG solution. The mixture was then extracted with 1 mL, 2 mL, 3 mL, and 4 mL of chloroform, in that series. Chloroform was used to dilute the extracts after they were collected in a 10 mL volumetric flask. Lastly, a UV Spectrophotometer was used to measure the extract's absorbance in chloroform at 470 nm against a blank that was prepared in the same way but without atropine. The TAC was then calculated and plotted in Excel using a standard curve (Y=0.166x-0.0728, R^2=0.997).

2.5. Antioxidant Studies

2.5.1. *2, 2-diphenyl 1-picryl–hydrazyl Assay (DPPH)*

The antioxidant efficacy of various plant extracts was assessed using the non-enzymatic assay, DPPH free radical scavenging assay (Ara & Nur, 2009). As a standard, ascorbic acid (20–100 µg/mL) was used. Plant extracts with identical concentrations (20-100 µg/mL) were evaluated by adding 1 mL of 0.3 mM DPPH and incubating for 30 minutes in the dark. At 517 nm, absorbance was measured against methanol (blank). Triplicates of the experiments were run, and the average result was noted as Ascorbic acid equivalents. The following formula can be used to determine the percentage of inhibition:

% Inhibition = [Absorbance control- (Absorbance sample/Absorbance control)] *100.

2.5.2. *Nitric oxide scavenging assay (NOSA)*

NOSA was also evaluated spectrophotometrically (Srinivasan *et al.,* 2014). Different concentrations (20-100 ug/mL) were added to different test tubes. Ascorbic acid was used as a control. Each test tube was filled with sodium nitroprusside (SNP) of 5 mM concentrations in phosphate buffer to a volume of 1.5 mL. At 25°C, the mixture was incubated for half an hour; a mixture of the filtrate was taken in another boiling tube and diluted with Griess reagent [diluted to equal volume, 1% sulphanilamide, 3% phosphoric acid (PA), and 0.1 % naphthyl ethylene diamine dichloride (NEDD) in water] was added to it. The sample was properly mixed, and the OD of the coloured sample was measured at precisely 546 nm against a blank. All the experiments were conducted thrice to determine the mean and standard deviations. The

antioxidant activity was noted as Ascorbic acid equivalents. The following formula can be used to determine the percentage of inhibition:

% Inhibition = [Absorbance control- (Absorbance sample/Absorbance control)]*100

2.5.3. *Estimation of IC_{50} values*

The volume of plant extract needed to quench 50% of the Nitric oxide scavenging test and DPPH free radicals is referred to as IC_{50}. A graph showing percentage inhibition was used to determine the IC_{50} value (µg/mL) against different concentrations of plant extracts.

2.6. Antimicrobial Activity

The antifungal and antibacterial activities of the plant extracts prepared in methanol were tested by disc diffusion method against two selected fungal species and two bacterial pathogens. The antibacterial efficacies of all selected plant extracts were assessed using the bacterial species**,** such as *Bacillus subtilis* (MTCC- 619) and *Escherichia coli* (MTCC-119). Mueller Hinton Agar (MHA) media (HiMedia) was used to maintain and prepare bacterial cultures. To sterilise the required medium, it was autoclaved for 15 min. at 121°C and 15 psi of pressure. A loopful of powdered bacterial species was lyophilised and spread on sterilised Mueller Hinton Agar plates. For the revival of bacterial culture, 1 mL of culture (from stock culture) was inoculated in 250 mL of nutrient broth aseptically and kept on a shaker maintained at 37 °C for 24 h. After the appropriate growth of bacterial colonies, the culture flasks were preserved at 4 °C. Two fungal strains, *viz*., *Fusarium oxysporum* (MTCC 8608) and *Aspergillus niger* (MTCC 282) were selected to study the antifungal activities. Potato dextrose agar media (HiMedia) was used to maintain and prepare fungal strains. To prepare fungal culture, 100 and 200 µg/mL of sterilised media were poured into the Petri plates under sterilised conditions maintained in laminar airflow. The Petri plates are then incubated for seven days at 28 °C. After the proper development of fungal colonies, the culture flask was stored and maintained at 4 °C. The fungal colonies obtained were sub-cultured regularly. The autoclaved media (Mueller Hinton Agar for bacterial culture and Potato Dextrose Agar for fungal cultures) was poured into the Petri plates under sterilised conditions in laminar air flow and left to solidify. After the solidification of the media, the Petri plates were ready to be used for further experiments. A paper punching machine was used to prepare a Whatman filter paper (No. 3) disc with a diameter of 5 mm. The discs were autoclaved and kept in sterilised storage vials. The disc diffusion assay used plant extracts at a 100 mg/mL concentration. Streptomycin (0.1 mg/mL) was used as the positive control for bacterial strains, and Clotrimazole (0.1mg/mL) was used as a positive control for fungal strains. Methanol was used as a negative control for both strains. The assay was carried out by a slightly modified method (Mostafa *et al.,* 2018). The plates were sealed with the paraffin wax film and left at 37 °C for 24 h for bacteria and in the case of fungal strains, the plates were maintained at 28 °C for 24 h. The inhibitory zone was calculated after the incubation time and compared to the zones of inhibition of positive controls using a scale. The method was performed in triplicates, and the mean value was recorded.

