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EVALUATION OF ANTIBACTERIAL PROPERTIES OF LEAF AND STEM EXTRACTS OF *ANDROGRAPHIS ELONGATA* T. AND. – AN ENDEMIC MEDICINAL PLANT OF INDIA

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ABSTRACT

The point of the present study was focused on investigating the antibacterial property of the acetone and aqueous extract of the leaves and stem of *Andrographis elongata* T. And. (Acanthaceae) using agar well diffusion manner against human pathogenic bacterial species, namely *Bacillus cereus, Escherichia coli, Klebsiella pneumoniae* and *Staphylococcus aureus*. In the present investigation all the extracts were found to be efficient against four human bacterial species, *B. cereus*, *E. coli, K. pneumoniae* and *S. aureus* susceptible to all the plant extracts. Among various extracts studied, the aqueous stem extract showed highest antibacterial potential against *S. aureus* (16.41 mm) followed by acetone extract (14.40 mm). The maximum control extent noted for aqueous extract of *A.elongata* leaves against *B.cereus* was 12.06 mm followed by acetone extract (10.37mm). The study suggests that the extract of part of the plant restrain modern extensive wide range of antibacterial screening. The antibacterial potentialities of aqueous extracts was prove to be maximum than that of acetone extracts. However the stem extract showed more inhibitory determine than the leaf extracts. This research suggests that original products acquired from *A. elongata* may contribute to the expansion of recent antimicrobial agents. It is the first report on the antibacterial potential of *A. elongata*.

KEYWORDS: ***Andrographis elongata*, Antibacterial activity, Plant extracts, Agar well diffusion method.**

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INTRODUCTION

Medicinal plants have been used to treat a number of sicknesses. Though the recovery is gradual, the therapeutic application of medicinal plant is becoming familiar because of its disability to principle side effects and antibiotic resistant microorganisms (Rawat, 2003). Antibacterial potentialities of several plants parts such as rhizome, root, stem, leaves, flowers, seeds and fruits have been well accounted for some of the medicinal plants for the past two decades (Leven *et al.,* 1979). Medicinal and aromatic plants and substances are luxuriant in antibacterial compounds could be an interchange method to contest against bacterial illness (Samy *et al.,* 1998; Meera *et al.,* 1999). The phytochemical constituents of plant performance an important role in recent medicine after profiling against various biological properties. Antibiotics brought about a revolt to control pathogenic disorders and infections. But these synthetic remedies are out of extent to millions of people. Those people who live in distant places depend on traditional healers whom they recognize and confidence (Bhattacharjee, 2001). India is endowed with a wealthy property of medicinal plants. Microbes are closely associated with the healthy and welfare of human beings. Some are serviceable and some are harmful. An antimicrobial is substances that eradicate or inhibits the development of microorganisms such as bacteria, fungi or protozoan's as well as destroying viruses (Chan-Bacab and Pefia–Rodriguez, 2001). Microorganisms have enlarged resistance to several antibiotics and this has produced immense clinical difficulty in the treatment of infectious disorders (Davis, 1994). The extend in resistance of microorganisms due to indiscriminate benefit of commercial antimicrobial drugs encouraged scientists to investigation for modern antimicrobial substances from several sources including medicinal plants (Karaman *et al.,* 2003). Another driving factor for the renovated attention in past twenty years has been the speedy ratio of plant species extinction. Around 12,000 plant secondary metabolites of antimicrobial significance have been isolated. These compounds drop in one of the superior groups of compounds such as alkaloids, tannins, flavonoids, phenols, terpenoids, quinines and other varieties (Schultes, 1978).

Herbal medication is peaceful the mainstay of about 75-80 percentage of the complete population and the superior part of traditional therapy includes the benefit of plant extract and their active phytoconstituents. Following the advent of novel medicine, herbal medication suffered a setback, but during latest two or three decades, proposes in phytochemistry and in identification of plant compounds, productive against particular disorders have renewed the attention in herbal medicines (Arora and Kaur, 1999). This position forced to investigation for modern antimicrobial substances. Therefore, there is a demand to advance substitute antimicrobial drugs for the cure of contagious ailments from medicinal plants. Antimicrobials of plant source have vast therapeutic potentialities (Werner *et al.,* 1999). Several plants have been used because of their antimicrobial traits, which are chiefly synthesized during secondary metabolism of the plant. *Andrographis elongata* T. And. is a member of the Acanthaceae family and has been widely used in healthcare traditions. Species of *Andrographis* Wallich ex. Nees (Acanthaceae) are used in the Indian systems of medicine like Siddha, Ayurveda, Unani, Amachi, Yoga, Naturopathy and Homeopathy (Alagesaboopathi and Balu, 1999). The genus *Andrographis* as a entire is of influential importance to India. The genus exhibits antipyretic activities (Kirtikar and Basu, 1975). This genus consists of 40 species distributed in Tropical Asia (Anonymous, 1948). About 21 species are distributed in India (Gamble, 1982) and all of them are obtainable in Tamilnadu (Henry *et al.,* 1987). Among the 21 species, 18 species are declared to be endemic to India (Ahmedullah and Nayar, 1986). *Andrographis elongata* T.And. is an endemic medicinal plant (Ahmedullah and Nayar, 1986) found in wild in Pachamalai Hills of Eastern Ghats of Tamilnadu, India (11^0 09'00" to 11^0 27'00" N latitude; 78^0 28'00" to 78^0 49' 00" E longitude). It is used in the treatment of antipyretic, snake bite, skin

