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PHYTOCHEMICAL SCREENING OF DIFFERENT ROOT EXTRACTS OF *Ageratum conyzoides* AND THEIR POTENTIAL BIOACTIVE PROPERTIES

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ABSTRACT

The present study was conducted to determine different classes of secondary metabolites of *Ageratum conyzoides* root extracts and to evaluate their antioxidant, antibacterial, and antifungal potentialities using various pathogenic fungal and different gram-positive/gram-negative bacterial strains. The roots powder was subjected to ultrasonic-assisted extraction with *n*-hexane, acetone, ethanol, and EtOH:H₂O(1:1). The screening of phytochemicals indicated the existence of terpenoids, alkaloids, coumarins, sterols, flavonoids, and glycosides in the root extract of *A. conyzoides*. However, the absence of saponins, tannins, anthocyanidins, anthraquinones, and phlobatannins was observed. The results indicated a reasonable antibacterial (against gram-negative and gram-positive bacteria), and antifungal potential. The antibacterial activity of the ethanolic extract was highest against all four strains of bacteria and was also comparable to the standard medicines used. However, antifungal activity was highest in EtOH:H₂O (1:1) extract. Moderate antioxidant properties were also demonstrated, favoring the importance of the roots of this plant from a medicinal point of view.

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1 Introduction

Ageratum conyzoides is a weed commonly present in cultivated lands, e.g., wastelands, grasslands, pastures, and even in forests areas (Batish et al., 2006; Harjanti et al., 2019). This plant is commonly found in subtropical and tropical regions of the world. It has spread to Southeast Asia, West Africa, South America, Southeastern North America, Central America, the Caribbean, and Florida (USA) (Okunade 2002; Bamidele et al., 2010). In Pakistan, it is widely distributed in Islamabad, Rawalpindi, and Changa Manga Forest near Lahore (Zafar et al., 2006). The roots and leaves of *A. conyzoides* are of great medicinal value. They are used for the treatment of an array of disorders such as burns and wounds. Due to excellent antioxidant, antibacterial, and antifungal properties, medicinal plants have been used for several bacterial infections, dyspnea, arthrosis, headaches, analgesic, anti-inflammatory, pneumonia, anti-asthmatic, anti-spasmodic, and haemostatic effects, stomach ailments, gynaecological diseases, and other skin disorders (Sachin et al., 2009; Kamboj et al., 2010; Al-Kaabi et al., 2021). In a murine ascites Dalton's lymphoma *in vivo*, the aqueous extract of roots of *A. conyzoides* declined glutathione in the liver and the lymphoma cells of the tumor-bearing mice. In the opinion of the authors, this could be one step in producing the anti-tumor effect (Rosangkima & Prasad, 2004).

There is a growing scientific concern in finding plants having high medicinal potential. Due to their chemical structure, plant extracts have been shown to exhibit improved capability to substitute these mediators of their multi-step processes for the efficient treatment of painful and inflammatory processes (Wang et al., 2013; Khalid et al., 2017a,b,c; Bilal et al., 2018; Shafiq et al., 2021). Nowadays, diarrhea is a very common disease in several countries, and it is the leading cause of morbidity and mortality, especially among the lower-age population (Wendel et al., 2008; Muhammad et al., 2012). Medicinal plants have a central role in the advancements of modern technologies on anti-diarrheal activities of naturally extracted compounds (Panda et al., 2012; Rawat et al., 2017). *S. aureus*, also known as golden staph causes pimples, scalded skin, pneumonia, and toxic shock syndrome. *Pseudomonas aeruginosa* is a casual microorganism of the urinary tract, pulmonary tract, and blood infections, while *Klebsiella pneumonia* causes pneumonia, wound infection, diarrhea, urinary tract infections, and upper respiratory tract infection. The research for efficient and safer agents for biomedical purposes has continued to be a hot area among the scientific community (Ishaq et al., 2019; Khalid et al., 2019; Bilal et al., 2020; Munir et al., 2021). Therefore, the WHO has encouraged scientific work on the evaluation of traditional medicinal plants for the treatment of common health-causing issues worldwide (Uddin et al., 2009; Al-Kaabi et al., 2021). This study was designed to determine the phytochemicals present in the root extract of *A. conyzoides*. Moreover, the evaluation of their

antioxidant, antibacterial and antifungal potential was a key purpose of broadening the role of *A. conyzoides* chemicals in the modern biomedical industry.

