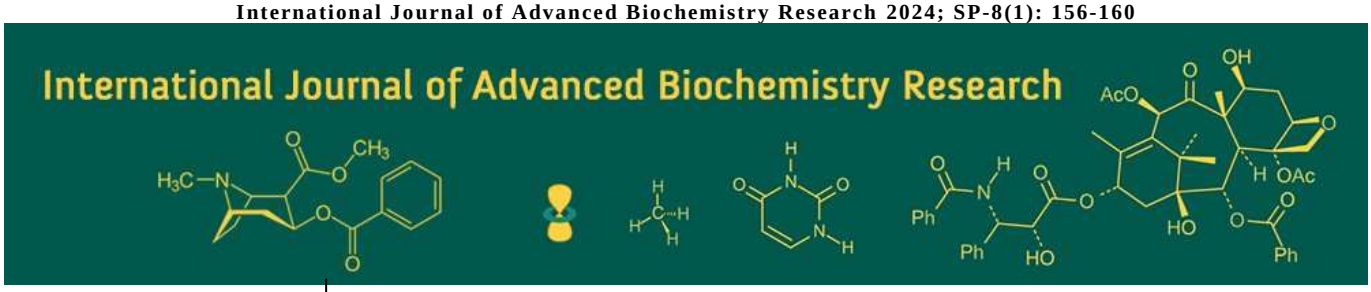

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The antimicrobial and antioxidant activity of different extracts of Kalmegh, *Andrographis paniculata* (Burm.f.) Nees

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Abstract
Andrographis paniculata (Burm.f.) Nees also known as Kaalmegh, is a medicinal plant that has been shown to have immunological, antibacterial, anti-inflammatory, anti-thrombotic, and hepatoprotective activities in human being. The therapeutic potential of such medicinal plants has recently been brought to light by some recent sudies, thus highlighting the need for a chemical assessment of this valuable medicinal plant. The antibacterial and antioxidant properties of A. paniculata were examined in the current study against some microbes. Plant extracts of A. paniculata in methanol, ethanol, hexane, and distilled water were tested against Pseudomonas sp. and Escherichia coli. The antioxidant activity was measured using the DPPH antioxidant test. The Distilled Water extract exhibited the highest antibacterial activity (17.00 mm) against both species of test microbes, whereas the hexane extract shown the lowest (10.67 mm). In the antioxidant experiment, distilled Water provided the highest activity (97.80%), while hexane extract given the lowest (75.59%). Both ethanol and methanol were moderate in term of antibacterial and antioxidant activities. Conclusively, A. paniculata is a very valuable medicinal plant that can be utilised to produce medications and can treat a wide range of illnesses in human being.

Keywords: Antimicrobial, antioxidant, activity, extract, *Andrographis paniculata*

Introduction
Medicinal plants produce a wide range of bioactive compounds, making them a valuable source for a variety of treatments. Chemicals produced by these therapeutic plants protect us from infections and subsequent illnesses inflicted by pathogens. Medicinal plants are being used in pharmaceuticals, cosmetics and nutraceuticals manufacturing also. The use of medicinal plants is an important aspect of primary healthcare because of their accessibility, acceptance, compatibility and affordability (Da Silva *et al.*, 2022) [6]. More than 80,000 plant species are thought to have been recognised and used as medicines around the world (Mathew *et al.*, 2005) [15]. India is identified as a major global source of traditional and complementary medical knowledge (Patel *et al.*, 2021) [21].

Andrographis paniculata (Burm.f.) Nees is an annual, herbaceous plant of the family Acanthaceae. It is known by different local names, such as "Kalmegh" in India, "Chuan-Xin-Lian" in China, "Fah Tha Lai" in Thailand, "Hempedubumi" in Malaysia, "Senshinren" in Japan, and "green chiretta" in Scandinavian countries (Kumar *et al.*, 2004; Hamid *et al.*, 2023) [14, 9]. This plant is also known as "King of Bitters" because to its exceptionally bitter flavour. Kalmegh is one of the most extensively used therapeutic herbs in Unani and Ayurvedic medicine (Akbar, 2011) [2].