disease, antidiabetic, malarial fever, diuretic, constipation, diabetes and also veterinary medications have been attributed to this plant in that traditional usage of Indian medicine (Subramaniam *et al.,* 1995; Alagesaboopathi *et al.,* 2007; Alagesaboopathi and Balu, 1999; Alagesaboopathi, 2010; Alagesaboopathi, 2012). It is used to manage antipyretic and earache (Subramaniam *et al.,* 1995; Neelima *et al.,* 2011). The new 2-oxygenated flavones were isolated from the entire plant extract (Jayakrishna *et al.,* 2001). There is no former announce on antimicrobial potentialities of *A. elongata,* therefore, it is needed to prove the scientific support for therapeutic activity of this plant. The present investigation is an attempt to evaluate the antibacterial properties of the leaves and stem of *A. elongata.* This is the first declare on the antibacterial properties of the leaf and stem extracts of *A. elongata* in world.

MATERIALS AND METHODS

Collection and identification of plant materials

Fresh leaves and stem of *Andrographis elongata* (Family: Acanthaceae) were collected from the Pachamalai Hills of Eastern Ghats of Tamilnadu, Southern India, during the month of December 2011 for the investigation and is identified and authenticated by botanist of Government Arts College (Autonomous), Salem, Tamilnadu. A voucher specimen (No.25/18/12/2011 CA) has been deposited in the Department of Botany, Government Arts College (Autonomous), Salem for the future reference.

Test microorganisms

The antibacterial potential was done by using four bacterial strains such as *Bacillus cereus, Staphylococcus aureus, Klebsiella pneumoniae* and *Escherichia coli* were collected from Microbiology Lab, Biomedical Engineering Research Foundation, Salem, Tamilnadu, South India. All the test bacterial strains were preserved on nutrient agar media.

Preparation of plant extracts

A. elongata leaves and stem collected were washed individually under running tap water to eliminate soil particles and other filthiness. The leaves were air dried in the laboratory at room temperature (30±1°C) for 8 days, while the stem materials were dried at 60±1°C for 60–72 h in an oven. The dried leaves and stem samples were ground well into a thin powder with a mixer grinder. The powder was stored in airtight bottles at room temperature before extraction. The manner of Alade and Irobi (1993) was adopted preparation of plant extracts. The powdered leaves and stem (25 g) were taken and extracts were prepared with Soxhlet using 150 ml each of acetone and aqueous for 72 h. Each mixture was stirred at 24 h interval using a sterile glass rod. At the completion of the extraction each extract was passed through Whatman no.1 filter paper (Whatman England) and the filtrate obtained was concentrated in vaccum using evaporator. Then the extracts were used for antibacterial action.

Antimicrobial assay

The agar well diffusion manner was employed for the determination of antibacterial property 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 then added into it. The plates were incubated at 37°C for 24 h. The diameter of the inhibition zone was measured on millimeters (mm). Each test was performed in triplicates, repeated thrice and were tabulated.

Antimicrobial activity

The media and experiment bacterial species were poured into dishes (Muller – Hinton Agar media). The experiment strain (0.2 ml) has an inoculum size (108 cells/ml) when the temperature reached 35 to 39°C. Care was taken to guarantee correct homogenization. The plant extracts were tested for antibacterial activity in the agar well diffusion action (Perez *et al.,* 1990) against *Bacillus cereus, Staphylococcus aureus, Klebsiella pneumonia* and *Escherichia coli.*

Agar well diffusion method

The antibacterial activity was tested against (acetone and aqueous) leaves and stem of *A. elongata*. The inoculation of microorganisms was prepared from bacterial strains (Parihar and Bohar, 2006). About 20-25 ml of Muller – Hinton Agar medium was poured in the sterilized Petridishes and allowed to solidify. One drop of bacterial strains was spread over the medium by a rod. Wells of 5 mm in diameter and about 2 cm apart were punctured in the culture medium using sterile cork borers. About 75 ml of the plant extracts were added to the wells. The plates were incubated for 24 h at 37°C (Esimone *et al.,* 1998). The diameter of the inhibition zones were measured on millimeters (mm). Each experiment was performed in triplicates, repeated twice and were tabulated. 25 µg/ml of Ciprofloxacin served as control.