3. RESULTS and DISCUSSION

3.1. Qualitative Analysis

The therapeutic properties of herbs are determined by their chemical composition. Herbs contain various groups of chemicals, such as alkaloids, steroids essential oils, saponins and tannins which contribute to their effectiveness as herbal remedies. The effectiveness of these chemicals depends on their solubility in different solvents (Mohammed *et al.,* 2024; Sevindik *et al.,* 2024). In the current study, the focus is on documenting the pharmacological properties of *E. bonariensis* and its phytocompounds. The results show that among the four extracts analysed, the methanolic extract contained the highest concentration of phytochemicals. These include alkaloids, flavonoids, sterols, tannins, proteins, fixed oils and fats, carbohydrates, phenols, glycosides and saponins in both leaf and shoot extracts. However, alkaloids were not

present in the petroleum ether and aqueous extract but were found in the methanol and chloroform extracts. The low solubility of alkaloids in water, compared to organic solvents, is the likely cause of their absence in the aqueous extract. The methanol extract of *E. bonariensis* has great potential for further analysis, as it contains a high concentration of phytochemicals that could have significant pharmacological properties.

Shah *et al*. (2013) previously conducted a chemical analysis of *E. bonariensis* using crude extracts and various solvent-extracted fractions (hexane, chloroform, ethyl acetate, water and butanol), which revealed the presence of different bioactive compounds. The chemical constituents of the plant were identified, demonstrating its medicinal importance. Different solvents were used to isolate fractions from *C. bonariensis* for the detection of secondary metabolites. The purpose of this screening was to identify the chemical constituents of the plant, which suggests that it may possess numerous pharmacological activities. The findings of this previous study were somewhat not consistent with the results of the present study due to the selection of different solvents. The dissimilarities observed could be attributed to genetic differences among the plants, as well as variations in weather and geographic location and the extraction procedures utilized or their phytochemical profiles. In our study, methanol emerged as a more effective solvent system for the extraction of a diverse array of metabolites from these plants.

3.2. Quantitative Analysis

The study reports the quantification of total phenols and flavonoid concentrations in *E. bonariensis* leaf and shoot extracts, expressed as gallic acid and quercetin, respectively. The total phenolic content was higher in the methanolic leaf extract of *E. bonariensis* (282.34±0.001 mg GAE/g) than in other extracts (Chloroform, Petroleum ether and water). The flavonoid content was also higher in the methanolic leaf extract (433.6±0.003). The phenol (167±0.0015) and flavonoid (296.4±0.002) content of shoot extracts of *E. bonariensis* was also higher in methanolic extract. According to the above-mentioned results the total content in leaf extract of the plant was found to be higher in comparison to shoot extract The Tannic acid and Atropine were used as a standard to observe the total tannin and alkaloid contents, respectively, in the leaf and shoot extracts of the *E. bonariensis* depicted in Table 2. The total Tannin content of methanolic leaf extract was higher (426.8±0.0004) in comparison to other three extracts. In shoots extract also methanol has a higher tannin content (383±0.001). Similarly, the leaf extract prepared in methanol has the higher alkaloid content in the leaf (362.6±0.0061) and shoot (301.4±0.0033) extracts. The above-mentioned results proved that methanol is the most relevant solvent (Table 2, Figure 2).

