PHYTOCHEMICAL SCREENING, GC-MS ANALYSIS, ANTIOXIDANT ACTIVITY AND ANTIMICROBIAL ACTIVITY OF *Andrographis paniculata* (KING OF BITTER) LEAF EXTRACTS

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Abstract : Medicinal plants contain active constituents that are used in the treatment of many human pathogenic diseases. The systemic screening of these constituents or phytochemicals helps in development of certain bioactive compounds.The present study describes the phytochemical screening and antimicrobial activity of *Andrographis paniculata* leaf extracts. These result revealed the presence of various secondary metabolites. Extracts showed both antibacterial and antifungal potential against the selected microorganisms. We recommend further research on this plant for possible isolation and characterization of various phytochemicals present in *Andrographis paniculata* leaf extracts.

Key words:Andrographis paniculata,Phytochemicals,GCMS analysis,Antimicrobialactivity

I. INTRODUCTION

World is endowed with rich wealth of medicinal plants.80% of the world population depends on plant-derived medicine for human alleviation because of its fewer side effects. In the last century roughly 121 pharmaceutical products were formulated based on the traditional knowledge obtained from various sources. Man cannot survive on this earth for long life without the plant kingdom because the plant products and their active constituents plays an important role. In response to the increased popularity and great demand for medicinal plants, a number of conservation groups are recommending that wild medicinal plants to be brought into cultivation (1). A large number of medicinal plants and their purified constituents have shown beneficial therapeutic potentials various herbs and spices have been reported to exhibit antioxidants activity and antibacterial activity (2).

Andrographis paniculata is a medicinal plant that has been effectively used in traditional Asian medicines for centuries. The plant belongs to the family of Acanthaceae. *Andrographis paniculata* commonly known as 'King of bitter.This herb is main source of the bitterness.The extremely bitter and characterisitic taste of *Andrographis paniculata* gives it the term kings of bitters (3). *Andrographis paniculata*, known as *Kalmegh* in the Indian system of Ayurveda, is a widely used home remedy for the common cold.The effectiveness of this herb has been widely recognized and its demand is on the rise. It is an herbaceous plant native to India and Srilanka. *Andrographis paniculata*, an herbaceous plant commonly known as the King of bitters belongs to the family *Acanthaceae*. This plant also referred to as Bile of the earth due to its bitterness. It is a plant with characteristic white-purple or spotted purple flowers. The plant grows in waste grounds and prefers moist habitat (4).

II.MATERIALS AND METHODS

2.1 COLLECTION OF PLANT MATERIALS

Fresh leaves of medicinal plant, *Andrographis paniculata* were collected from kerala. The plant materials were taxonomically identified and authenticated from Botanical survey of India,Tamil Nadu Agricultural university. The plant materials were dried until all the water molecules evaporated and plants became well dried for grinding. After drying, the plant materials were ground well using mechanical blender into fine powder labeling for future use.

2.2 METHODOLOGY

2.2.1 PREPARATION OF LEAF EXTRACT

Crude plant extracts were prepared by Fresh leaves of *Andrographis paniculata* plant powder were kept in contact with methanol and chloroform separately in conical flasks for a defined period with continuous agitation. The extracts is then filtered and condensed extracts were kept in a refrigerator at 4ºC for their future use in analysis.

2.2.2 QUALITATIVE PHYTOCHEMICAL ANALYSIS

The extracts were tested for the presence of bioactive compounds by using standard methods.

Test for Alkaloids

Crude extract was mixed with 2ml of 1% HCl and heated gently. Mayer's reagent was then added to the mixture. Turbidity of the resulting precipitate was taken as an evidence for the presence of alkaloids.

Test for Flavonoids

Crude extract was mixed with 2ml of 2% solution of NaOH. An intense yellow colour was formed which turned colourless on addition of few drops of dilute acid which indicated the presence of flavonoids.

Test for Glycosides

2ml of concentrated H_2SO_4 was added carefully and shaken gently. A reddish brown colour indicated the presence of steroidal ring ie., glycone portion of the glycoside.

Test for Phenols

Crude extract was mixed with 2ml of 2% solution of $FeCl_3$. A blue green or black coloration indicated the presence of phenols.

Test for proteins

When crude extract was boiled with 2ml of 0.2% solution of Ninhydrin, violet colour appeared suggesting the presence of proteins.

Test for saponins

Crude extract was mixed with 5ml distilled water in a test tube and it was shaken vigorously. The formation of stable foam was taken as an indication for the presence of saponins.