RESULTS AND DISCUSSION

The results of antimicrobial screening of acetone and aqueous extracts of the leaves and stem of *A. elongata* are given in Table 1. The acetone and aqueous extracts of the leaf and stem of *A. elongata* showed considerable antibacterial properties. All the extracts exhibited a wide spectrum of potential. When the two extracts were compared with each other and with that of standard antibiotic Ciprofloxacin, the aqueous stem extract showed the highest potential compared to that of the acetone extract. The inhibitory zone for aqueous extracts of the stem showed highest antibacterial activity against *S. aureus* (16.41 mm), *E. coli* (11.27 mm) and *K. pneumoniae* (9.38 mm). Least inhibition zone was observed against *B. cereus* (9.02 mm). The research made on acetone extracts of stem highest activity against *S. aureus* (14.40 mm), *E. coli* (10.63 mm) and *B. cereus* (9.22 mm). Minimum inhibition zone was observed against *K. pneumonia* (8.11 mm) (Fig. 1). The leaf extracts of aqueous showed positive significant antibacterial potential against *B. cereus* (12.06 mm), *S. aureus* (10.13 mm), and *E. coli* (7.69 mm), while decrease in activity against *K. pneumonia* (7.05 mm). The acetone extract of the leaves showed notable and highest antibacterial activity against *B. cereus* (10.37 mm), *S. aureus* (9.41 mm), and *E. coli* (8.20 mm). Whereas moderate degree of activity was recorded against *K. pneumonia* (6.08 mm) (Fig. 2).

The antibacterial potential of another species of this family (Acanthaceae) has been reported (Abubacker and Vasantha, 2010; Vishnu Kirthi *et al.,* 2010; Sukesh *et al.,* 2011). In another study, the species of *Andrographis* like *Andrographis lineata*, *Andrographis echioides*, *Andrographis neesiana*, *Andrographis affinis*, *Andrographis paniculata* were reported as worthiness antimicrobial medicines (Santhi *et al.,* 2006; Abubacker and Vasantha, 2010; Vishnu Kirthi *et al.,* 2010; Alagesaboopathi, 2011a; Alagesaboopathi and Sivakumar, 2011; Vijayakumar Arul Doss and Kalaiselvan, 2012). In another investigation methanol, acetone, ethanaol, petroleum ether and chloroform extracts *Andrographis alata* was found to be worthy antimicrobial properties (Alagesaboopathi, 2011b). The antimicrobial phytoconstituents may be found as alkaloids, tannins, saponins, steroids, phenolic compounds, triterpenoids and flavonoids whose presence may be attributed to the biotherapeutic potentialities of plants (Santhi *et al.,* 2006; Merlin Rose and Cathrine, 2011). Several plants and phytoconstituents isolated from plants have been reported to control antimicrobial activities (Khan *et al.,* 2011; Samiullah *et al.,* 2011). The inhibition activity of plant extracts against the development of microorganisms was attributed to the presence of antioxidants (Puupponen *et al.,* 2001; Prabu *et al.,* 2011; Vijayakumar Arul Doss and Kalaichelvan, 2012). The results of the current investigation are found to be directly correlated with the observations of earlier workers (Sumathi and Parvathi, 2010; Hosamani *et al.,* 2011; Abubacker and Vasantha, 2010). Other details are wanted to isolate and characterize the bioactive methods to create recent antimicrobial drugs. This antimicrobial study of the plant extracts described that ethnomedicine can be serviceable as modern medicine to contention pathogenic microorganisms, such as bacteria, viruses and fungi etc.

Table 1
Antibacterial potential of the leaf and stem extract of Andrographis Elongate T. And. by agar well diffusion method

Plant part	Plant extracts	Zone of inhibition (in mm)			
		Bacillus cereus	***Escherichia coli***	***Klebsiella pnemoniae***	***Staphylococcus aureus***
Leaves	Acetone	10.37±0.08	8.20±0.25	6.08±0.10	9.41±0.16
	Aqueous	12.06±0.20	7.69±0.13	7.05±0.11	10.13±0.02
Stem	Acetone	9.22±0.12	10.63±0.28	8.11±0.37	14.40±0.18
	Aqueous	9.02±0.17	11.27±0.24	9.38±0.01	16.41±0.04
	Ciprofloxacin 25 µg/ml	21.0±73	20.0±0.60	19.0±30	22.0±0.15

Data given are mean of triplicates ± standard error.

