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A study on the phytochemical's characterization and antimicrobial potential of *Andrographis paniculata*

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Abstract
Andrographis paniculata is an herbaceous plant of the family of Acanthaceae, found in tropical and subtropical regions and has wide application in herbal medicine preparation. Stem and leaf and even whole plant are to cure various ailments in human beings. In fact, plants produce a diverge range of bioactive principles, making them a rich source of different types of medicines researches. Bioactive substances lead to the discovery of new compounds that could be used to formulate new and most potent antimicrobial drugs to overcome the problem of resistant to the currently available medicines. Acetone, alcohol, benzene, chloroform, and water extracts of leaf and stem were used for phytochemical screening and antimicrobial activity.

Keywords: *Andrographis paniculata*, phytochemical, bioactive substance, antimicrobial activity

1. Introduction
Nature has provided a complete store house of remedies to cure all ailment of mankind. Use of plants as a source of medicine has been inherited and is an important component of the healthcare system. The aims of this paper were to evaluate the preliminary phytochemical character such as determination of pharmacognostic and screening of bioactive principles of *Andrographis paniculata*.
Now a day's multiple drug resistance has developed due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious disease. In India, addition to this problem, antibiotics are sometimes associated with adverse effects on the host including hypersensitivity, immune suppression and allergic reactions. This situation forced scientists to search for new antimicrobial substances. Antimicrobial activity of plants can be detected by observing the growth response of various microorganisms to plant tissue or extracts which are in contact with them. Screening different plant species for bioactive principles confirm the therapeutic potency of plants used in traditional medicines.
Andrographis paniculata, the Kalmegh of Ayurveda is an erect annual herb extremely bitter in taste in each and every part of the plan body. It is widely cultivated in southern Asia, where it is used to treat infections and some diseases, often being used before antibiotics were created. The main objectives of this study to evaluation of phytochemical characters, Screening of bioactive principles and testing the bioactive principles against bacteria of *Andrographis paniculata*.

2. Material and Methods
Preparation of Plant Extracts
Andrographis paniculata was collected from Botanical garden of Govt. Science College, Rewa (M.P.) and authentically identified. The plant materials were washed with water to remove shade dried at room temperature. Extracts were prepared from the method of Auda *et al*. (2000) [1]. The dried plant materials were ground into fine powder in an electric blender and subsequently sieved using a sieve for obtaining fine powder. Thereafter 3 gm of fine powder sample was weighed and soaked separately in 15 ml of different solvents (acetone, alcohol, chloroform, benzene and distilled water) in the ratio of 1:3 weights for volume (w/v). These were allowed to stand for 24 hrs at ambient room temperature. The soaked plant powder was filtered and used as crude extract. Different crude extract of these plants were stirred in refrigerator and used as such for qualitative, phytochemical analysis and antibacterial assays.

Preliminary phytochemical screening
Phytochemical screening was carried out to assess the qualitative chemical composition of crude extracts using commonly employed precipitation and coloration reaction to identify the major natural chemical groups such as steroids, reducing sugars, alkaloids, phenolic compounds, saponins, tannins, flavonoids, amino acids and anthracene glycosides. General reactions in these analyses revealed the presence or absence of these compounds in the crude extract tested (Brindha and Saraswathy, 1981) [2]. Crude extracts of the plants previously prepared and stored in a refrigerator were used for the phytochemical tests.

Antibacterial activity
The leaf and stem extracts of *Andrographis paniculata* were tested for the antibacterial activity against *Bacillus subtilis, Escherichia coli, and Salmonella typhi*. Authentically identified clinical isolates obtained from the Department of Microbiology, Awadhesh Pratap Singh University, Rewa (M.P.) India. All strains were maintained on nutrient agar slopes (Hi-Media Laboratories Pvt. Limited, Mumbai, India) at room temperature and were sub-cultured, every two-weeks. These bacteria served as test pathogens for the assay. 0.1 ml of diluted bacterial cultures was spread on sterile

nutrient agar plates. The pre-soaked crude extracts and dried discs of 6 mm diameter of filter paper No. 1 were then placed on the seeded plates and gently pressed down to the ensure contact. The plates were incubated at 37°C for 24 hours. After the incubation period the diameter of incubation zone around the plant extract saturated discs were measured. The inhibited zone around the discs were measured and recorded as the difference in diameter between the discs (6 mm) and growth free zone.

