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Evaluation of phytochemical and antimicrobial activity of *Andrographis paniculata* nees (Acanthaceae) aerial parts

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Abstract

The present study has been designed with the objective to examine the petroleum ether, acetone, chloroform and methanol extracts of *Andrographis paniculata* leaves and stems, in order to evaluate the chemical composition, investigate its *in vitro* antimicrobial potential against strains of *Enterococcus faecalis, Streptococcus pyogenes, Klebsiella pneumonia*, *Proteus vulgaris*, *Candida albicans and Aspergillus flavus*. Phytochemical analysis revealed the presence of flavonoids, alkaloids, glycosides, steroids, phenols, tannins and saponins. The antibacterial activity is more significant against Gram positive bacterium *Enterococcus faecalis* whereas the antifungal activity is more significant against *Aspergillus flavus.* These results may justify the popular use of this species as it has antimicrobial activity. However, in order to evaluate possible clinical application in therapy of infectious diseases, further clinical trials are required.

Key-Words: Chemical composition, Antimicrobial potential, Clinical application and infectious diseases.

Introduction

Andrographis paniculata Nees (Family Acanthaceae) is available abundantly in India, Pakistan and Srilanka, growing in hot and shade places. It is cultivated in certain parts of India, East and West Indies and Mauritius. *Andrographis paniculata* or kalmegh is one of the most widely used plants in Ayurvedic formulations[1]. *A. paniculata* was recommended in Charaka Samhita dating to 175 BC for treatment of jaundice along with other plants in multi plant preparations[2]. It has also been used traditionally for sluggish liver as antidote in case of colic dysentery and dyspepsia[3]. It is used as bitter tonic, antispasmodic, antiperistatic, stomachic and also an anthelmintic. It has been employed with benefit in case of general debility in convalescence after fever, disorders of liver and advanced stages of dysentery[4].The juice of fresh leaves is a domestic remedy in the treatment of colic pain, loss of appetite, irregular stools and diarrhea[5].

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Long known in traditional Asian medicine as an immune system booster, *Andrographis* has demonstrated significant activity in fighting common cold, flu and upper respiratory infections[6-7].The pharmacological studies suggest anti-inflammatory [8-9], antipyretic[10], anti-viral[11], immunostimulatory[12], potential cancer therapeutic agent[13], anti-hyperglycemic[14] and antioxidant[15]properties.

In recent years focus on use of non-traditional approaches to treat diseases has been revived world wide. The evidence collected till now shows immense potential of medicinal plants used in traditional systems. The herb, *Andrographis paniculata* is the main source of the bitter principle. The extremely bitter and characteristic taste of *A. paniculata* of the Acanthaceae family, gives it the term "kings of bitters". Several recent studies have validated some of the medicinal properties of this plant and its use in traditional medicine, such properties include its antimicrobial activity [16], hepatoprotective capacity[17], antimalarial activity[18] and antidiarrhoeal potential[19].

Hence, in the present study, the phytochemical and antimicrobial activities of extracts of two parts of *A. paniculata* (stem and leaves) were evaluated.

Material and Methods

Plant collection

The plant materials were collected from Keeriatti, Athur Taluk, Salem District. The collected plants were identified and authenticated by Dr. V. Balasubramaniam, Associate Professor of Botany, Kongunadu Arts and Science College, Coimbatore.

Preparation of extracts

The plant parts were collected and shade dried for about two weeks and ground into coarse powder. About 50g powder extracted with 250ml of petroleum ether using soxhlet apparatus. The same powders were also extracted with chloroform, acetone and methanol. The extracts were concentrated to dryness to yield crude residue. These residues were used preliminary phytochemical and antimicrobial activity. In the present study, all preliminary phytochemical screening was carried out using the following methodologies[20].

Target Microorganisms

Authentic pure cultures of human pathogenic bacteria *like Enterococcus faecalis, Streptococcus pyogenes, Klebsiella pneumonia and Proteus vulgaris* and fungi such as *Candida albicans and Aspergillus flavus* were obtained from University of Madras, Mycology lab, India.