Test for steroids

Crude extract was mixed with 2ml of chloroform and concentrated H_2SO_4 was added sidewise. A red colour produced in the lower chloroform layer indicated the presence of steroids.

Test for tannins

Crude extract was mixed with 2ml of 2% solution of $FeCl_3$.A blue – green or black colouration indicated the presence of tannins.

Test for terpenoids

Crude extract was dissolved in 2ml of chloroform and evaporated to dryness. To this 2ml of concentrated H_2SO_4 was added and heated for about 2 minutes.

2.2.3ANTIMICROBIAL ACTIVITY OF *ANDROGRAPHIS PANICULATA* LEAF EXTRACTS

Culture media and strains:

Nutrient agar medium and potato dextrose agar medium were used for antibacterial and antifungal study respectively. Pathological strains such as *Escherichia coli, Staphylococcus aureus, Pseudomonas aeroginosa, Bacillus subtilis and Salmonella typhi* were used for testing antibacterial activity of extracts. For antifungal study *Aspergillus niger, Aspergillus fumigates, Aspergillus parasitis, Monococus puricurus and Candida albicans* were selected.

Disc diffusion method

Antimicrobial activity of the *Andrographis paniculata* leaf extracts obtained by different solvents was determined by Disc diffusion method. Microbial inoculums were spread on the solidified agar plates. The 4mm diameter sterile Whatman No.1filter paper discs were soaked and saturated in extracts. Prepared discs were placed on the inoculated agar plate. Pencillin (for bacteria) and Chloramphenicole (for fungi) (1mg/ml) discs were used as positive control. The antimicrobial assay plates were incubated at 37ºC for 24 hrs. The zone diameters were measured in mm and the results were tabulated.

The plant leaves were washed thoroughly two to three times using tap water followed by distilled water.25g of leaf sample was boiled with 100 ml of double distilled water, at 60 ^{0}C for about 5 minutes **(Fig. 2)**

3. RESULT AND DISCUSSION

3.1 PHYTOCHEMICAL ANALYSIS

The results of the preliminary phytochemical analyses were carried out in extracts of *Andrographis paniculata* medicinal plant. The experiment showed the presence of secondary metabolites such as alkaloid,glycosides, flavonoids, saponins, tannins,steroids, protein, amino acid and phenol.

The results of the phytochemical analyses of *Andrographis paniculata* are shown in **Table.1**. Hence the phytochemical screening reveals that Methanol and Chloroform extract shows high secondary metabolites. Thus the preliminary screening analysis is helpful in the detection of bioactive compounds and lead to the discovery and development of novel drugs.

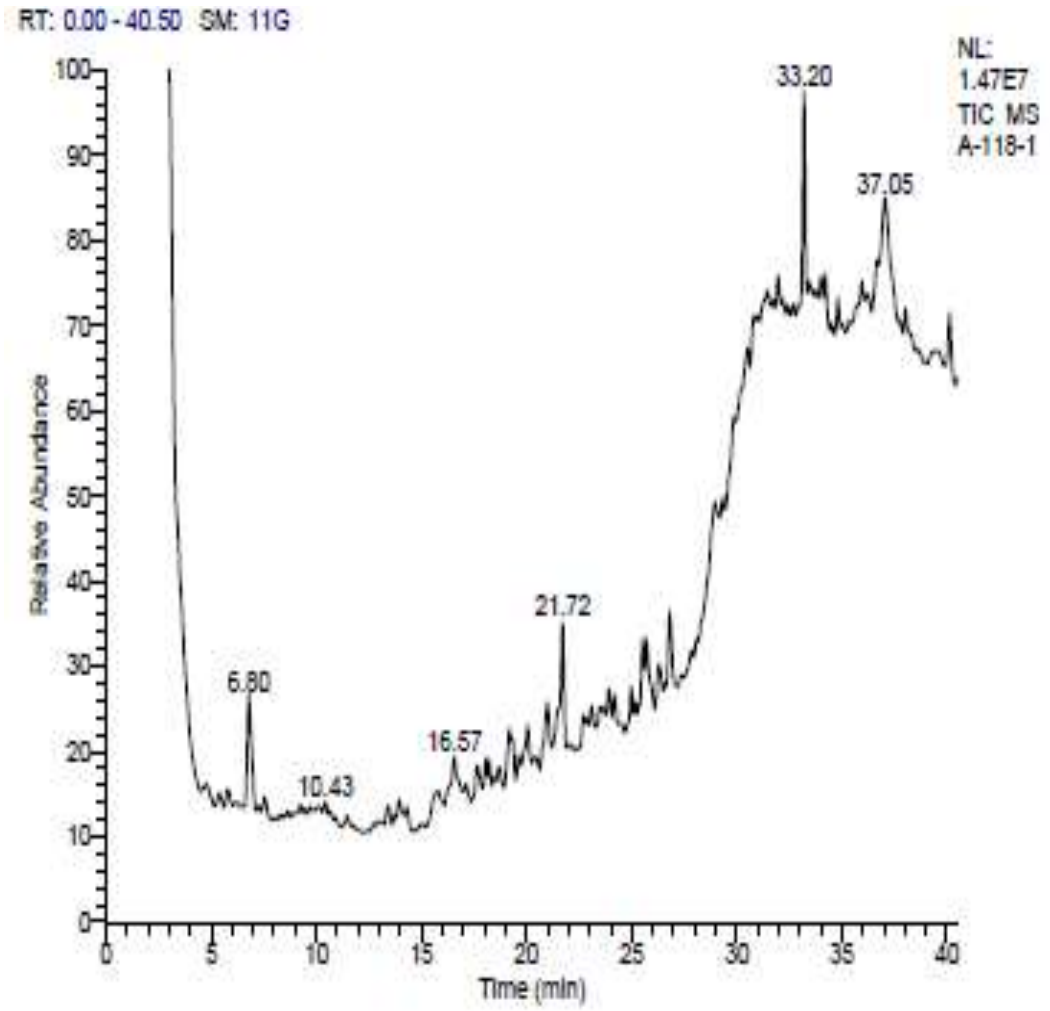
Table.1 Phytochemical screening of *Andrographis paniculata*