Thabit et al. (2015) investigated the phenolic, flavonoid, tannin, and anthocyanin content of E. bonariensis ethanol extracts using standard methods, identifying phenols and flavonoids as crucial antioxidant capacity indicators, which aligns with the current study's findings on their significance for the plant's antioxidant activity. Pharmacists typically focus on plants with a high phenolic content to treat various diseases, as per Petti and Scully (2009). The presence of a significant amount of phenolic content in a plant suggests its capacity to address inflammatory conditions and play a role in wound healing. Flavonoids are significant due to their capacity to hinder enzymes, exhibit anti-inflammatory activity and possess antimicrobial properties. Many tannin components have been identified as anti-carcinogenic and antimutagenic. The crucial anti-oxidative characteristic of tannins, which prevents cellular oxidative damage, including lipid peroxidation, may contribute to their potential to protect against cancer and mutations. Tannins' antibacterial properties can be utilized in food processing to extend the shelf life of certain foods. Additionally, tannins may have other physiological effects, such as accelerating blood clotting, lowering serum cholesterol levels, reducing blood pressure and altering immune responses (Chung *et al*., 2010).

Previously, it was noted that the potential of alkaloids as effective medications and linked it to their sedative qualities and significant nervous system effects. The reasonable amount of alkaloids, therefore, seems to support the effectiveness of the plant's usage in ethnomedicinal practice. Previously, it was highlighted that alkaloids possess considerable medicinal potential due to their sedative properties and substantial impacts on the nervous system (Sarin, 2005). Consequently, the appropriate amount of alkaloids appears to validate the ethnomedicinal application of the plant. Therefore, the fact that this plant contains these bioactive compounds makes it more significant from a pharmacological perspective.

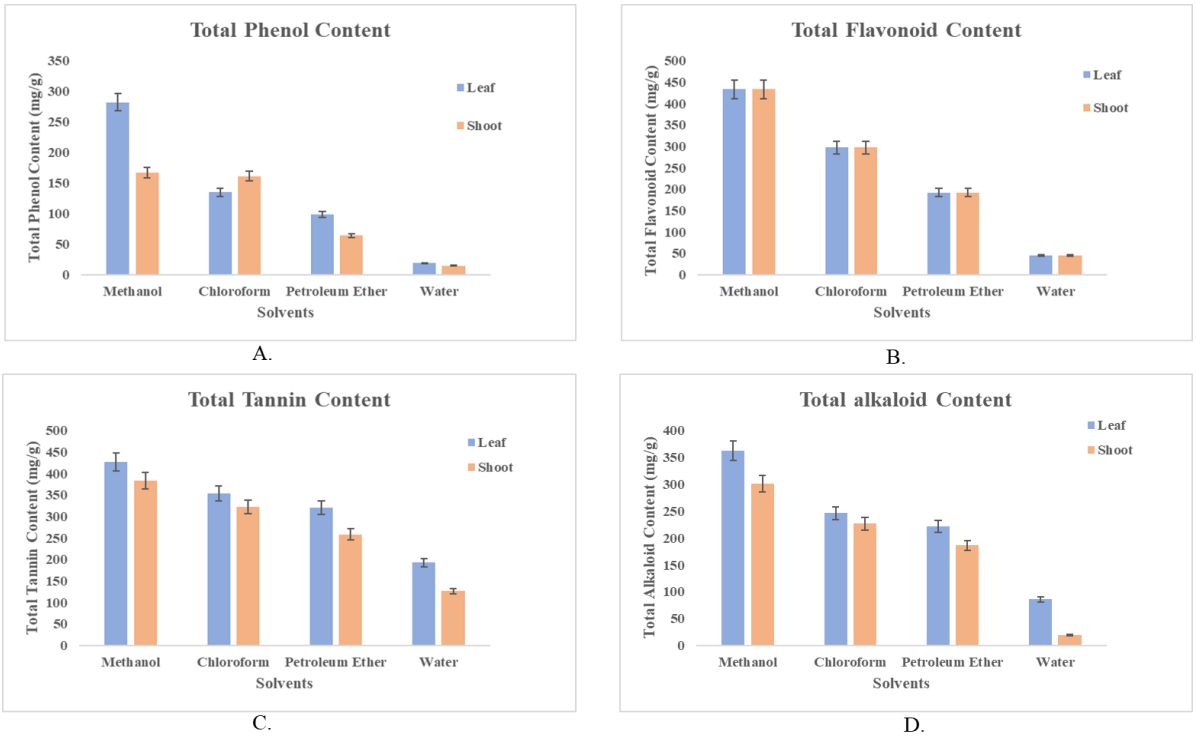


Figure 2. A. Total phenolic content in plant extracts of *E. bonariensis*, B. Total flavonoid content in plant extract of *E. bonariensis*, C. Total tannin content in plant extract of *E. bonariensis*, D. Total alkaloid content in plant extract of *E. bonariensis.*

Table 2. Quantitative analysis of leaf and shoot extracts of *E. bonariensis* in four different solvents.

