How to Cite:
Rawat, D., Keerti, K., & Panwar, M. (2022). Antimicrobial activity of A. paniculata against isolated MDR strains. *International Journal of Health Sciences*, *6*(S4), 10618–10630. https://doi.org/10.53730/ijhs.v6nS4.11382

Antimicrobial activity of A. paniculata against isolated MDR strains

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***Abstract*---**Antibiotic resistance is a major problem and the greatest challenge to both hospitals and in the community. Initially, antibiotic-resistant strains were found to the hospital environment only, but now they can be present everywhere. It was therefore decided to conduct a short-term study on Andrographis paniculata's ability to inhibit the growth of some of the most resistant bacteria in the Doon Valley. Antimicrobial activity of *Andrographis paniculate* of leaf and steam extract was performed against different MDR isolates. The VITEK2 COMPACT device with a consistent inoculum was used to identify and evaluate the antimicrobial susceptibility of clinical samples obtained in suspected critical patients at Indresh hospitals in Dehradun District. Clinical and laboratory guidelines were followed during the testing process (Shri Mahent Indresh Hospital). Steam and leaf extract from *A. paniculata* leaf was tested for antibacterial activities using the disc diffusion method. The present study showing that the leaf extract and steam extract petroleum ether, acetone and ethyle acetate extract are most active against *K. pneumoniae, E. coli, Proteus mirabilis, Pseudomonas aeruginosa, Acetinobacter, Pseudomonas sp. , Enterobacterium (MDR strains)* and *Candia albugin, Aspergillus niger, Fusarium, penicillium (pathogenic fungus.* The result of the present research is the high potency of extracts to stop the growth of MDR strains and this extract can be further suggest for as natural antimicrobial source.

***Keywords*---**Andrographis paniculata, antimicrobial activity, MDR strains.

Introduction

Plants have been a source of safe and effective medicines since the dawn of mankind. Many countries' primary healthcare has relied heavily on herbal remedies. In the world, almost 80% of people rely on traditional medicine. To put it simply, herbal medicines are any finished, labelled medicinal products containing active ingredients that come from plants in the form of extracts of the aerial or underground parts of plants, as well as any combination of the two. Essential oils, juices, gums, fatty acids, and other compounds derived from plants are all examples of plant materials. Excipients may also be found in herbal medicines, along with the active ingredients. Chemically defined bioactive molecules in combination with plant materials, such as chemically defined isolates of plants, are not herbal medicines (WHO, 1998). [1]

Plants as Alternative Source of Antimicrobials

Biochemical and medicinal stores have never been found on our planet larger than those found in plants. As long as there are living organisms around, they can produce infinite biological molecules. Only 1 to 10 percent of the plants on Earth are used by humans and animals for their daily needs (250,000 to 500,000 species). A wide range of secondary metabolites with antimicrobial characteristics can be found in medicinal plants. These include saponines, tannins, alkaloids, sesquiterpene lactones, glycoalkaloids, flavonoids, terpenoids and phorbol esters [2]. A noted mega-diversity centre, India is home to a large number of medicinal plant species, each of which is likely to have genetic and chemical variants of commercial relevance. Ayurveda, Siddha, and Unani Andrographis paniculate are just few of the thousands of plant species that have been utilised in traditional Indian medicine for ages.

Andrographis paniculate

Kalmegh (Andrographis paniculata), often known as "king of bitter," is a major annual medicinal herb found throughout Madhya Pradesh, India. It is a member of the Acanthaceae family. In tropical moist deciduous forests, this tall plant grows mostly as an under shrub. In Ayurvedic formulations, it is one of the most commonly utilised plants [3]. Sannipata heat, difficulties in breath, hemopathy burning pain and cough are some of the symptoms that might be alleviated with this remedy. Acidity and liver problems can also benefit from its use [4]

