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Full Length Research Paper

Phytochemical screening and antimicrobial potential of *Andrographis ovata* Clarke

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The aim of the present study was to investigate phytochemical and antimicrobial (antibacterial and antifungal) properties of various leaf extracts of *Andrographis ovata.* Ethanol, methanol, acetone, chloroform and petroleum ether extracts of this plant were evaluated against the bacterial strains *Staphylococcus aureus, Proteus vulgaris, Escherichia coli, Pseudomonas aeruginosa* and *Klebsiella pneumoniae* and the fungal strains *Penicillium notatum, Penicillium pinophilum, Aspergillus flavus, Aspergillus niger* and *Aspergillus nidulans.* Among the five extracts evaluated, chloroform extract was found to have the most significant activity followed by the petroleum ether, acetone, ethanol and methanol extracts against Gram-positive bacteria. Antifungal activity assessment revealed that the tested fungal strains are more susceptible to petroleum ether extract followed by methanol, chloroform, acetone and ethanol extract. Phytochemical analysis showed that the extract contain flavonoids, triterpenoids, glycosides, gums and mucilages, sterols and steroids. This is the first report on antibacterial, antifungal and phytochemical screening of *A. ovata*. This study scientifically validates the use of this plant as a potent antibacterial and antifungal agent.

Key words: Medicinal plant, *Andrographis ovata, Acanthaceae,* leaves extracts, antifungal activity, phytochemical, antibacterial activity, agar well diffusion method.

INTRODUCTION

India is endowed with a wealth of medicinal plants, which have been a valuable source of natural products for maintaining human health. Plants produce a diverse range of bioactive molecules, making them rich source of various types of medicines. Medicinal plants are an important source of therapeutic remedies for various ailments. Scientific experiments on the antimicrobial properties of plant components were first documented in the late 19th Century (Zaika, 1975). Natural antimicrobials have been often derived from plants, microorganisms or animal tissues (Gordon, 2001). Microbes are closely associated with the health and welfare of people. Some are beneficial, while others are detrimental. Due to the increasing therapeutic problem in the treatment of infectious diseases, the search for new drugs from natural sources becomes imperative, because most ram-pant killer diseases in developing countries are of microbiological origin (Gundidza and Gaza, 1993). Natural products from plants may offer new agents for antibiotic use.

A special feature of higher plants is their ability to produce a large number of organic chemicals of high structural diversity of the so-called secondary metabolites (Evans et al., 1986). Such metabolites are divided into three different categories based on their mechanism of function viz., bacteriostatic, antimicrobial and chemo-therapeutic (Castello et al., 2002). The antibacterial and antifungal studies of the plant extracts and pure compounds have been carried out by the agar well diffusion method (Bauer and Kirby, 1966). In this way, several studies on antibacterial and antifungal substances from plants have been conducted by a number of researchers (Barnabas and Nagarajan, 1988; Aday et al., 1989; Krishnakumar et al., 1997; Sattar et al., 2004; Ehsan et al., 2009; Wang et al., 2010). Medicinal plants occupy a distinct role in the life of people since ancient times (Latha and Pari, 2003).

Species of *Andrographis* Wallich ex Nees (Acanthaceae) are used in the Indian systems of medicines such as Ayurvedha, Homeopathy, Naturopathy,

Table 1. Antibacterial activity of various leaf extracts of *A. ovata* by agar well diffusion method.

Microorganism	Ethanol ZOI (mm)	Methanol ZOI (mm)	Acetone ZOI (mm)	Chloroform ZOI (mm)	Petroleum ether ZOI (mm)	Ciprofioxacin ZOI (mm)
S.aureus	7.28±0.05	7.0±0.04	9.0±0.2	14.6±0.12	9.13±0.1	23±0.15
P. vulgaris	9.14±0.15	8.11±0.06	-	12.41±0.06	11.7±0.04	26±0.20
E. coli	-	-	11.7±0.03	10.0±0.15	13.06±0.05	24±0.07
P. aeruginosa	8.0±0.30	9.33±0.06	9.12±0.07	9.51±0.1	10.22±0.06	28±0.40
K. pneumonia	10.5±0.2	-	12.3±0.1	11.43±0.06	-	21±0.5