3. Results and Discussion

The phytochemical analysis of *Andrographis paniculata* presented in Table 1. The quantitative determination is helpful in evaluating the pharmacognostic\ value of the medicinal plant.

Table 1: Physico-chemical screening of leaf and stem of *Andrographis paniculata.*

S. No.	Particulars of parameter	Percentage	
		Leaf	Stem
1.	Loss of weight on drying	73.6	75.1
2.	Total ash	87.8	92.01
3.	Acid soluble ash	17.34	15.32
4.	Water soluble ash	10.02	9.34
5.	Extracts		
6.	Benzene	73	75
7.	Chloroform	83	86
8.	Ethanol	96	73
	Methanol	60	56
	Water	90	92

The moisture content of *Andrographis paniculata* leaf and stem was 75% and 86.6 respectively. The total ash content of leaf was 87.8 and stem was 92.01%. The acid insoluble ash found to be higher in leaf (17.34%) than stem (15.32%). Higher amount of water soluble ash was recorded in leaf (10.02%) than stem (9.34%). Higher extractive value was found in ethanol extract of leaf, stem when compared to other solvents.

Screening of Phytocompounds

Phytochemical screening of the various extracts of the leaf and stem of *Andrographis paniculata* for the presence of steroids, triterpenoids, sugars, alkaloids, phenols, saponins, aminoacids, tannins, flavonoids, anthracene glycoside were screened and presented in Table 2 and 3.

Table 2: Preliminary phytochemical screening of leaf extracts of *Andrographis paniculata*

S. No.	Name of Extract	Steroids	Triterpenoids	Sugar	Alkaloids	Phenols	Saponins	Amino acids	Tannins	Flavonoids	Anthracene glycosides
1.	Benzene	+	-	+	+	+	+	+	+	+	+
2.	Chloroform	+	-	+	+	+	+	+	+	-	+
3.	Ethanol	+	-	-	-	+	+	+	+	+	+
4.	Acetone	+	+	+	+	+	+	+	+	-	-
5.	Water	-	-	+	-	+	+	-	+	-	-

Table 3: Preliminary phytochemical screening of Stem extracts of *Andrographis paniculata*

S. No.	Name of Extract	Steroids	Triterpenoids	Sugar	Alkaloids	Phenols	Saponins	Amino acids	Tannins	Flavonoids	Anthracene glycosides
1.	Benzene	+	-	+	+	+	+	+	+	+	+
2.	Chloroform	+	-	+	+	+	+	+	+	-	+
3.	Ethanol	+	-	-	-	+	+	+	+	+	+
4.	Acetone	+	-	+	+	+	-	+	+	-	-
5.	Water	-	-	+	-	+	+	-	+	-	-

Leaf

The Benzene extract of leaf showed in presence of steroids, sugars, alkaloids, phenols, saponins, amino acids, tannins, glycosides and anthracene glycosides. Chloroform extracts of leaf showed the presence of steroids, sugars, alkaloids, phenols, saponins, tannins, anthracene glycosides. Steroids, phenols, tannins, amino acids, tannins, anthracene glycosides were screened in ethanol extract of leaf. Acetone extract of leaf showed the presence of steroids, triterpenoids, sugars, alkaloids, phenols, saponins, amino acids and tannins. Sugars, phenols, saponins, tannins were found in water extract. Phenol, tannin, and saponin were predominanty found in all the five extracts of the leaf followed by steroids and sugars which were found in four extracts. Flavonoids and terpenoids were found only in benzene and acetone extracts. From the above results that have been observed that the extraction of phytocompound depended on the type of solvent used for the extraction flavonoids