Disc diffusion method

The Petroleum ether, Chloroform, Acetone and Methanol extracts of *A. echioides* leaf and stem extracts were screened for antimicrobial activity using by disc diffusion method according to[21]. The overnight culture grown in broth was used for inoculation. The plant extracts to be tested were prepared in various concentrations i.e. 25%, 50%, 75% and 100%. The sterile impregnated discs with plant extracts were placed on the agar surface with framed forceps and gently pressed down to ensure complete contact of the disc with agar and dextrose surfaces. Positive control discs were also prepared in the same manner using Ampicillin, a bactericide. But it was not used for fungi. The prepared control discs were placed using respective solvents. All the plates including control plates were incubated at 37°C for 24 hours. After incubation, the size (diameter) of the inhibition zones was measured. Triplicates were maintained for each sample of the extract respectively. The results were expressed in terms of the diameter of the inhibition zone: <9 mm - inactive; 9-12mm - partially active; 13-18mm - active; >18mm - very active [22-23].

Statistical analysis

All the data were subjected to Duncan's Multiple Range Test (DMRT). It was done using the SPSS Version 2007 Winsat software.

Results and Conclusion

In the present study, the phytochemical screening, antibacterial and antifungal activities were performed with petroleum ether, acetone, chloroform and methanol extracts of the leaves and stems of *Andrographis paniculata*. The study was made against four pathogenic bacteria and two fungi using the standard disc diffusion method.

The leaves and stems of *A. paniculata* were rich in flavonoids, alkaloids, glycosides, steroids, phenols, tannins and saponins. These phytochemicals confer antimicrobial activity on the plant extract (Table 1).

Table 1: Preliminary phytochemical components of leaf and stem extracts of *Andrographis paniculata*

Components	PE		CH		A		M	
	L	S	L	S	L	S	L	S
Alkaloids	+	++	+	++	-	+	-	+
Flavonoids	-	+	+	+	-	-	-	-
Glycosides	+	++	++	++	+	++	++	++
Steroids	-	-	+	+	+	++	+++	++
Phenols	+	++	+	+++	+++	++	++	++
Tannins	++	++	-	+	+	+	+	+
Saponins	-	+	+	++	-	+	+	-
Resins	-	-	-	-	-	-	-	-

Abbr.: PE= Petroleum ether, CH=Chloroform, A= Acetone, M= Methanol, L=Leaves, S=Stem- =absent, + =Presence, ++ = Moderate, +++ = Maximum

The various phytochemical compounds detected are known to have beneficial importance in medicinal sciences. For instance: flavonoids have been referred to as nature's biological response modifiers, because of their inherent ability to modify the body's reaction to allergies and virus and they showed their anti-allergic, anti-inflammatory, anti-microbial and anti-cancer activities[24]. Alkaloids have been use to treat diseases like malaria, pain killers and managing heart diseases[25]. Generally, glycosides are non volatile and lack fragrance cleaving the glycosidic bond yields the aglycone, which itself may be volatile and fragrant. Glycosides serve as defense mechanisms against predation by many microorganisms, insects and herbivores [26].

It should be noted that steroidal compounds are of importance and interest in pharmacy due to their relationship with such compounds as sex compounds[27]. Plant steroids are known to be important for their cardiotonic activities, possess insecticidal and anti-

microbial properties. They are also used in nutrition, herbal medicine and cosmetics [28]. Phenols are a class of low molecular weight secondary metabolites found in most land plants. Phenolic compounds are the largest group of phytochemicals and accounts for most of the antioxidant activity in plants or plant products [29]. At low concentration tannins can inhibit the growth of microorganisms and act as an antifungal agent at higher concentration by coagulating the protoplasm of the microorganism[30]. Saponin is used as mild detergent and in intracellular histochemical staining. It is also used to allow antibody access in intracellular proteins. In medicine, it is used in hypercholesterolemia, hyperglycaemia, antioxidant, anticancer, anti-inflammatory, weight loss, etc. it is also known to have antifungal properties [31].

In the present study 25%, 50%, 75% and 100% acetone and methanol extracts of leaves and stems of *A. paniculata* showed greater inhibitory effect (30.33±0.88mm) on the growth of *Enterococcus faecalis* (Table 2 and 3).