2 Materials and Methods

2.1 Collection of raw materials

The root samples of *A. conyzoides* were obtained from I-9/4 Islamabad, Punjab-Pakistan. The plant species (specimen number–84902) was authenticated by the Department of Plant Sciences, Quaid-i-Azam University, Islamabad, Pakistan. The samples were preserved in the herbarium of the Department of Plant Sciences. The plant specimen was cleaned with tap water and air-dried before preservation.

2.2 Development of plant extract

Powdered (1.5 kg) roots were successively extracted with the increased polarity of solvents such as *n*-hexane, dimethyl ketone (Me₂CO), ethanol (EtOH), and EtOH:H₂O (1:1). At each stage, a sufficient amount of solvent was added, and the resulting slurry was subjected to mechanical stirring for at least 24 h. The slurry was also irradiated with ultrasonic waves for efficient extraction. The extract was decanted, and the solvent was driven off under the vacuum. Plant extracts with 1 mg/ml concentration were used for the determination of bioactivities.

2.3 Screening of phytochemicals

The phytochemicals screening of the above-mentioned plant extracts was performed by following the previously reported protocols of Trease & Evans (1989) and Cannell, (1998). A brief description of methodologies has been given in sub-sections below to determine the secondary metabolites such as alkaloids, tannins, saponins, flavonoids, terpenoids, sterols, glycosides, coumarins, anthraquinones, anthocyanidins, and phlobatannins (Okamoto et al., 2006; Tiwari et al., 2011; Ganesh et al., 2011; Mulugeta et al., 2021)

2.3.1 Alkaloids (Dragendroff's test)

For the determination of alkaloids, plant extracts (0.2 g) were added with 2% sulfuric acid and incubated for 2 minutes. The mixtures were filtered using filter paper, and a few drops of Dragendroff's reagent were added. The formation of orange-red precipitates indicated the existence of alkaloids in root extracts.

2.3.2 Tannins (FeCl₃ test)

The plant extracts (0.5g) were added to a test tube containing 10 mL of H₂O. The samples were boiled and filtered. Few drops of FeCl₃ (0.1%) were added. The appearance of blue-black or brownish-green coloration confirmed the existence of tannins in the root extracts of the plant.

2.3.3 Saponins (Frothing test)

The plant extracts (0.5g) were dissolved in boiling water. The mixtures were gently shaken for mixing and allowed to cool for a few minutes. Persistent froth appeared indicating the existence of saponins in the extracts.

2.3.4 Flavonoids (Alkali test)

The plant extracts (0.2g) were dispersed in dilute sodium hydroxide and added with the dilute HCl. The yellowish color of the solution was disappeared indicating the existence of flavonoids in the plant extracts.

2.3.5 Terpenoids (Salkowski test)

The roots extracts(0.5g) were added with CHCl₃(2 mL) along with concentrate H₂SO₄ (3 mL). The appearance of the reddish-brown color of the interface indicated the existence of terpenoids in plant extracts.

2.3.6 Sterols (Lieberman-Burchard reaction)

The concentrate H₂SO₄ (1 mL) was added to 1 mL of each extract of *A. conyzoides* roots using a test tube. The appearance of a red coloration indicated the existence of sterols in plant extracts.

2.3.7 Glycosides (Keller-Killiani test)

The root extracts (0.5g) were diluted by the addition of H₂O (5 mL), followed by the addition of glacial HOAc (2 mL). The mixture was added with one drop of FeCl₃ solution and 1 mL of concentrate H₂SO₄. The interfacial brown ring formation showed the existence of deoxy-sugar properties of glycosides.

2.3.8 Coumarins (NaOH test)

The root extracts (0.5g) were taken in test tubes, wrapped with filter paper, and soaked with NaOH (1M). Test tubes were kept in a water bath for few minutes. The test tubes were examined under UV light for yellow fluorescence which showed the existence of coumarins.

2.3.9 Anthraquinones

Plant extracts (0.5 g) were placed in 10% of HCl and boiled for few minutes in the water bath. After gentle filtration, the mixtures were allowed to cool. The mixture was equilibrated with CHCl₃ along with a few drops of 10% solution of NH₃. The mixture was heated, and the formation of rose-pink color confirmed the presence of anthraquinones.