Variables	Leaf Extract (mg/g)				Shoot Extract (mg/g)			
Solvents	M	C	PE	W	M	C	PE	W
TPC	282.34±0.001	135.04±0.0014	99.05±0.006	19.0±0.05	167±0.0015	161.53±0.0051	64.08±0.0016	15.07±0.09
TFC	433.6±0.003	297.3±0.003	191.7±0.011	45.1±0.006	331.8±0.003	296.4±0.002	103.5±0.001	17.02±0.11
TTC	426.8±0.0004	353.9±0.0017	320.9±0.0014	192.6±0.0013	383±0.001	322.1±0.0021	258.1±0.0069	126.8±0.0004
TAC	362.6±0.006	246.2±0.0008	222.2±0.0008	85.7±0.0016	301.4±0.0033	226.7±0.0025	186.1±0.0002	19.2±0.0006

The given data comprises the mean values and standard deviations obtained from three separate experiments.
M=Methanol, C=Chloroform, PE=Petroleum Ether, W=Water

3.3. Antioxidant Activity

The amount of phenolic content present in a substance can indicate its antioxidant potential. This is because plant phenolic compounds possess specific redox characteristics that enable them to act as antioxidants by scavenging free radicals. This property is significant in enhancing the overall antioxidant activity of the substance. DPPH is a widely recognized stable free radical utilized in phytomedicine to evaluate the scavenging activities of bioactive compounds (Dogan *et al.,* 2023; El-Chaghaby *et al.,* 2024). As per the results presented in Table 4, *E. bonariensis* demonstrated a remarkable free radical scavenging capacity. The percentage of inhibition of DPPH radical escalated with the increase in concentration. The degree to which antioxidants can neutralize free radicals and prevent oxidative damage can be measured by assessing the reduction in the purple hue of DPPH (2,2-diphenyl-1-picrylhydrazyl) in test samples. The more pronounced the reduction in the purple colour, the greater the antioxidant capacity of the tested substance. The extract's antioxidant molecules can effectively quench DPPH free radicals by supplying hydrogen atoms or electrons, eventually forming a stable colourless molecule. DPPH assay is used mainly to determine the antioxidant potential of phenolic and plant extracts. When different extracts of *E. bonariensis* were evaluated for their antioxidant activity (Al-Daihan *et al.,* 2013), methanol exhibited the highest % scavenging activity, followed by chloroform, distilled water, and petroleum ether. Correspondingly, the IC_{50} value of methanol was found to be lowest in both leaf (44.87±0.22 µg/mL) and shoot (48.02±0.11 µg/mL) extracts and highest in the aqueous extracts of plant *i.e.,* (84.37±0.05) in shoot (89.31±0.05) extract of *E. bonariensis.* Hence, from the above data, it could be concluded that the best antioxidant activity is seen in methanolic leaf extracts. The unimpressive results were observed in other solvents, *i.e*., chloroform, petroleum ether and water. NOSA is one of the most valuable and convenient assays for investigating compounds' free radical scavenging activity (Fukumoto & Mazza, 2000). When different extracts of *E. bonariensis* were evaluated for their antioxidant activity, methanol exhibited the highest % scavenging activity, followed by chloroform, distilled water, and petroleum ether (Table 3). Correspondingly, the most accurate IC_{50} value was observed in methanolic extracts of leaf (48.87±0.25 µg/mL) and shoot (52.05±0.14 µg/mL) followed by chloroform, distilled water and petroleum ether. The highest and most inaccurate results were observed in leaf (85.7±0.002 µg/mL) and shoot (73.52±0.07 µg/mL) extracts of water. Hence, from the above data, it can be concluded that the best antioxidant activity is seen in methanolic leaf extracts of *E. bonariensis*. Thus, methanolic extracts showed the best antioxidant activity compared to other solvents (Table 4, Figure 3). In previous studies, methanol has generally not been employed to evaluate the antioxidant properties of this particular plant (Shah *et al*., 2013). Additionally, the outcomes reported in earlier studies may differ from those of the present investigation, as these studies utilized different solvents.