Diameter of zone of inhibition of *A. elongata* stem extracts against various pathogenic bacterial strains

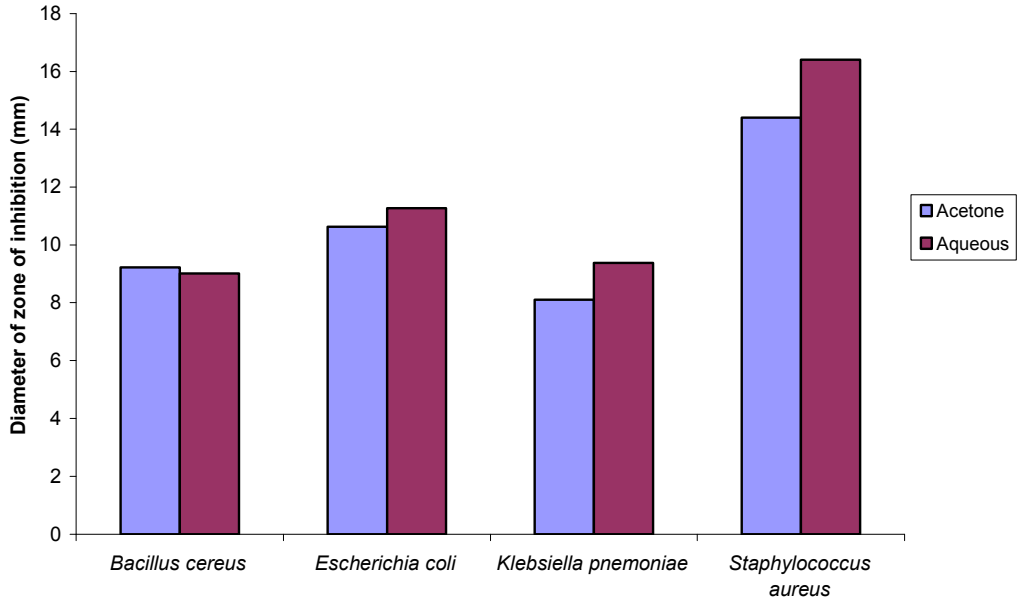

Figure 1
Antibacterial activity of stem extracts of Andrographis elongata against various pathogenic bacterial strains by agar well diffusion method.

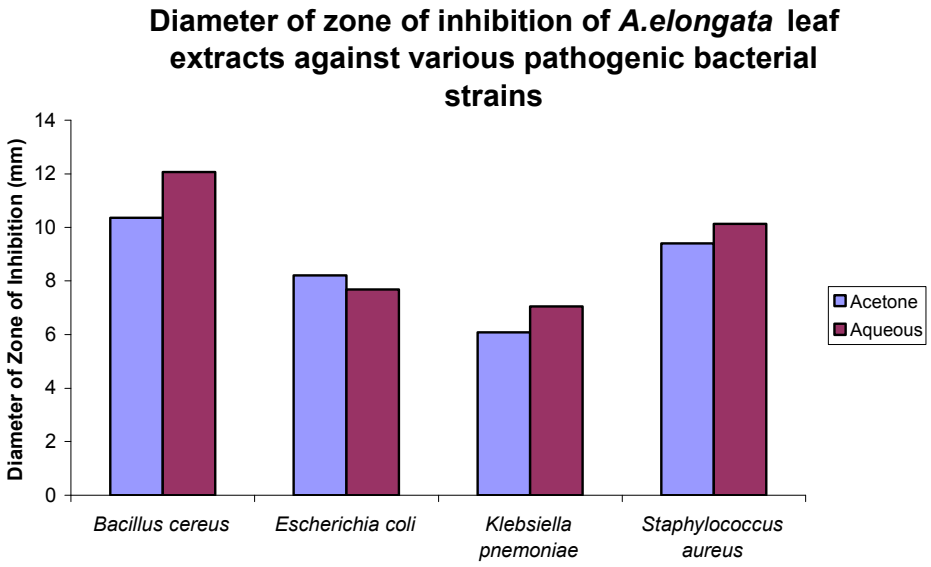

Figure 2
Antibacterial activity of leaf extracts of Andrographis elongata against various pathogenic bacterial strains by agar well diffusion method.

CONCLUSION

From this research, it can be achieved that, the stem of *Andrographis elongata* are excellent principle of antibacterial property. The effects of antibacterial activity of two solvents (aqueous, acetone) used in this investigation, aqueous was exhibited highest antibacterial screening. This determination may supply an essential for the isolation of bioactive compounds of from *Andrographis elongata.* Further studies are needed with this plant to isolate, characterize and elucidate the structure of the bioactive constituents of this plant for industrial drug formulation. From the above studies it can be concluded that the antimicrobial potential of the selected plant part extract would be beneficial in curing several kinds of disorders. The results of the investigation subsistence the traditional announce of this plant.

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