Table 2 Antibacterial activity of leaf extracts of *Andrographis paniculata*

MO	Con	Zone of inhibition in diameter			
		PE	CH	A	M
Enterococcus faecalis	Control	27.66± 3.84	17± 1.53	24.67± 1.45	23.67± 0.33
	25%	19.66± 0.88	25.67± 1.20	15.66± 1.15	17.33± 1.20
	50%	21± 1	28± 1.20	17.67± 1.20	10.67± 0.88
	75%	21.67± 1.20	24± 1.73	10.66± 0.88	27.67± 0.88
	100 %	19± 1.52	30.33± 0.88	14.33± 0.88	14.33± 0.88
Streptococcus pyogenes	Control	34.67± 2.60	27.67± 0.22	7.67± 0.88	6.33±0 .88
	25%	30.67± 0.88	18.33± 0.88	12.33± 0.88	6± 1.53
	50%	21± 1.15	13.33± 1.45	8± 0.58	7.67± 1.20
	75%	19± 1.15	14.67± 1.85	12.67± 0.88	6.67± 0.88
	100 %	14.67± 0.88	13.67± 1.76	10.67± 0.88	4.33± 0.88
Klebsiella pneumoniae	Control	20.33± 0.88	27± 1.53	18± 1.14	16.67± 0.88
	25%	19.33± 1.45	23± 1.15	17.67± 1.20	15.67± 1.20
	50%	23.33± 0.67	27± 1.15	21± 1.15	10.67± 0.88
	75%	20.67±	23.33±	21.67±	15.33±
		0.88	1.20	1.20	1.20
	100 %	20.33± 1.20	27± 1.15	25± 0.58	11.33± 0.88
Proteus vulgaris	Control	-	-	5.67± 1.20	11± 0.58
	25%	-	-	8.67± 0.88	13.67± 0.88
	50%	3.33± 1.20	4± 1.15	4.67± 0.88	18.33± 0.88
	75%	6.67± 0.88	6.67± 0.88	9.33± 0.88	10.67± 0.88
	100 %	7.33± 0.88	8± 0.58	11± 1.15	18.33± 0.88

Values are expressed as Mean ±Standard error of three replicates. Means within a column followed by same letter(s) do not differ significantly (P< 0.05) according to DMRT.

Table 3. Antibacterial activity of stem extracts of *Andrographis paniculata*

MO	Con	Zone of inhibition in diameter			
		PE	CH	A	M
.Enterococcus faecalis	Control	25± 1.15	21.67± 1.20	21.67± 4.48	25.33± 0.58
	25%	13± 1.15	9.33± 0.88	20.67± 0.88	19± 0.58
	50%	15.67± 0.67	11.67± 1.76	28.67± 1.20	27± 1.15
	75%	11.67± 1.20	10.67± 0.88	17± 1.15	23.33± 1.20
	100 %	13± 1.15	13± 1.15	17± 1.15	21.33± 1.45
Streptococcus pyogenes	Control	31.67± 1.16	34.33± 2.33	30.67± 0.88	28.33± 0.88
	25%	8.67± 0.88	17.33± 1.45	18± 1.52	15.67± 1.20
	50%	9± 1.15	18.33± 0.88	12.33± 0.88	18.33± 0.88
	75%	19.67± 0.88	14± 1.15	14.67± 0.88	20.33± 0.88
	100 %	18.67± 1.33	12.33± 0.88	9.33± 1.45	15.33± 0.88
Klebsiella pneumoniae	Control	13± 1.15	25± 1.73	23.33± 1.20	24± 0.58
	25%	10± 1.15	18.67± .0.88	22.33± 0.88	18± 1.15
	50%	13± 1.15	9.67± 0.88	27.33± 1.76	24.67± 0.88
	75%	11.67± 1.20	19.33± 0.88	23.67± 1.45	22± 0.58
	100 %	8.67± 0.88	12.67± 1.20	21.67± 1.20	23.33± 0.88
	Control	-	-	7± 1.15	9± 0.58

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Proteus vulgaris	25%	-	8.67± 0.88	4.67± 0.88	8± 1.15
	50%	6.33± 0.88	6.67± 0.88	10± 0.58	10.67± 0.88
	75%	7± 0.67	6.67± 0.88	6.67± 0.88	9± 0.58
	100 %	7± 1.73	4.33± 0.88	9± 1.15	12± 0.88

Values are expressed as Mean ±Standard error of three replicates. Means within a column followed by same letter(s) do not differ significantly (P< 0.05) according to DMRT.