ZOI = Zone of inhibition (mean ± S.D); - = Negative

Amchi, Modern, Siddha and Unani, and exhibit antipyretic properties (Kirtikar and Basu, 1975). This genus consists of 40 species distributed in Tropical Asia. Among these species, 24 have been found mainly in the hilly areas of the districts of Tamilnadu, India (Gamble, 1982), in which 18 species are reported to be endemic in India (Ahmedullah and Nayar, 1986). *Andrographis ovata* Clarke is a medicinal herb (Ahmedullah and Nayar, 1986) found wild in Shevaroy Hills of Salem District, Tamilnadu (11°45′ and 11°55′ N and 78°11′ to 78°20′E) up to 1400 m. There is no report available on this plant to date. The leaves of *A. ovata* are used in Folk medicine for the treatment of snake bite, fever, diabetes, and skin diseases. Therefore, it is necessary to establish the scientific basis for therapeutic action of this plant. The present study was an attempt to evaluate the phytochemical, antibacterial and antifungal activities of the leaves of *A. ovata.*

MATERIALS AND METHODS

Collection of plant material

A. ovata leaves were collected in November 2009 from Shevaroy Hills (Eastern Ghats) of Salem district of Tamilnadu and dried at 31°C for 10 days. The plant specimens were identified and confirmed with the Flora of Tamilnadu and voucher specimen (No. 25/14.11.09 CA) deposited in Department of Botany, Government Arts College (Autonomous), Salem for the future reference.

Preparation of extracts

The solvents used were petroleum ether, chloroform, acetone, methanol and ethanol. The powdered leaves (15 g) were taken and the extracts were prepared with soxhlet using 150 ml of each solvent. The extract was filtered through membrane filter (0.45 μm size) with the aid of a suction pump. The obtained filtrate was evaporated to dryness at 38°C. The extract was then weighed, dissolved in the minimal volume of dimethyl sulphoxide (Silva et al., 1997) and used for phytochemical, antimicrobial and antifungal activity.

Microorganisms used

In the experiments, five bacteria (*Staphylococcus aureus, Proteus vulgaris, Escherichia coli, Pseudomonas aeruginosa* and *Klebsiella pneumoniae*) and five fungi (*Pencillium notatum, Pencillium pinophilum, Aspergillus flavus, Aspergillus niger,* and *Aspergillus nidulans)* were used. All the cultures were obtained in pure form from the field strain of Biomedical Engineering Research Foundation, Salem, Tamilnadu, India.

Antibacterial screening

The agar well diffusion method was employed for the determination of antimicrobial activity of the extracts. The petriplates containing 20 ml of Muller Hinton Agar medium were seeded with 24 h culture of the microorganisms. The wells (6 mm in diameter) were cut from the agar and the extract solution (5 mg/ml) was delivered into them. The plates were incubated at 37°C for 24 h. The diameter of the inhibition zones were measured in millimeters (mm). Each experiment was repeated three times, and the average values were calculated.

Antifungal activity

Antifungal properties of *A. ovata* were proved in a radical growth inhibition activity. A fungal plug was placed in the center of the Potato Dextrose Agar plate. Extracts (30 mg/ml) was applied into the wells. The petriplates were incubated in the dark at 23°C. Antifungal properties was observed as a crescent shaped zone of inhibition at the mycelial form. The effect on fungal growth was expressed qualitatively. Ciprofloxacin and fluconazole were used as positive controls for bacteria and fungi, respectively. .

Preliminary phytochemical screening

The preliminary phytochemical investigation was carried out by the methods described by Harborne (1998) and Kokate et al., (2003). The leaf extracts were assayed for the presence of flavonoids, triterpenoids, glycosides, gums and mucilages, sterols and steroids.

RESULTS AND DISCUSSION

In the present study, antibacterial properties of *A. ovata* against *S. aureus, P. vulgaris*, *E. coli*, *P. aeruginosa* and *K .pneumoniae* were evaluated by agar well diffusion method (Table 1). The *in vitro* antibacterial activity revealed that the chloroform extract had significant activity against all the microorganisms tested, mainly *S. aureus*, *P. vulgaris* and *K. pneumonia* (zone of inhibition >10 mm). The petroleum ether and acetone extracts possessed moderate activity. Petroleum ether extract

Table 2. Preliminary phytochemical screening of the various leaf extracts of *A. ovata.*

Phytoconstituent	Ethanol extract	Methanol extract	Acetone extract	Chloroform extract	Petroleum ether extract
Flavonoids	+	+	+	+	+
Triterpenoids	+	+	+	+	+
Alkaloids	-	-	-	-	-
Glycosides	+	+	+	+	+
Carbohydrates	-	-	-	-	-
Gums and mucilages	+	+	+	+	+
Protein and amino acids	-	-	-	-	-
Steroids and Sterols	+	+	+	+	+

+ Denotes the presence of the respective phytoconstituents; - denotes the absence of the respective phytoconstituents.