2.3.10 Anthocyanidins

The root extracts (0.5g) were added with HCl (1 mL). The presence of red coloration indicated the existence of anthocyanidins.

2.3.11 Phlobatannins

The root extracts (0.5g) were mixed in dH₂O and filtered. The filtered solution was boiled with 2% of HCl. The formation of red precipitates confirmed the existence of phlobatannins.

2.4 Determination of antibacterial activity

Antibacterial activities of different root extracts were analyzed against four different bacteria (*Bacillus subtilis*–ATCC 6059, *Streptococcus aureus*–ATCC 6538, *Klebsiella pneumonia*–ATCC 4352, and *Pseudomonas aeruginosa*–ATCC 7221) using the previously described protocol (Mehreen et al., 2016). For this purpose, samples were prepared by mixing crude extracts (1 mg) in 1 mL of dimethyl sulfoxide (DMSO) as a negative control, whereas two different antibiotics were used as a positive control e.g., Chloramphenicol (positive control-1) and Streptomycetes (positive control-2). The nutrient agar media was prepared by the standard protocol. Seeded agar plates were prepared by pouring 75 mL of media into the petri dish. When the agar was solidified, the sterile cork borer (5 mm) was used for the formation of four wells per plate. The samples (100 µL) were poured along with the positive as well as a negative control in the respective wells of the petri dish with the help of a micropipette. The plates were placed at 37 °C for incubation. After 24 hrs, the zone of inhibition was calculated with the help of a scale.

2.5 Determination of antifungal activity

Antifungal activities of crude root extracts were analyzed using the agar-tube-dilution process (Washington & Sutter, 1980). Two toxic fungal strains (*Fusarium moniliforme* and *Helminthosporium sativum*) were used for the evaluation of anti-fungal potentialities. The strains were obtained from the Microbiology Department of Quaid-i-Azam University, Pakistan. Both fungal cultures were maintained on Sabouraud dextrose agar (SDA) media at kept at refrigerator temperature (4 °C). The samples were prepared from the initial stock of 1 mg of crude extracts in 1 mL of DMSO. Another tube from each specimen was prepared without plant extract which acted as a negative control. While standard fungicide Chloramphenicol (1 mg/ml) was kept as a positive control. The test tubes were placed at 28 °C for seven days. Inhibition of fungal growth was measured by the length of fungal growth, and the growth inhibition was analyzed according to positive and negative controls. Percent inhibition of fungal growth for each sample was determined by the following formula:

$$\text{Percent inhibition of fungal growth} = \frac{100 - \text{Linear growth in sample test tube (mm)}}{\text{Linear growth in control test tube (mm)}} \times 100$$

2.6 Determination of antioxidant activity

For the determination of antioxidant potentialities, different concentrations of the test sample and standard (ascorbic acids) were prepared e.g., 100, 50, 25, 12.5, 6.25, 3.15, and 1.56 µg/mL, respectively. A set of three clean and dry test tubes were taken and 2.7 mL of DPPH solution was added to each test tube. Ascorbic acid solution (0.3 mL) in EtOH was added in another three test tubes including 2.7 mL of DPPH solution. Twenty-one clean and dry test tubes were taken in which 2.7 mL of DPPH reagent and 0.3 mL of different concentrations of test sample (100, 50, 25, 12.5, 6.25, 3.15, and 1.56 µg/mL) concentrations were added and mixed thoroughly. Seven test tubes were taken in which 0.3 mL of various concentrations of test samples (100, 50, 25, 12.5, 6.25, 3.15, and 1.56 µg/mL) and 2.7 mL EtOH solvent were added and run in UV as blank. After 30 min incubation period at 37 °C in dark, the absorbance of the standard solution and the resulting mixtures was determined at 517 nm with UV/vis spectrophotometer (model UV-1700 (E) 23 OCE, Shimadzu, Japan).