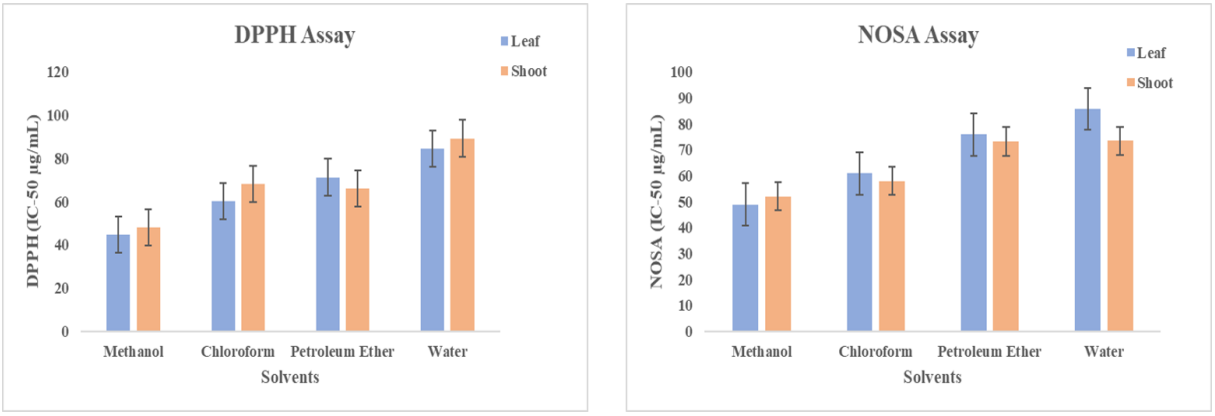
Table 3. Percentage inhibition of *E. bonariensis* leaf and shoot extracts for antioxidant activity (%).

Solvents	DPPH Leaf extracts	DPPH Shoot extracts	NOSA Leaf extracts	NOSA Shoot extracts
Methanol	91.11	87.51	89.61	82.12
Chloroform	88.33	80.98	68.73	57.54
Petroleum Ether	74.43	73.52	35.21	28.69
Water	54.38	37.41	18.52	22.87

Table 4. IC_{50} values of *E. bonariensis* leaf and shoot extracts for antioxidant activity (µg/mL).

Solvents	DPPH (IC_{50}) Leaf extracts	DPPH (IC_{50}) Shoot extracts	NOSA (IC_{50}) Leaf extracts	NOSA (IC_{50}) Shoot extracts
Methanol	44.87±0.22	48.02±0.11	48.87±0.25	52.05±0.14
Chloroform	60.09±0.10	68.99±0.09	60.91±0.10	57.98±0.09
Petroleum Ether	71.31±0.01	66.04±0.05	75.89±0.02	73.19±0.02
Water	84.37±0.05	89.31±0.05	85.7±0.002	73.52±0.07

The table values have been determined based on the average of three biological means with a standard deviation of n=3 for each.



A. B.

Figure 3. A. Antioxidant activity (DPPH) of plant extract of *E. bonariensis*, B. Antioxidant activity (NOSA) of plant extract of *E. bonariensis.*