The principal active element in *A. paniculata* is andrographolide (Weiming & Xiaotian, 1982) [32]. The aerial portion of the plant has a large number of chemical compounds, primarily lactones, diterpenoids, diterpene glycosides, flavonoids, and flavonoid glycosides (Sharma & Sharma, 2013; Hong *et al.*, 2021) [26, 11]. *A. paniculata* possesses a remarkably wide range of pharmacological actions, such as anti-inflammatory (Shen *et al.*, 2002; Sheeja *et at.*, 2006) [28, 27], antidiarrheal (Gupta *et al.*, 1990) [8], antiviral (Misra *et al.*, 1992) [17], antimalarial (Rahman *et al.*, 1999) [24], hepatoprotective (Handa & Sharma, 1990) [10],

cardiovascular (Zhang &Tan, 1997) [33], anticancer (Matsuda *et al.*,1994) [16] and immune stimulatory effects (Calabrese *et al.*, 2000) [5].

The extract obtained from the leaf of Kalmegh using methanol showed highest activity against pathogen like *Pseudomonas aeruginosa* (Alagesaboopathi, 2013) [3]. Results of various studies conducted during recent past regarding antimicrobial and antioxidant efficacy of *A. paniculata* against some bacteria have provided insight that this plant has potential to inhibit certain bacteria like *Escherichia coli and Pseudomonas* spp. under laboratory conditions (Mishra *et al.*, 2009; Nagaraja & Chandrashekhar, 2014; Kuan, 2015; Adaramola *et al.*,2018; Dedhia *et al.*, 2018) [17, 19, 13, 1, 7]. The leaf extract of *A. paniculata* have great potential as antimicrobial compounds against bacteria like *E. coli*, *S. aureus*, and *Salmonella typhimurium* (Singh *et al.*, 2017) [29]. Various solvent fractions of crude methanol extract of *A. paniculata*; especially the ethyl acetate fraction could be considered a remedy for various infections and diseases which are associated with both the test organisms and free radicals (Adaramola *et al.*, 2018) [1]. Nancy and Banu (2017) [20] have proven Inhibition of antibiofilm mediated Virulence Factors in *P. aeruginosa* by *A. paniculata*.

The extracts obtained from amaranth in vegetative, early flowering and grain fill stages due to high content of hydroxycinnamic acid derivatives and rutin can be a valuable source of antioxidants that can be exploited for the production of nutraceuticals or used as a functional food ingredient (Karamac *et al.*, 2019) [12]. The percentage of DPPH scavenging activity reported in *Amaranthus spinosus* (Rajasekaran *et al.*, 2014) [25], and in *A. paniculata* (Thoo *et al.*, 2013) [31] have revealed that these plants have excellent antioxidant qualities and may be useful in the treatment of disorders caused by free radicals.

The primary objective of present research work is to investigate the antibacterial capabilities of *A. paniculata* against some bacterial species, along with assessment of antioxidant activity of this novel plant extracts in hexane, distilled water, ethanol and methanol to keep the health of human being at the best.

Materials and Methods

The study was carried out at Biotechnology laboratory, SGRR University, Dehradun, Uttarakhand, India. Dehradun experiences a humid subtropical climate. The average elevation is 450 m above sea level. The city is situated between latitudes 30°01' N and 31°2'N and longitudes 77°34' E and 78°18'E.

Collection of plants

The plant of *A. paniculata* was collected from the herbal garden of SGRR University, Dehradun, during year 2022 and 2023. The plant was washed in tap water and then dried in a shady place for a few days for removal of moisture content. After removal of moisture, plant was powdered in the mixer-grinder. The powdered material was then stored in an airtight container.