3 Results and Discussion

3.1 Screening of phytochemicals

Phytochemical screening of four different extracts i.e., *n*-hexane, acetone, ethanol, and EtOH:H₂O (1:1) of *A. conyzoides* was carried out to determine the detailed information regarding phytochemical constituents of *A. conyzoides* roots, which may provide an incentive for proper evaluation of this plant for its medicinal potential. Table 1 summarizes the results obtained after the screening of secondary metabolites whose presence was indicated by the above-mentioned phytochemical tests. According to the results obtained phlobatannins, anthraquinones, anthocyanidins,

saponins, and tannins were absent in all extracts. However, flavonoids, glycosides, sterols, terpenoids, and alkaloids were found in all plant extracts except for flavonoids which were absent in *n*-hexane extract and cardiac glycosides in EtOH:H₂O (1:1) extract. Coumarins were also absent in *n*-hexane and EtOH:H₂O (1:1) extracts but present in acetone and ethanol extracts. Therefore, it is concluded that phytoconstituents isolated from *A. conyzoides* roots possess a wide range of biological activities. However, their effects on the cardiovascular system, diuresis, anti-viral, spasmolytic, anti-inflammatory, and other biomedical properties are yet to be studied. Phytochemical constituents and their biological activities showed the medicinal importance of this plant (Kamboj et al., 2011; Al-Kaabi et al., 2021). Parveen et al., (2014) studied the efficiency of *Artemisia absinthium* and *A. conyzoides* in-vitro on *Rhipicephalus microplus* using the AIT test. Five different concentrations of plant extract from 1.25–20% were used in the evaluation process. The results revealed 66.7% and 40% of mortality at 20% concentration of *A. absinthium* and *A. conyzoides*, respectively. *A. conyzoides* reduced 90% of egg hatching; however, *A. absinthium* indicated 100% inhibition. The study revealed that *A. absinthium* presents higher acaricidal characteristics. Chah et al., (2006) reported excellent antibacterial and wound healing properties of *A. absinthium*.

3.2 Antibacterial activities

Antibacterial activity of different root extracts was determined against four different bacteria [Gram-positive e.g., *B. subtilis* (ATCC 6059), *S. aureus* (ATCC 6538), and Gram-negative e.g., *K. pneumonia* (ATCC 4352) and *P. aeruginosa* (ATCC 7221)] using agar-well-diffusion method. Bioassays of four different extracts of roots of *A. conyzoides* was carried out to compare the relative activities of these extracts. In the current study, the results

Table 1 Screening of phytochemicals of the different root extracts of *A. conyzoides*.

S. No.	Metabolites	Extracts			
		<i>n</i> -hexane	Me ₂ CO	EtOH	EtOH:H ₂ O(1:1)
1	Alkaloids	+	+	+	+
2	Tannins	-	-	-	-
3	Saponins	-	-	-	-
4	Sterols	+	+	+	+
5	Flavonoids	-	+	+	+
6	Terpenoids	+	+	+	+
7	Glycosides	+	+	+	-
8	Coumarins	-	+	+	-
9	Anthocyanidins	-	-	-	-
10	Anthraquinones	-	-	-	-
11	Phlobatannins	-	-	-	-

signified that all the crude extracts have exhibited strong in-vitro antibacterial activities against the different bacteria that was employed in the experiments in various degrees. The bioassays findings have been represented in Figure 1. These results showed that the antibacterial activity of EtOH extract was highest against all four strains of bacteria and was also comparable to the standard medicines used. The activity of EtOH:H₂O extract was found relatively less than EtOH extract but greater than *n*-hexane and acetone extracts, whereas the activity of *n*-hexane extract was the least. From the obtained results it can be concluded that slightly more polar compounds present in EtOH extract possess superior antibiotic character in nature in comparison to non-polar components of *n*-hexane extract and highly polar components of EtOH:H₂O extract. The graphical representation of these results has been shown in Figure 1.

3.3 Antifungal activities

Antifungal activities of crude plant extracts were determined using

the agar-tube-dilution method. Two toxic fungal strains (*F.moniliforme* and *H.sativum*) were used to analyze the antifungal activities of these *A. conyzoides* extracts. *A. conyzoides* very strongly suppressed the growth of the fungal strains used, therefore this plant is anticipated for the isolation of the compounds with excellent antifungal characteristics. Results obtained after antifungal activities have been shown in Figure 2. In the present study, the obtained results illustrated that all the crude extracts have demonstrated strong in-vitro antifungal activities against two toxic fungal strains that were used in the experiments in various degrees. Results of antifungal activity indicated the highest activity of EtOH:H₂O extract against both fungal strains. Whereas activity of acetone and ethanol extracts were also appropriate and comparable to standard drugs against *F. moniliforme*. These results indicated that polar compounds extracted by polar solvents were more antifungal. Therefore, the extract of highest polarity i.e., EtOH:H₂O extract showed the highest activity and the extract of lowest polarity i.e., *n*-hexane extract, showed the lowest activity. Results of antifungal activities have been shown in Figure 2.