Table 3. Antifungal activity of the various leaf extracts of *A. ovata.*

Fungal strain	Ethanol extract	Methanol extract	Acetone extract	Chloroform extract	Petroleum ether extract	Fluconazole
A. flavus	+	+	-	+	-	+++
A. niger	+	++	++	+	+++	++++
A. nidulans	+	-	++	+	+++	++++
P. notatum	-	++	+	-	+	+++
P. pinophilum	-	+	+	++	+	++++

+++, High; ++, moderate; +, slight; - , negative.

exhibited the maximum inhibitory effect against *E. coli* and considerable inhibitory activity against *P. vulgaris, P. aeruginosa* and *S. aureus*, but was inactive against *K. pneumonia*. Acetone extract exhibited the maximum inhibitory effect against *K. pneumoniae* and *E. coli* and moderate inhibitory activity against *P. aeruginosa* and *S. aureus*. The methanol extract exhibited only weak activity against *P. aeruginosa, P. vulgaris* and *S. aureus*, while ethanol extracts had significant inhibitory activity against *K. pneumoniae* and moderate activity against *P. vulgaris*, *P. aeruginosa* and *S. aureus*. The results of preliminary phytochemical analysis of all extracts revealed the presence of various phytoconstituents like flavonoids, triterpenoids, glycosides, gums and mucilages, steroids and sterols and absence of alkaloids, carbohydrates, protein and amino acids (Table 2).

The results of antifungal activity are given in Table 3. The tested fungal strains were most susceptible to petroleum ether followed by acetone, chloroform, methanol and ethanol extracts. Petroleum ether and acetone extracts were most effective against *A. niger,* and *A. nidulans*, weakly active against *Penicillium* spp. and inactive against *A. flavus.* The chloroform extract showed moderate activity against *P. pinophilum,* and slight activity against *Aspergillus* spp. The methanol extract expressed better activity against *P. notatum*, but was only slightly active against *A. niger*. The ethanol extract was slightly active against *Aspergillus* spp. In the recent years, there were many reports on the antimicrobial activity of plant extracts against human pathogenic bacteria (Srinivasan et al*.,* 2001; White et al*.,* 2002; Williams and Heyamnn, 1998; Mishra et al., 2009; Harish Kumar et al., 2010).

Shanthi et al. (2006) reported the antimicrobial activity of ethanol extract of leaf of *Andrographis lineata* and *Andrographis echioides*, which showed the maximum activity against *Shigella dysenteriae* and *Salmonella typhi.* Suresh et al. (2008) reported the best antimicrobial activity of ethanol extract obtained from *Rauvolfia tetraphylla*, which showed maximum activity against *E. coli* and *Enterobacter aerogenes*, and various tested fungi such as *A. niger* and *Penicillium* spp, were found to be more sensitive to crude extract when compared to others. Several phytoconstituents such as terpenoids (Scortichini and Rossi, 1991), flavonoids (Tsuchiya et al., 1966) and tannins (Ya et al., 1988) are effective anti-bacterial against a wide range of microorganisms. The results of the present investigation clearly demon-strate the antibacterial and antifungal activities of the ethanol, methanol, acetone, chloroform and petroleum ether extracts of the leaves.

Conclusion

A. ovata leaf extracts possess antibacterial and antifungal activities against tested microorganism. These activities may be due to the presence of various active principles in

the leaves. This plant could be a useful source of new antibacterial and antifungal agents. The present investigation is the first report of antibacterial, antifungal and phytochemical potential of the leaf of *A. ovata,* and supports the use of *A. ovata* leaf as medicinal plant by traditional healers. However, further work is needed to isolate and identify the bioactive (s) principle, in order to develop new antibacterial and antifungal drugs.

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