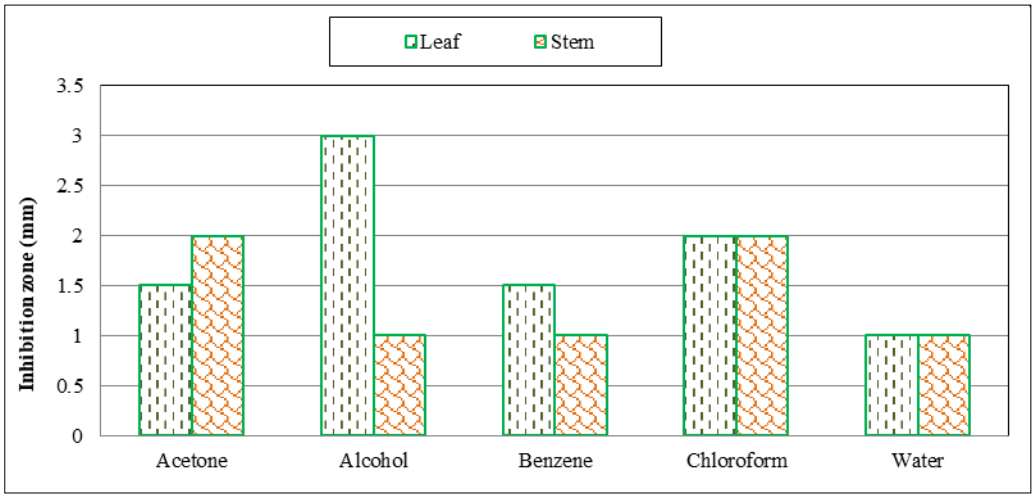

Fig 1: Graphics analysis of Antibacterial activity of crude extract of *Andrographis paniculata against B. subtilis.*

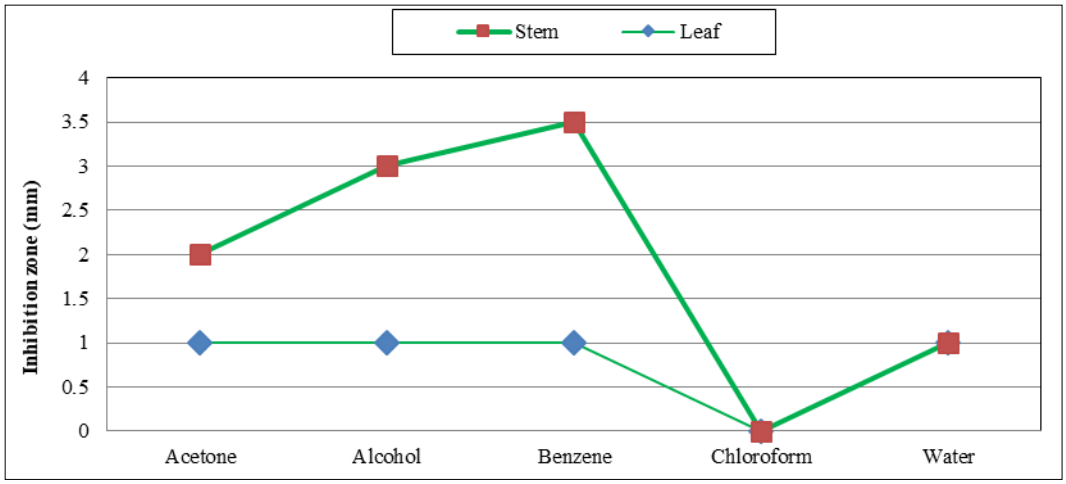

Fig 2: Graphics analysis of Antibacterial activity of crude extract of *Andrographis paniculata against E. coli*..