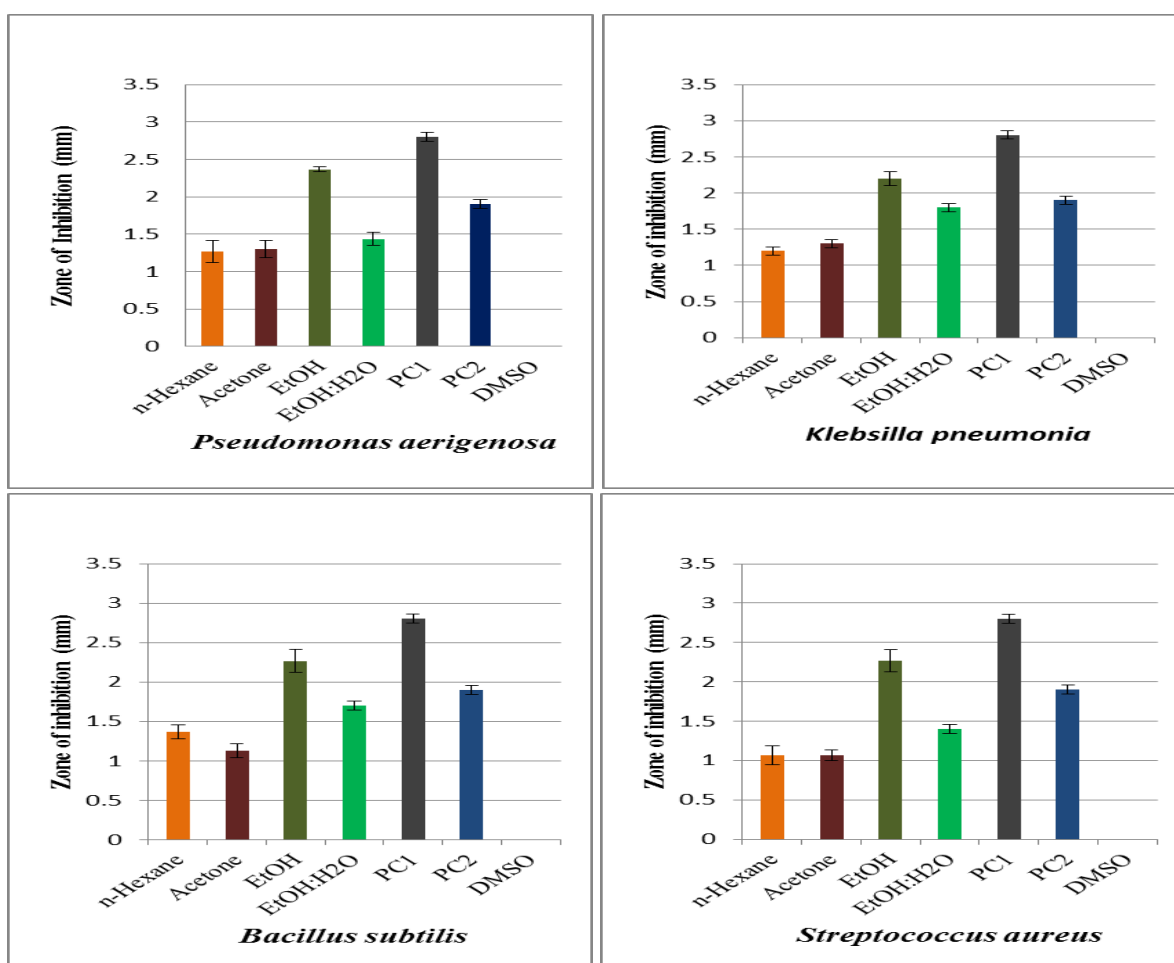


Figure 1 Graphical representation of antibacterial activities of four different extracts of *A. conyzoides* against different bacterial strains.