Preparation of extracts

Five gram dried powdered of *A. paniculata* was added to 100 ml solvent (distilled water, Methanol, Ethanol, Hexane each) and left in a conical flask for a about 48 hrs at room temperature on a shaker. This was done using all four extractants. The extract solutions were filtered using Whatman No. 1 Filter paper into a clean beaker and subjected to drying. The extracts obtained were then collected in the vials and stored in a refrigerator at 4 °C for antimicrobial and antioxidant analysis.

Test organisms

The test organisms used were collected from the microbiology laboratory of SGRR University, Dehradun, Uttarakhand, India. The two bacterial cultures selected for the study were *Escherichia Coli* and *Pseudomonas* spp.. A nutrient broth was prepared for the inoculation of bacterial culture. Prepared nutrient broth was transferred into sterile test tubes, so that, each tube was filled with 20 ml broth. All work is done in laminar air flow to maintain sterilized conditions. Different bacterial cultures were taken from the already cultured plates by using sterile inoculating loops and inoculated into the separate test tubes. Inoculated test tubes were incubated for 24 hours at 37 °C in a B.O.D. incubator.

Antimicrobial activity by Kirby-Bauer method

The antimicrobial activity of the plant extract was detected by the Kirby-Bauer Method. The 75 ml nutrient agar medium was sterilized and poured into 3 sterilized petri-plates. Approximately 100 µL of inoculum (*Pseudomonas* spp.) was poured onto the plates by micropipette. Culture was spread by a glass spreader. Discs were prepared from Whatman No. 1 Filter Paper and autoclaved. Ten different concentrations of each extract were made. Different concentrations taken were: 2mg/mL, 4 mg/mL, 6 mg/mL, 8 mg/mL, 10 mg/mL, 12 mg/mL, 14 mg/mL, 16 mg/mL, 18 mg/mL, and 20 mg/mL. A disc of control was placed and ten discs of different concentrations of sample were loaded on each plate. The antibiotic streptomycin disc (10 mcg) was used in the test system as a positive control. Five concentrations of extracts were made of these higher concentrations, and this was tested against *E. coli*. These different concentrations were: 10 mg/mL, 20 mg/mL, 30 mg/mL, 40 mg/mL, and 50 mg/mL. The procedure described above was repeated, but this time the antibiotic tetracycline disc was used as a positive control (To compare with both antibiotics). Plates were then incubated at 37 °C for overnight. The zone of inhibition was observed and recorded against control (Annonymus, 1993) [4].

Antioxidant activity by DPPH Antioxidant Assay

The antioxidant activity of plant extract was determined by the DPPH antioxidant assay. For this test, the first 45 ml of DPPH (7.89 mg/100 mL) solution was made in methanol in a conical flask. The flask was covered with aluminium foil and kept in a dark place for 2 hours. Then, we taken 1 ml of methanol and make four concentrations, i.e. 25 mg/mL, 50 mg/mL, 75 mg/mL and 100 mg/mL of each extract in methanol. After 2 hrs, we taken the DPPH solution and added 1 mL of the solution to each conc. The reaction mixtures were mixed and incubated in the dark at room temperature for 30 minutes. The absorbance data was then recorded at 517 nm. (Soler *et al.*, 2000) [30].

Results and Discussion

Antimicrobial activity of different extracts of *A. paniculata*

The results of the antimicrobial activity of *A. paniculata* are

shown in tables 1 and 2 respectively. The plant extracts of *A. paniculata* presented different degrees of inhibition against the test bacteria. The distilled water extract showed the best result when compared with the standard antibiotic, i.e., streptomycin. The methanol, ethanol, water and hexane extracts of *A. paniculata* indicated inhibition zone diameter of 15.33 mm, l5.00 mm, 17.00 mm, and l0.67 mm separately at a concentration of 20 mg /ml against *P. aeruginosa,* (Table 1). The methanol and water extract of *A. paniculata* possessed higher antibacterial activity against *P. aeruginosa*. Thus from the result it is revealed that the antibacterial activity is maximum at 50 mg/ml concentration. As the concentration of the extract increases, the diameter of the inhibition zone also increased. The antibacterial activity of the methanol and water extract of *A. paniculata* might be due to the presence of flavonoids, terpenoids and polyphenols.