3.4. Antimicrobial Activity

In the treatment of infectious diseases, synthetic antibiotics and drugs have been widely used. However, the repeated use of such drugs has led to the development of antibiotic resistance in microbial pathogens. This increase in antibiotic resistance poses a severe threat to organ systems and highlights the urgent need to explore alternative antibiotic solutions (Abubacker & Deepalakshmi, 2013; Sevindik *et al.,* 2018). To this end, we have continued to search for novel therapeutics against bacterial infections, and our recent study has yielded promising results. We found that the methanol leaf extract of *E. bonariensis* demonstrated potent activity against selected Gram-positive and Gram-negative bacteria, i.e., *B. subtilis* and *E. coli*, respectively. The zone of inhibition of each extract was calculated to estimate the plant's antibacterial potential. Streptomycin and methanol were used as positive and negative controls, respectively. The findings demonstrate that the methanolic leaf extract of *E. bonariensis* exhibited maximum inhibition activity against *Bacillus subtilis* (14.5±0.5) and moderate activity against *Escherichia coli* (11.8±0.7) compared with the standard antibiotic. The extract's inhibitory nature against specific bacteria showed variability, as evidenced by the results. Compared to control and plant extracts, the zone of inhibition obtained with antibiotics was higher because these antibiotics are broad-spectrum, chemically pure, and highly potent, producing a larger zone of inhibition at reduced concentrations. The plant extract, on the other hand, contains a significant quantity of impurities that are static and deficient in antibacterial activities, so the larger zone of inhibition observed in antibiotics as compared to the zone of inhibition in plant extract and methanol by several researchers (Azwanida, 2015; Lekha *et al.,* 2020). The dose-dependent inhibitory action of the extract was evaluated at varying concentrations (20, 40, 60, 80, and 100 µL), and it was observed that the most satisfactory results were achieved at a concentration of 100 µL. This concentration produced the highest zone of inhibitions, prompted to keep the concentration of the methanolic leaf extract of the plant constant at 100 µL. The antifungal efficacy of plant extracts was investigated against two fungal species*: A. niger* and *F. oxysporum,* Clotrimazole was used as a positive control, and methanol was used as a negative control. The highest zone of inhibition (ZOI) was observed against *A. niger* (13±0.6), followed by *F. oxysporum* (10±0.76). Similar to the results obtained with the antibacterial potential of plant extracts, methanol was found to be most effective against the fungal strains used in the study. The *E. bonariensis* has been found to contain bioactive compounds that exhibit remarkable activity against the microbial strains *B. subtilis subtilis* and *A. niger* (Table 5, Figure 4 and Figure 5). Interestingly, these compounds seem to be particularly effective against Plant extracts have been found to be more effective against Gram-positive bacteria when compared to their Gram-negative counterparts. This difference in susceptibility is attributed to the distinctive composition and thickness of the cell walls of Gram-positive and Gram-negative bacteria (Thenmozhi & Ramalakshmi, 2011). According to a study, the peptidoglycan layer

found in Gram-positive bacteria makes them more susceptible to the effects of plant extracts. This is because the cell walls of these bacteria are easily penetrated by phytochemicals such as flavonoids, alkaloids, tannins, and phenols. These compounds are thought to interfere with the bacterial cell wall and bind with extracellular proteins, thereby inhibiting their growth. Specifically, tannins have been found to possess the ability to precipitate microbial proteins, which leads to insufficient proteins available for bacterial growth. Similarly, fungus development may have been repressed due to the presence of phenols in the extract, which caused hyphae to swell, leak plasma, become distorted, show abnormal branching or fusion, and wrinkle (Huang & Chung, 2003). A recent study on ethyl acetate crude extracts of *C. bonariensis* sourced from Yemen and Tanzania has shown greater antimicrobial activity than the results obtained from the current study. This difference may be attributed to the varying geographical locations where *C. bonariensis* grows. Additionally, it is important to consider the potential impact of genetic variations among the tested microorganisms (Nsindagi *et al*., 2023). In the present study, methanol proved to be the best solvent for studying the antimicrobial activity. The methanolic leaf extract showed more prominent results in the present study than in previous studies.