Morphology of *Andrographis paniculate*

Plant Andrographis paniculata in damp, shady areas to a height of 30–110 cm and it will thrive. A wide range of biological properties have been described as herbal treatments for the roots, leaves, stem, bark, and flowers of this plant including hepatoprotective, antitumor, immunomodulator, anti-inflammatory, wound closure, antioxidants, and antitubercular, and it is a healthy medicinal

plant [5]. Hydro-alcoholic leaf extracts of three species, A. paniculate, produced by the Soxhlet extractor were tested for their antibacterial properties against disease-causing bacteria. The goal of this research is to increase the antibacterial activity of plant materials against pathogenic microbes, which can be used in a variety of ways. A small winged petiole adorns each leaf. On both surfaces of the lamina, glandular and non-glandular hairs are present. White flowers with rose purple dots on the petals. Capsule-shaped, linearly-oblong fruits with pointy ends. They measure approximately 2 centimeters in length and a few millimeters in width. Numerous seeds, sub quadrate in shape and yellowish brown in colour, are contained within the fruit [6].

Andrographis paniculata: plant *Andrographis paniculata*: leaves

Andrographis paniculata used as crude drug

The Indian herbal treatment Andrographis paniculata is popular. It is called a crude medicine if the plant's leaves, stems, as well as other aerial components are consumed. There are times when the entire plant is used including the root. The plant's panchang (stems, leaves, flowers, roots, and seeds) is utilised in a variety of Indian medicine formulations to cure a wide range of ailments. There should be no more than 2% foreign organic materials in the medication [7].

Medicinal Use of _Andrographis Paniculata_

Andrographis paniculata has been utilised for a variety of medical purposes in traditional Indian siddha and ayurveda medicine, as well as tribal medicine, since ancient times. As a common household remedy, Kalmegh (also known as "green chiretta") contains this herb. Itching can be alleviated by mixing powdered plant with mustard oil [8]. As an over-the-counter medication for gastroenteritis as well as other gastrointestinal disorders, child's flatulence and diarrhoea could be treated with macerated leaves or juice, along with certain spices, as well. It is prescribed for hepatic torpor, nerve pain, and during the recovery period following a fever. An infusion of the flower is used to treat fever, while a decoction is used to purify the blood. As a decoction and infusion of a leaves can be helpful in cases of general exhaustion and dyspepsia It is also used as a febrifuge, a tonic, an

anthelmintic, a stomachic, and a cholagogue [9]. One of the most commonly utilised species in ayurvedic formulations is Andrographis paniculata, or Kalmegh. In the Charaka Samhita, which dates back to 175 BC, Andrographis paniculata and other plants were advised for the treatment of jaundice. [10]. Also traditionally used as an antidote in cases of colic dysentery or dyspepsia for liver sluggishness [11].

Material and Methods

Collection of plant *A. paniculate*

A. Paniculate is collected from the Himalayan region of Uttrakhand. The morphology characteristics were observed and recorded in the gardern. For further investigation. The plant were further identified by Botanical survey of India (BSI) Dehradun (Acc.No.507).

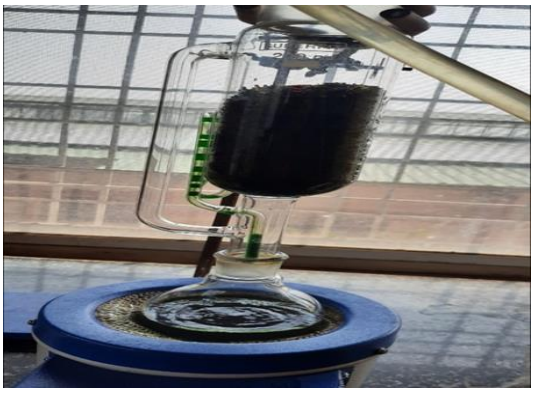
Preparation of plant extract

Using a mechanical grinder, we ground up 100 grammes of fresh Andrographis paniculate leaf material. These leaves were dried in the shade for three weeks before being surface sterilised with 70 percent ethanol [10].