In the present study resistant nature of *E. coli* may be due to the presence of andrographolide. Results of various studies conducted during recent past regarding antimicrobial and antioxidant efficacy of *A. paniculata* against some bacteria have provided insight that this plant has potential to inhibit certain bacteria like *Escherichia coli and Pseudomonas* spp. under laboratory conditions. Our results are in the line of findings reported by Mishra *et al.*, 2009 [17]; Adaramola *et al.,* 2018 [1]; Dedhia *et al.*, 2018 [7]; Kuan, 2015 [13] and Nagaraja & Chandrashekhar, 2014 [19]. Studies conducted by Nancy and Banu, (2017) [20] also supported our work.

Antioxidant activity of different extracts of *A. paniculata*

After monitoring the ODs at 517 nm, we can conclude that plant extracts have strong antioxidant activity. The graph in the results shows that with increasing optical density, the antioxidant activity decreases and vice-versa. The distilled water extract showed the best results for antioxidant activity, while the hexane showed the least activity. The results of the test are shown in tables 3 and 4, and the graphs are shown in figs. 1 and 2.

DPPH scavenging activity

DPPH Scavenging activity has been used to analyze the free radical scavenging capability of the different extracts of *A. paniculata*. DPPH is one of the stable organic lipophilic radical. When the antioxidant reacts with DPPH, the molecule transfers an electron or hydrogen atom to DPPH, by neutralizing it (Pattanayak *et al.*, 2012) [22].

The DPPH scavenging activity helps to evaluate the antioxidant potentiality in a very short time. In the present study, the distilled water extract showed higher level of free radical scavenging activity compared to methanol, ethanol and hexane extracts.

The increasing concentration of the extracts raised the potential to scavenge the free radical. The ethanol, methanol, hexane and water extract showed significant percentage of inhibition over DPPH such as 85.88%, 83.91% and 75.59% and 97.80% respectively at 100 mg/ml concentration. Among these the water extract showed highest DPPH free radical scavenging activity (Table 3 and 4 and Fig.1 and 2). The free radical scavenging activities might be due to the presence of phenolics. The percentage of DPPH scavenging activity was comparable to the earlier results found after screening of *Amaranthus spinosus* for anti-oxidant test conducted by Rajasekaran *et al.*, 2014 [25], and results of studies on *A. paniculata* Ireported by Thoo *et al.*, 2013 [31].

Tables and Graphs

Table 1: Antimicrobial activity of different extracts of *A. paniculata* against *Pseudomonas sp.* showing zones of inhibition

Test Organism:- *Pseudomonas sp.*						
Control ZOI (mm)	Streptomycin 22	Zone of Inhibition (mm) mean value				Control
Sample Concentrations		Meth. Ext.	Eth. Ext.	Wat. Ext.	Hex. Ext.	DMSO
1.	2 mg/mL	-	-	-	-	Nil
2.	4 mg/mL	-	-	-	-	Nil
3.	6 mg/mL	8.33	-	-	-	Nil
4.	8 mg/mL	9.00	7.00	8.67	-	Nil
5.	10 mg/mL	10.00	7.67	9.33	-	Nil
6.	12 mg/mL	10.67	9.67	9.33	-	Nil
7.	14 mg/mL	12.00	11.67	10.67	-	Nil
8.	16 mg/mL	13.33	12.33	11.00	9.67	Nil
9.	18 mg/mL	14.00	13.67	14.67	10.00	Nil
10.	20 mg/mL	15.33	15.00	17.00	10.67	Nil

Table 2: Antimicrobial activity of different extracts of *A. paniculata* against *E. coli* showing zones of inhibition

Test Organism:- *Escherichia coli*						
Control - Tetracycline ZOI (mm)- 26.75 (mean)		Zone of Inhibition (mm) mean value				Control
Sample Concentrations		Meth. Ext.	Eth. Ext.	Wat. Ext.	Hex. Ext.	DMSO
1.	10 mg/mL	-	-	-	-	Nil
2.	20 mg/mL	-	-	11.67	-	Nil
3.	30 mg/mL	-	10.00	13.00	-	Nil
4.	40 mg/mL	12.67	11.33	13.67	-	Nil
5.	50 mg/mL	14	16.33	16.00	13.67	Nil