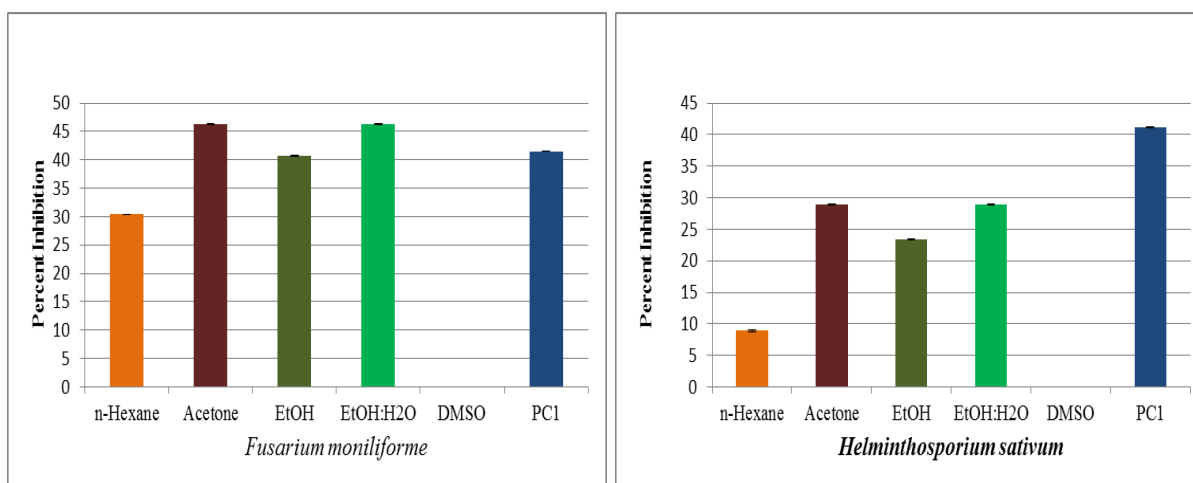


Figure 2 Graphical representation of the antifungal activity of four extracts of *A. conyzoides* against *Helminthosporium sativum* and *Fusarium moniliforme*.

Table 2 DPPH Radical Scavenging Activity of Me₂CO extract of roots of *A. conyzoides*.

S.N.	DPPH	AA	100 µg	50 µg	25 µg	12.5 µg	6.25 µg	3.12 µg
1	0.723	0.024	0.378	0.488	0.545	0.594	0.624	0.63
2	0.736	0.024	0.381	0.485	0.561	0.609	0.631	0.648
3	0.735	0.023	0.388	0.495	0.567	0.601	0.634	0.65
4	2.194	0.071	1.147	1.468	1.673	1.804	1.889	1.928
Mean	0.731	0.024	0.382	0.489	0.558	0.601	0.630	0.643
Test sample + Solvent	-	-	0.028	0.013	0.006	0.002	0.001	0.000
Final absorbance	0.731	0.024	0.354	0.476	0.552	0.599	0.629	0.643
Inhibition (%)	-	-96.72	-51.57	-34.88	-24.48	-18.058	-13.95	-12.04

3.4 Antioxidant activities

The DPPH free radical scavenging activities were presented as inhibition percentage and results are exhibited in Table 2. The obtained results varied from 12.04 to 96.72 % for the acetone extract. The antioxidant activity was considerably observed in the Me₂CO extract as compared to other extracts. The IC₅₀ values of standard ascorbic acid and Me₂CO extract were calculated with the help of EZ fit™ enzyme kinetic software (statistical software) (Table 2). From the IC₅₀ value, it could be observed that Me₂CO extract exhibits moderate antioxidant activity. Acetone extracts showed moderate hydroxyl free radical scavenging activity. This study leads to the conclusion that the polar constituents of roots of this plant exhibited higher antioxidant activity as compared with non-polar constituents.

Conclusion

The phytochemical screening exhibited the presence of various useful compounds in the root extract of *A. conyzoides* including

terpenoids, alkaloids, coumarins, sterols, flavonoids, and glycosides. Moreover, the results indicated reasonable antibacterial (against both gram-positive and gram-negative bacteria), antifungal, and antioxidant potentialities, thus favoring the importance of the roots of this plant from a medicinal point of view. Keeping in view the biological activities of the phytochemicals identified in the roots of this plant, it can be stated that this plant could be a potential candidate for modern medicinal formulations.

Conflict of interest

The authors declared that they have no conflict of interest.

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