Extraction of *Andrographis paniculate*

These seven solvents are employed in the extraction development of plant leaves and steam parts: petroleum ether, ethanol, benzene, methanol, ethyl acetate, methanol, and distilled water. Using a soxhlet extractor setup, which includes an open-bottom flask, a syphon tube and other distillation-related components like expansion adapters and condensers as well as heat sources and thimbles, we were able to extract the active ingredient. Soxhlet's thimble chamber is filled with an Andrographis paniculate leaf powder, which is held in place by a porous bag or "thimble" consisting of strong filter paper or cellulose. The heating mantle was used to warm 350 ml of petroleum ether in a round bottom flask. The extraction solvent affects the temperature of the heating process. Heated condenser returns evaporating solvent to sample thimble from which it was drawn out in the bottom flask. Siphon tube clean solution signifies that a process has completed. This process is employes for all the remaining solvents.

Andrographis paniculata: shead dried leave



Soxhlet apparatus: extraction process

Tested Bacterial strains

Total number of 6 bacterial strains i.e. *K. pneumoniae, E. coli, Proteus mirabilis, Pseudomonas aeruginosa, Acetinobacter, Pseudomonas sp. , Enterobacterium* and 4 fungus strains i.e. *Candia albugin, Aspergillus niger, Fusarium, penicillium* were used in this study. These MDR strains were isolated from Indresh hospital Dehradun with the approval of ethical committee ECR/710/Inst/UK/2015/RR-18

Screening for Antibacterial Activity

The antibacterial activity of *A. paniculata* extracts was determined to use the agar disc diffusion. 6 bacterial strains i.e. *K. pneumoniae, E. coli, Proteus mirabilis, Pseudomonas aeruginosa, Acetinobacter, Pseudomonas sp. , Enterobacterium*and 4 fungus strains i.e. *Candia albugin, Aspergillus niger, Fusarium, penicillium* were used. After 24 hours of growth at 37 degrees Celsius, all bacteria were transferred to tissue culture flasks. For each bacterium, 2 to 3 colonies were transferred to an appropriate liquid medium (Mueller Hinton Broth) then cultured until adequate development of turbidity equal to the McFarland standard was achieved. The Mueller Hinton plates were inoculated with the bacteria's inoculum. Ten percent DMSO was used to dissolve all dried plant extracts and sterilise them by filtering them through 0.45-mm membrane filters. Filter paper plates (6 mm) (Whatman No. 1) were perforated and soaked with 100 l of the extract, then dried at room temperature. All of the test strains were inoculated on Mueller-Hinton agar plates. Incubation at 37°C for 24 hours at room temperature was the final step. Antibacterial activity was measured by measuring the size of the inhibitory zone around the disc (in millimetres). The inhibition zone generated by the discs around which antibacterial activity was measured was used to make the determination. We reported the average values from our three experiments. Ten percent DMSO was employed as a negative control, while tetracycline and gentamicin were utilised as positive controls.

Phytochemical evaluation of the most active extracts

For the presence of Phenolic acids, Flavonoids, Carboids, Alkaloids, Tannins, Anthroquinons, Terpenes, Steroids, Glycosides, Cardic glycosides, and Xanthoproteins, different extractions were analysed using established techniques.

Test for Anthraquinones

Anthraquinones were detected in the ammonia phase by observing the colour of the filtrate, which was pink, violet, or red, after 10 minutes of soaking in benzene and 10 ml of 10% ammonium hydroxide, which was forcefully shaken for 30 seconds.

Test for Tannins

The 0.5g of extraction was treated with 10 ml liquid bromine water. Tannins were detected through the discoloration of bromine water.

Test for Saponins

In a test tube, aqueous crude plant material was combined with 5.0 ml distilled water and thoroughly mixed. Saponins were detected in the foaming when it was blended with some drops of olive oil.

Test for Phenols

FeCl3 solution was added to the crude extract at a volume of 1.0 ml. Flavonoids were found to be present because of the dark green tint.

Test for Steroids

Chloroform was used to dissolve the crude extract. Sulphuric acid, H2SO4, will be gently added to create a lower layer that is yellow in colour and emits green fluorescence, as shown in the following image. A steroid ring was thought to be visible because of the reddish brown coloration on the upper layer.