The 25% petroleum ether extract showed higher activity against *Streptococcus pyogenes* (30.67±0.88). The methanol and chloroform extract of leaves and stems showed higher activity against gram negative bacterium *Proteus vulgaris*. The gram negative bacterium *Klebsiella pneumoniae* was highly controlled (27.33±1.76mm) by the methanol extracts taken from the leaves[32] took an aqueous and two ethanolic extracts of *A. paniculata* which were used in Chinese and also Indian medicines. Andrographolide, an active principle of *A. paniculata*, was also investigated for their antimicrobial activity against nine bacterial species including *Salmonella typhimurium, E.coli, Shigella sonnei, Staphylococcus aureus, Pseudomonas aeruginosa, Streptococcous pneumonia, S. pyogenes, Legionella pneumophila* and *Bordetella pertussis*. Of all the tested concentrations, direct antimicrobial activity of the two ethanolic *A. paniculata* extracts were observed for only two human pathogens. Given that the TLC of *A. paniculata* extracts showed that andrographolide was present in all the three extracts, it was concluded that the observed antimicrobial activity was done to other active principle present in the extracts used in[32]. *A. paniculata* extracted components like neoandrographolide[33], andrographolide[34] and andrograpanin[35] that are reported to have medicinal usages. The yeast, *Candida albicans* showed susceptibility to 75% of chloroform extracts of the leaves (23.33±1.20mm). The acetone extracts of stems showed inhibitory effect on the growth of the fungus, *Aspergillus flavus* (23.67±0.88) (Table 4 and 5).

Extracts from this plant showed varying antimicrobial activities when compared to the standard antibiotic. The results suggest that the antimicrobial activity of this plant may contribute to its claimed activity as an antidote for snake bite.

Table 4: Antifungal activity of leaf extracts of *Andrographis paniculata*

MO	ConC.	Zone of inhibition in diameter			
		PE	CH	A	M
Candida albicans	Control	-	8.66 ±0.88	11.33 ±1.20	11 ±0.57
	25%	21 ±0.57	12.66 ±0.88	21 ±0.57	7.66 ±0.88
	50%	21.33 ±1.45	20 ±0.57	18 ±0.57	7.33 ±1.66
	75%	7.67 ±0.33	23.33 ±1.20	12.33 ±0.67	18 ±1.15
	100%	22.33 ±1.20	17.33 ±0.88	15 ±0.57	15.66 ±1.20
Aspergillus flavus	Control	-	8.67 ±0.88	11.33 ±1.20	6.67 ±1.45
	25%	-	17.63 ±0.88	20.33 ±0.67	5.67 ±0.88
	50%	-	19.33 ±0.88	16.33 ±1.76	6.33 ±1.20
	75%	-	18.67 ±1.20	14.33 ±0.88	18.33 ±0.88
	100%	-	15.67 ±1.20	9.33 ±0.88	19.33 ±0.88

Values are expressed as Mean ±Standard error of three replicates. Means within a column followed by same letter(s) do not differ significantly (P< 0.05) according to DMRT.

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Table 5: Antifungal activity of stem extracts of *Andrographis paniculata*

MO	Conc.	Zone of inhibition in diameter			
		PE	CH	A	M
Candida albicans	Control	-	9.33± 0.88	3.33± 1.20	4.66± 0.33
	25%	-	3.33± 0.88	7± 1.15	4± 1.15
	50%	-	4± 0.57	5.66± 0.66	6.66± 1.20
	75%	-	-	8.66± 0.66	8.66± 0.88
	100%	-	4± 1.15	7± 1.15	8.66± 0.88
Aspergillus flavus	Control	-	-	8.33± 0.88	7.33± 0.88
	25%	-	-	6.67± 0.88	6.33± 0.33
	50%	2.3± 0.88	-	11.3± 0.88	6.33± 0.33
	75%	6± 0.57	-	20± 0.57	7.33± 1.20
	100%	-	-	23.6± 0.88	5.33± 2.03

Values are expressed as Mean ±Standard error of three replicates. Means within a column followed by same letter(s) do not differ significantly (P< 0.05) according to DMRT.

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