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Research Article

COMPARATIVE ANTIMICROBIAL ACTIVITY AND TLC-BIOAUTOGRAPHIC ANALYSIS OF ROOT AND AERIAL PARTS OF *ANDROGRAPHIS SERPYLLIFOLIA*

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ABSTRACT

In present study, hexane, chloroform and methanol extracts of root and aerial parts of *Andrographis serpyllifolia* were tested for antimicrobial activity. *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa, Escherichia coli* growth were used as test organisms. In addition, the MIC's of the extracts were also determined. Methanol extracts of both the parts has shown significant activity against bacteria compare to other solvent extracts. Both methanolic extracts were analyzed with TLC-bio autographic analysis and antimicrobial components were identified by their R_f values.

Keywords*: Andrographis serphyllifolia*, antimicrobial activity, TLC-bio autographic analysis

INTRODUCTION

Medicinal plants are an important therapeutic aid for various ailments. Scientific experiments on the antimicrobial properties of plant components were first documented in the late 19th century[1]. In India, from ancient times, different parts of medicinal plants have been used to cure specific ailments. Today, there is widespread interest in drugs derived from plants. This interest primarily stems from the belief that green medicine is safe and dependable, compared with costly synthetic drugs that have adverse effects. Natural antimicrobials can be derived from plants, animal tissues, or micro-organisms[2]. The shortcomings of the drugs available today, propel the discovery of new pharmacotherapeutic agents in medicinal plants[3]. To determine the potential and promote the use of herbal medicine, it is essential to intensify the study of medicinal plants that find place in folklore[4,5].

Andrographis serphyllifolia (Vahl) Wight is a common small shrub belongs to Family: Acanthaceae. The root extract of the plant is used to cure fever[6]. The plant extract is used in treating wounds and also effective in the treatment of Jaundice[7]. This plant was reported to possess Serpyllin, apiginin 7, 4' -dimethyl ether and tectochrysin[8] and Acylated flavone glycosides and andrographidine C[9].

MATERIAL AND METHODS

Collection of plant material

The Plant was collected in December month 2006 from Campus surroundings, P.G. Centre, Hassan, and Karnataka, India. The plant was identified by using Flora of Hassan District and voucher specimen (No. 12 / 2-12-06 PS) deposited in Department of Bioscience, P.G. Centre, Hassan.

Preparation of extracts

The powdered plant parts were extracted successively by using solvents of varying polarities such as n-Hexane, chloroform and methanol by using soxhlet apparatus. The anti-microbial activity was evaluated by using disc diffusion method[10].

TLC-bio autographic analysis

Thin layer chromatography for both aerial and root parts were performed on Merck TLC F_{254} plates, with chloroform: Methanol (95:5) as mobile phase. The separated components were visualized under visible and ultraviolet light (254 and 360 nm) [11].

Bioassay

The antimicrobial activity was studied by agar paper disc method and the minimum inhibitory concentrations (MIC) was determined by 2-fold serial dilution of extracts beyond the level where no inhibition of growth of *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus* and *Bacillus subtilis* was observed.

For bio autographic analysis, developed TLC plates were dried overnight and sprayed with a concentrated suspension of actively growing *S. aureus* cells. Before incubating at 38°C in a chamber at 100% relative humidity, Plates were sprayed with a 2 mg/ml solution of *MTT* (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide). All the bacteria were obtained from stock cultures maintained at Department of Microbiology, National College of Pharmacy, Shimoga, India.

RESULTS

Both the root and aerial part of the plant has shown the varying degree of antimicrobial activity as demonstrated by paper disc method and MIC studies.

Different extracts have exhibited different activity with different organisms (Table 1 and 2). The methanolic extracts of both root and aerial parts have shown highest activity among all the extracts.

Table 1: Antimicrobial activity of *Andrographis serpyllifolia*

Micro organisms	Zone of inhibition (mm)						
	Root			Aerial part			STD
	HE	CE	ME	HE	CE	ME	
E. coli	NA	7	10	6	NA	10	10
P. aeruginosa	NA	8	6	7	NA	12	12
S. aureus	10	10	14	NA	8	9	12
B. subtilis	NA	NA	9	NA	NA	12	12

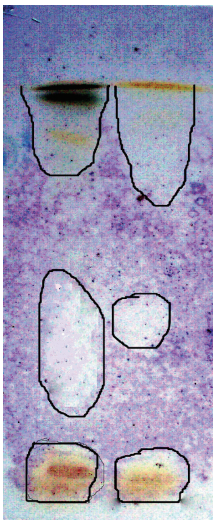
Zone of inhibition is the mean of five readings, HE- hexane extract, CE-chloroform extract, ME-methanol extract (100 mg/ml), STD - Standard Chloramphenicol (10mg/disc), NA - No activity

Table 2: MIC values for the *Andrographis serpyllifolia* extracts

Micro organisms	MIC values* (against 10^7 cells/ml) mg/ml					
	Root part			Aerial part		
	HE	CE	ME	HE	CE	ME
E. coli	NA	7.35±0.36	2.13±0.23	NA	NA	2.02±0.26
P. aeruginosa	NA	7.68±0.31	3.12±0.21	NA	NA	1.56±0.32
S. aureus	4.23±0.23	5.26±0.25	1.26±0.39	4.26±0.25	4.65±0.32	2.62±0.36
B. subtilis	NA	NA	2.06±0.25	NA	NA	1.98±0.27

Values are mean of five readings ± SEM, HE- hexane extract, CE-chloroform extract, ME-methanol extract (100 mg/ml)

MIC - Minimum inhibitory concentration, NA- No activity



(A) (B)

Fig. 1: Bio-autographic analyses of extracts

A -Aerial part & B- Root part of *Andrographis serpyllifolia*

The thin layer chromatography mobile phase that we developed ($CHCl_3$: MeOH 95: 5) separated compounds over a wide range of R_f values. The R_f values of the inhibiting components were 0.85, 0.73, 0.60, 0.24 for root part and 0.85, 0.70, 0.64, 0.52, 0.20 for aerial part and (fig. 1).

The three different solvents used to extract the components of the finely ground aerial and root parts of *A. serpyllifolia*, methanol extracted the largest number of inhibitory compounds from both parts. In addition to the components with antimicrobial activity, there were also several compounds on the chromatogram visible in UV light at 235nm and others that quenched fluorescence at 254 nm many of these compounds did not coincide with the antimicrobial components.

DISCUSSION AND CONCLUSIONS

Methanolic extract of both parts showed strong to moderate inhibitory action on all the tested bacterial with MIC of 3.125-6.250 mg/ml for root and 3.639-4.235 mg/ml for aerial part (table. 1). With the techniques used, it could be demonstrated that there

are at least 5 to 6 components in aerial part and 3 to 4 components in root part present that inhibited the growth of *S. aureus*.

The polar components of plant may responsible for its anti-microbial effect as showed maximum activity in methanolic extract.

The large number of antimicrobial components may explain why this plant showed very good anti-microbial effect. The wide diversity in polarity of the antimicrobial components may provide clinically useful leads. Characterization of active components is required and their activity has to be evaluated in further work

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