Test for Sugar

Mixtures of plant extract and water were prepared by diluting the mixture to 1.5%. Then, the extract was subjected to equal volumes of boiling Fehling A and B solutions in separate test tubes. Because of the oxidizing and reducing sugars, the brick red precipitate was assumed to form.

Test for Terpenoids

Chloroform was diluted with 0.4 cc of plant extract. Two layers will be generated by adding 0.6 ml of concentrated H2SO4. Coloration inside the interface indicates the presence of terpenoids, as shown by the reddish brown hue.

Test for Flavonoids

Boiling water will be added to the extract and the mixture will be cooked for 5 minutes. There will be three drops of a 20% NaOH solution added. The transformation from a colourless state to a golden one. Next, 5 drops of HCl at a concentration of 1% will be applied. The decolorization of a yellow colour was used to determine the presence of flavonoids.

Result

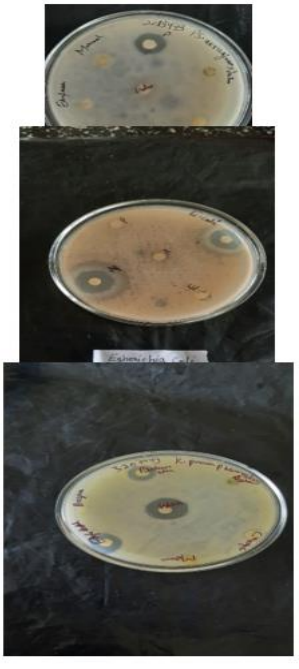
Petroleum ether of leave extract shows maximum result and acetone and ethyle acetate extract of steam extract of *Andrographis paniculata* against microorganisms. Among the several extracts of the *A. paniculata* leaves and steam, the quantitative antibacterial assay found that the Petroleum ether (leaf extract) and ethyl acetate (from the steam extract) showed the greatest antibacterial activity against MDR strains. In table 2 indicates that the ethyl acetate extract of A. paniculata have higher activity against *P.aeruginosa.*

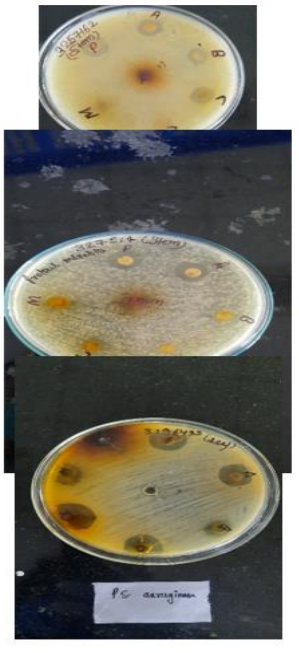
Table 1: Bacterial strains activity in leaf extract

Bacterial strains	Zone of inhibition (mm)							
	Petroleum ether	Acetone	Benze-ne	Chlo ro-form	Ethyl acetate	Methanol	Distilled water	STM
E. coli	15	15	13	8	-	24	-	-
Proteus mirabilis	19	16	-	10	20	-	-	-
P.aeruginosa	28	22	10	12	18	11	-	-
Acibaumanni	16	12	14	10	-	8	16	-
Pseudomonas sp.	19	-	-	11	-	-	-	-
Enterobacterium	22	-	-	14	-	-	-	-
K. pneumonae	30	16	-		22	-	22	-

Table 2: Bacterial strains activity in steam extract

Bacterial strains	Zone of inhibition (mm)							
	Petroleum ether	Acetone	Benze-ne	Chloro -form	Ethyl acetat e	Methanol	Distilled water	STM
E. coli	-	10	15	14	22	-	-	-
Proteus mirabilis	15	25	18	11	14	-	24	-
P.aeruginosa	-	23	22	16	28	-	-	-
Acetinobacter	-			24	-	-	-	-
Pseudomonas sp.	-	18	16	-	18	22	-	-
Enterobacteriu m	-	-	-	-	-	-	12	-
K. pneumonae	-	26	-	22	25	-	-	-