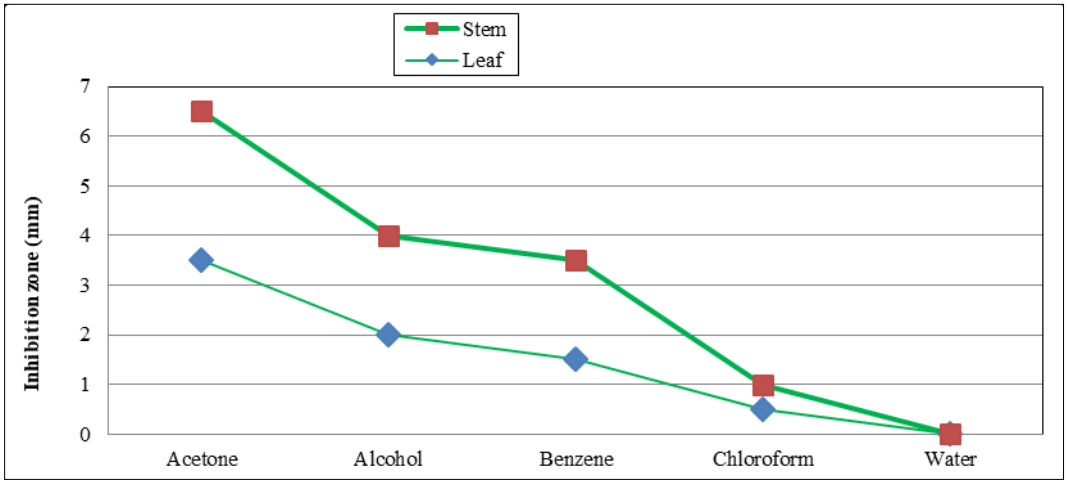

Fig 3: Graphics analysis of Antibacterial activity of crude extracts of *Andrographis paniculata* against *S. typhi*

Stem

Benzene and chloroform extract of stem of *Andrographis paniculata* showed the presence of steroids, sugars, alcohols, phenols, saponins, amino acids, tannins, flavonoids and anthracene glycosides. The ethanol extract of stem showed the presence of steroids, phenols, saponins, amino acids, tannins, flavonoids and anthrance glycosides. Steroids, sugars, alkaloids, phenols, saponins, amino acids, tannins were found in acetone extract. The aqueous extract of stem exhibited the presence of sugars, alkaloids, phenols, saponins, amino acids and tannins. These phytocompounds were predominantly found in all the five extracts used for screening of phytocompounds. Flavonoids and anthracene glycosides were seen only in benzene, chloroform and ethanol extracts. Triterpenoid was not found in all the five extracts used for phytochemical screening. Nkere and Iroeghbu (2005) [3] reported that the ethanolic and aqueous of stem and bark of *Picralima nitida* showed the presence of alkaloids, tannins, flavonoids, proteins, carbohydrates and ascorbic acid. The presence of linolenic acid in *Ocimum*

sanctum fixed oil has been reported by Singh (2005) [5].

Antibacterial Activity

The leaf and stem extracts of *Andrographis paniculata* were tested for their antibacterial activity against *B. Subtilis, E. coli and S. typhi* the results are presented in Fig. 1, 2 and 3. The crude extracts of leaf and stem showed varying degree of antimicrobial activity against all the tested bacteria. Acetone and alcohol extracts of leaf showed higher inhibitory action against *S. typhi* and *B. subtilis* respectively. Acetone extracts of stem showed maximum inhibitory activity against *S. typhi* and benzene extracts of stem show moderate inhibitory action against *E. coli*. Similar results were also reported by Venkatesan *et al*. (2005) [7], Prescott *et al*. (1999) [4] and Stainer *et al*. (1986) [6], who reported diseases such as typhoid, urinary tract infection, caused by *S. typhi* and *E. coli* respectively

4. Acknowledgement

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