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In vitro evaluation of antibacterial potential of *Andrographis paniculata* against resistant bacterial pathogens Methicillin Resistant *Staphylococcus aureus* (MRSA) and Multiple Drug Resistant *Escherichia coli* (MDR *E. coli)*

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Abstract: Rise of antibiotic resistant pathogenic bacteria namely Methicillin Resistant *Staphylococcus aureus* (MRSA) and Multiple drug resistant *Escherichia coli* (MDR *E. coli* results in reduced efficacy of currently used antibacterial agents. Medicinal plants serve as potential targets for biologically effective antibacterial agents. The present study determined the phytochemical and *invitro* antibacterial activity of ethanol, chloroform, hexane and water extracts of whole plant of *Andrographis paniculata* against MRSA and MDR *Escherichia coli.* Zone of inhibition diameters were measured. Compared to all the extracts, ethanolic extract showed highest activity. The antibacterial activity was absent in hexane and water extracts. Chloroform extracts showed moderately good activity. The antibacterial compounds found in ethanolic extract were flavanoids, saponins and alkaloids.

Key words: *Andrographis paniculata*; Antibacterial activity; phytochemical analysis

Introduction

Discovery of antibiotics have paved the way to treat several bacterial infectious diseases. However, their indiscriminate use has lead to an alarming increase in antibiotic resistance among micro organisms, giving rise to multi resistant strains which has become a global concern. [1]. As occurrence of multidrug resistant bacteria is increasing, it is necessary to probe new sources for identifying antimicrobial compounds. [2] Medicinal plants represent a rich source of antimicrobial agents. Plants are used medicinally in different countries and a source of many potent, powerful drugs [3]. The antibacterial compounds found in plants may prevent bacterial infections by different mechanisms than the commercial antibiotics and therefore may have clinical value in treating resistant microorganisms. [4]. Increase in the incidence of multidrug resistant *Escherichia coli* has been reported in various studies. [5]. *Escherichia coli* is the most common Gram negative bacterial pathogen causing community and hospital acquired urinary tract infections. [6]. Several recent reports have noted increased prevalence of Methicillin Resistant *Staphylococcus aureus* (MRSA) as a common multidrug resistant bacterial pathogen [7,8,9].

Andrographis paniculata (Burm. F) Nees, commonly known as "King of bitter" is a herbaceous plant belonging to Acanthaceae and is found throughout tropical and subtropical Asia, South east Asia and India. Several broad range biological activities such as anti-inflammatory, antibacterial., antitumour, antidiabetic, antimalarial and hepatoprotective activities have been documented. [10]. Due to its blood purifying property, *Andrographis paniculata* plant is recommended for the use of boils, skin eruptions, chronic and seasonal fevers. [11,12]

Andrographis paniculata has been reported to exhibit antibacterial activities (Leelarasamee *et al.,* 1990, Singha *et al.,* 2003, Mishra *et al.,* 2009). [13,14,15]. Considering the promising therapeutic potential of the individual plant parts and limited availability of data with respect to whole plant, our present study was designed to investigate the antibacterial activity of the whole processed plant specifically against MRSA and MDR *E. coli* followed by preliminary phytochemical analysis of plant extract responsible for antibacterial activity.

Materials and Methods

Plant collection and sample preparation: *Andrographis paniculata* plants were collected from Thiruvallur District in Tamilnadu. (Voucher number qmc/2009/1321). The plant materials were washed thoroughly, shade dried for about 15 days and grounded into a powder. About 25g of plant powder was extracted with 100 ml solvents by using Soxhlet apparatus. Solvents used for extraction were ethanol, chloroform, hexane and water. The resultant crude extracts were filtered by using Whatman No 1 filterpaper and then concentrated in a rotary evaporator and were stored in a refrigerator at 4^0C in small sterile glass bottles for further investigations.

Antibacterial activity: The dried plant extracts were dissolved in Dimethyl sulfoxide (DMSO) separately at the concentration of 1mg/ml for antibacterial assay. The bacterial culture used in the study was pure clinical isolates of Methicillin resistant *Staphylococcus aureus* (MRSA) and Multiple

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drug resistant (MDR) *Escherichia coli* obtained from Private Hospital, Chennai. Muller Hinton Agar (MHA) medium was used to study antibacterial activity. Prior to antibacterial screening, the bacterial culture was cultured in Muller Hinton Broth for about 4 hrs at 37^{0}C. Antibacterial sensitivity testing was carried out with standard antibiotics following Kirby Bauer disc diffusion method (Bauer *et al*., 1986) [16]. The bacterial culture was inoculated as lawn culture using sterile swab over the agar surface. The filter paper discs impregnated with 100 microl of plant extract (1mg/ml) were placed on the seeded agar plates. Dimethyl sulfoxide (DMSO) served as negative control and Streptomycin (10 microg) as reference. The plates were then labelled and incubated at 37^{0}C for 24 hours. After incubation, the plates were examined for clear inhibition zone and zone of inhibition diameters were measured and recorded.