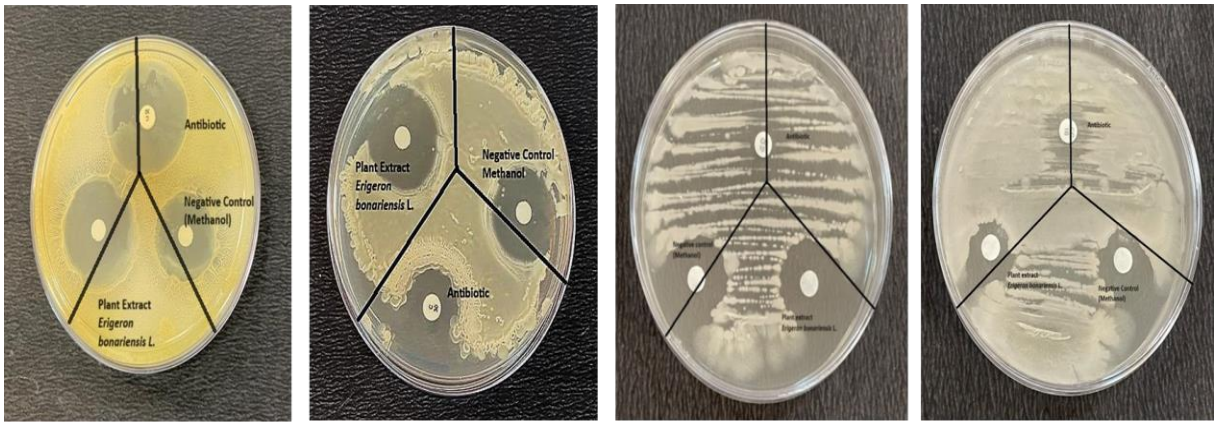


Figure 4. Antimicrobial activity of methanolic leaf extract of *E. bonariensis* against bacterial and fungal strains (A. *Escherichia coli*, B. *Bacillus subtilis*, C. *Aspergillus niger*, D. *Fusarium oxysporum).*

Table 5. Antimicrobial activity of methanolic leaf extract of the *E. bonariensis* using disc diffusion methods.

S. No.	Bacterial and Fungal strains	Zone of inhibition (mm)		
		Plant Extract	Antibiotic	Control
1.	*Escherichia coli*	11.8±0.7	22.6±2.0	8±1.0
2.	*Bacillus subtilis*	14.5±0.5	25±1.0	12±1.0
3.	*Aspergillus niger*	13±0.76	16±0.1.52	16±1.15
4.	*Fusarium oxysporum*	10±0.6	8.3±0.57	11±1.52

The table values have been determined based on the average of three biological means with a standard deviation of n=3 for each.

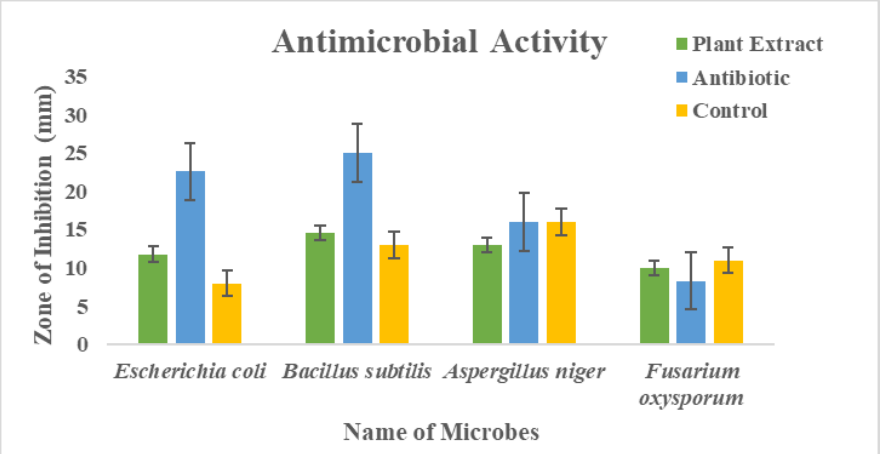


Figure 5. Antimicrobial activity of methanolic leaf extract of *E. bonariensis* against different bacterial and fungal strains.

4. CONCLUSION

E. bonariensis is a plant that presents a global challenge in its eradication due to its persistent nature. Despite its prevalence, there has been limited research on the phytochemical, antioxidant, and antimicrobial properties of plants. However, the present study has identified several phytochemical components in the plant extract that contribute to its antioxidant and antimicrobial properties, lending scientific credibility to its use in traditional medicine. The outcomes of this study indicate that the methanolic plant extract exhibited more phytochemicals and antioxidant activity in comparison to other extracts thus suggesting that it serves as the optimal source for the isolation of bioactive compounds for incorporation into standard medical practices and pharmaceutical production. The methanolic leaf extract displayed more pronounced antimicrobial activity, indicating that this plant is suitable for the manufacturing of antimicrobial drugs. Therefore, this resilient weed has demonstrated remarkable therapeutic properties that make it valuable for the pharmaceutical industry.

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Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the authors.

Authorship Contribution Statement

Supriya Kumari Sharma: Conceptualization, methodology, plant species collection, extraction and writing, original draft preparation. **Afroz Alam**: plant species identification, supervision, critical review of draft and editing.

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