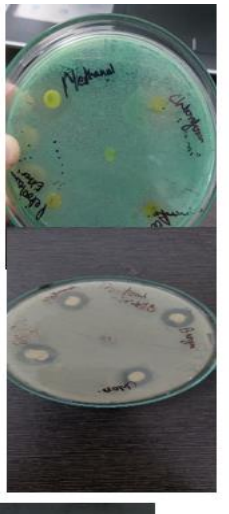


Table 3: Fungus strains activity in different solvents

	Zone of inhibition (mm)							
	Petroleum ether	Acetone	Benzene	Chloro-form	Ethyl acetate	Methanol	Distilled water	afflatoxin
Candia albugin	-	-	-	-	-	-	-	
Aspergillus niger	-	-	-	-	-	-	-	
Fusarium	11	-	22	23	22	-	-	
Penicillium	-	-	-	-	-	-	-	

Phytochemical assisment of most active extract

Presence of alkaloids, flavonoids, steroids and tannins were found in petroleum ether leaf extracts of *A. paniculata* leaves. The presence of penols, steroids, flavonoids, found in the benzene leaf extract of leaves of *A. paniculata*. The presence of flavonoids, glycosides, saponins, phenols, tannins and steroids in acetone extract of *A. paniculata* steam. And the presence of alkaloids, phenol, glycoside, terepenoid, charbohydrates were found in the ethyleacetae of A. *paniculata* steam.

Table 4: Qualitative analysis of the phytochemicals in the methanolic and aqueous extracts of *Andrographis paniculates*

	Petroleum ether leaf	Benzene leaf	Acetone steam	Ethyleacetae steam
Test for alkaloids				
Mayers test	+ve	+ve	+ve	+ve
Wagners test	+ve(Ring formation red and brown precipitate)	+ve	+ve	+ve
Test for phenol				
Ferric chloride test	-ve	+ve (greenish black colour)	+ve	+ve
Test for flavonoids				
Test for glycoside	-ve	+ve	+ve	+ve
Killer killani test	-ve	+ve (brown ring formation)	+ve (brown ring formation)	+ve(brown ring formation)
Test for protein				
xanthoprotein	-ve	-ve	-ve	-ve
Millons				
ninhydrine	-ve	-ve	-ve	-ve

Test for terponoids	+ve(interface brown ring)	+ve (interface brown ring)	+ve(interface brown ring)	+ve (interface brown ring)
Test for tannin	-ve	-ve	-ve	-ve
Test for saponin	-ve	-ve	-ve	-ve
Test for carbohydrates				
Molish	+ve (orange ring)			+ve (orange ring)
Iodine	+(purple colour)	+ve (purple colour)	-ve	-ve
Benedict	-ve	-ve	-ve	-ve
Fehlings	-ve	-ve	-ve	-ve

Conclusion

Andrographis paniculate leaf was compared to conventional antibiotics for their antimicrobial properties against clinical strains. The hydro-alcoholic extract demonstrated an inhibitory zone that was at least as large as the antibiotic inhibitory zone for the pathogens examined, indicating that these leaf extracts definitely block the development of germs even at low doses of these substances. Although there are many antibacterial, antifungal, and antihelminthic medications on the market, they have numerous side effects; thus, various maladies of seed extract like Andrographis paniculate is very useful to improve the state of therapy. There are no poisons left behind, and the clean, pleasant environment is created by all of the leaf extracts that are used to treat common disorders. Preliminary findings from this study suggest that hydro-alcoholic leaf extract from Andrographis paniculate has the potential to treat infectious diseases.

Acknowledgment

The authors would like to express their gratitude to professor and Head Department of Microbiology at Shri Guru Ram Rai University Dehradun, Uttarakhand, who assisted in numerous ways during this investigation.

Author contribution

The experiments were designed and conceived by KS. DR performed the experiments and analyzed the data. The manuscript was drafted by KS. It was critically reviewed by KS for its intellectual content. The final version to be published was approved by KS and DR.

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