Phytochemical screening: Based on the results in the antibacterial activity, the concentrated crude ethanolic extract was subjected to preliminary qualitative phytochemical screening method as per standard procedures. [17,18,19]. The plant extracts were investigated for tannins, alkaloids, flavanoids and saponins.

The test of tannins was carried out by boiling 0.5g of sample in 20 ml distilled water followed by addition of 3 drops of 5% ferric chloride to the filtrate. Development of dark green colouration indicated positive by the presence of tannins. The flavanoids was determined using 0.2 g of plant extract was dissolved in dilute sodium hydroxide and adding drops of dilute hydrochloric acid. The development of yellow colouration was taken positive for flavanoids. The test for alkaloids was carried out by treating 1 g of plant extract with 5 ml methanol and 5ml of 2N HCl and then the filtrate was treated with Mayer's reagent. Development of precipitate indicated the presence of alkaloids. Saponins were detected by boiling 1 g of the sample with 10 ml distilled water for 15 minutes and the cooled extract was shaken for froth formation.

Results and Discussion

In the present study ethanol extract showed maximum antibacterial activity against both the antibiotic resistant strains, Gram positive bacteria MRSA and Gram negative bacteria MDR *E. coli* visibly observed with zones of inhibition. (Fig.1). It is noteworthy that ethanolic extracts showed more zone of inhibition compared to standard antibiotics.

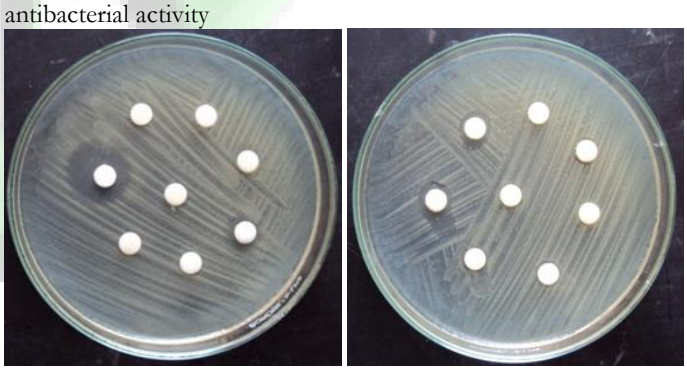
Table 1: Comparative Antibacterial activity profile of *Andrographis paniculata* on MRSA and MDR *E. coli*.

Concentration of the Plant Extracts	Solvents	Positive Control (Streptomycin 10 Micro gram) ZOI (18 mm)			Positive Control (Streptomycin 10 Micro gram) ZOI (20 mm)		
		Andrographis paniculata on MRSA			*Andrographis paniculata* on MDR *E.coli*		
		ZOI (mm)			ZOI (mm)		
		1	2	3	1	2	3
1 mg/ml	Ethanol	30	28	30	40	41	40
	Chloroform	18	16	18	20	18	20
	Hexane	--	--	--	--	--	--
	Water	--	--	--	--	--	--

No Inhibition: ---
ZOI – Zone of inhibition diameter.

Larger zone of inhibition of *Andrographis paniculata* ethanolic extract was observed for MDR *E. coli* (41mm) followed by MRSA (30mm). Chloroform extracts showed moderate activity. Hexane and water extracts did not inhibit both MRSA and MDR *E. coli*. Interestingly, our results corelates with the result of Sharma *et al.,* who also found antibacterial activity in ethanol extracts of whole processed plant against urinary tract E. coli. [20]. The result coincides with the observations of Leelarasamee *et al.,* also reported no in vitro antibacterial activity of *Andrographis paniculata* extracts against *Staphylococcus aureus* and *Escherichia coli*. [13]. Slightly different results were obtained by Zaidan *et al.,* who reported no activity of crude aqueous extract of *Andrographis paniculata* leaves but exhibited significant antibacterial activity against *Staphylococcus aureus*. [21].

Figure 1: Zone of Inhibition results of antibacterial activity



Preliminary phytochemical analysis showed the presence of flavanoids, saponins and alkaloids in both ethanolic extracts of *Andrographis paniculata*. The data is shown in Table 2.

Table 2: Qualitative Phytochemicals screening of *Andrographis paniculata* ethanolic extract.

S. No	Phytochemicals	Presence
1	Tannins	--
2	Flavonoids	+
3	Saponins	+
4	Alkaloids	+

Our present study revealed better antibacterial activity in ethanolic extracts which may be due to presence of more active phytochemicals in the extracts. Previous researchers have reported that alkaloids and flavanoids are known to possess curative activity against several pathogens and hence could be used for treatment of various diseases. [22]. Saponins have been reported to possess ability to cause leakage of proteins and certain enzymes of the cell [23].

Conclusion

Our present study demonstrated promising antibacterial activity of ethanolic extracts of *Andrographis paniculata* against MRSA and MDR *E. coli*. The encouraging results prospects us further to next line of directed efforts towards isolation and identification of active phytocompounds and their mechanism of action to be considered for better treatment of infectious diseases.

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