Table 3: ODs of the four extracts of *A. paniculata* at different concentrations recorded at 517nm wave length

Concentration (mg/ml)	Ethanol	Methanol	Hexane	Distilled water
25	0.433	0.425	1.308	0.280
50	0.413	0.402	0.862	0.226
75	0.269	0.329	0.664	0.054
100	0.251	0.286	0.434	0.039

-OD of control (Methanol + DPPH) - 1.778

Table 4: DPPH assay showing antioxidant activity (%) of the four extracts of *A. paniculata*

Concentration (mg/ml)	Ethanol (%)	Methanol (%)	Hexane (%)	Distilled water (%)
25	75.64	76.09	26.43	84.25
50	76.77	77.39	51.51	87.28
75	84.87	81.49	62.65	96.96
100	85.88	83.91	75.59	97.80

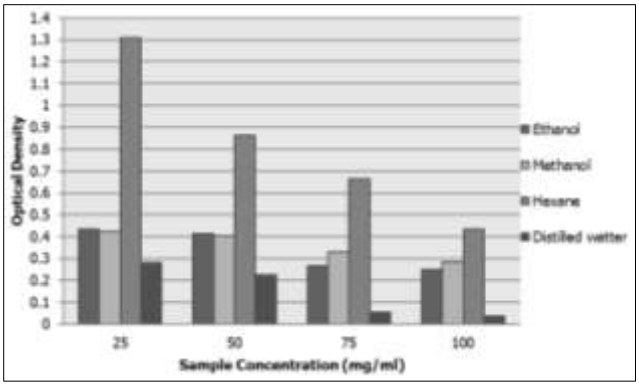

Fig 1: Line graph showing the optical density of extracts observed at 517nm

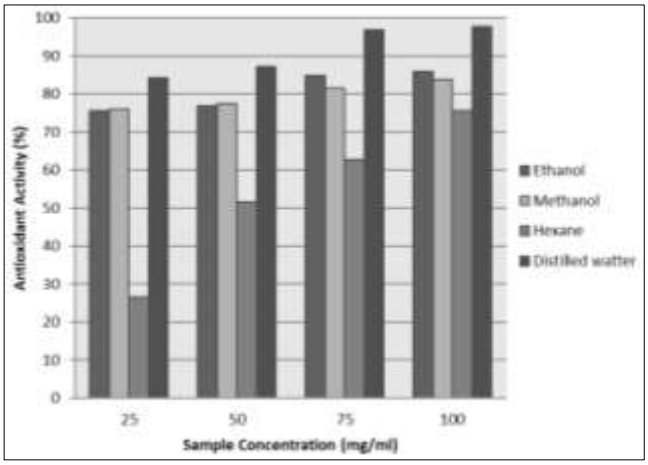

Fig 2: Antioxidant activity of the different extracts of *A. paniculata*

Conclusion

According to the study's findings, *A. paniculata* extracts had a significant inhibitory effect on the bacterial species like *E. coli* and *P. aeruginosa*. This finding conclude that *A. paniculata* can be used as an antibacterial agent. Again it is imperious to state that *A. paniculata* is also an effective antioxidant agent. Taking into account all of these findings, we can conclude that *A. paniculata* is a very valuable medicinal plant that can be utilised to produce herbal medicines and can treat a wide range of illnesses in human being.

Future Scope

It is necessary to find out and isolate novel bioactive chemicals from medicinal plants such as *A. paniculata* due to the rising prevalence of drug-resistant diseases. In the future, medicinal properties of medicinal plants like *A. paniculata* will offer new, strategies against pathogenic microorganisms.

Conflict of interest

Authors have no conflict of interest in term of financial, personal or other relationships with any person, Institution or any other organization.

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