	Methanol extract	**Ethanol extract**
Alkaloids	+	+
Flavinoids	+	+
Glycosides	+	+
Phenols	+	+
Proteins	+	+
Saponins	-	-
Steroids	+	+
Tannins	-	+
Terpinoides	+	-

+ = presence of compound, - = absence of compound

Gas chromatography mass spectroscopy analysis was carried out in methanol and chloroform extracts of *Andrographis paniculata* leaves. Phytochemical analysis conducted on the *Andrographis paniculata* extracts revealed the presence of constituents which are known to exhibit medicinal as well as physiological activities. Many of the previous reports suggest that there are numerous secondary metabolites present in *Andrographis paniculata* extracts. Sumathi.P and Isabella Rosaline (2013) which reported the presence of phytoconstituents like flavinoids, steroids and tannins. Meenu Sharma *et al.*, (2011) studied various secondary metabolites in methanol and chloroform extract of *Andrographis paniculata* extracts. In their study, terpinoids compound found in methanol extracts. Thus the preliminary screening analysis is helpful in the detection of bioactive compounds and lead to the discovery and development of novel drugs. This finding was consistent with several reports on the effectiveness of solvents in the extraction of bioactive compounds.

Graph 1: GC-MS CHROMATOGRAM OF THE METHANOL EXTRACT OF *Andrographis paniculata*



The GC-MS analysis of the *Andrographis paniculata* methanol extracts contains 6 major compounds.Compounds detected in *Andrographis paniculata* leaf extract are listed below. Bioactive components were identified and tabulated with compound name, retention time, molecular formula, molecular weight and peak area.

Table.2 Phytocomponents identified in the methanol extract of *A. paniculata* by GCMS.

Sl. no	Compound name	Retention time	Molecular formula	MW	Peak area (%)
1	Methyl 3-(cis-2,3-Epoxybutanoxy)propanoate	3.07	C8H14O4	174	1.28
2	(2R,4S)-(E)-4-(BENZYLOXY)-2-(but-2-enyl)-5,5-dimethoxypentanal	6.80	C28H26O4	306	6.14
3	2-tert-Butyl-4-isopropyl-5-methylphenol	13.93	C14H22O	206	1.77
4	Benzene,chloro(chloromethyl)(1-methylethyl)-(CAS)	15.71	C10H12CL2	202	2.19
5	Isopropyl(xylyl)(phenyl)acetate	16.57	C22H28O2	324	4.21
6	1,3-D5-HEXAN-2-ONE 2,4-DINITROPHENYLHYDRAZONE	17.64	C12H11D5N4O4	280	1.76
7	1,3-Dimethyl-2(2,4,6-trimethylphenyl)-1,3-cyclopentadiene	18.06	C16H20	212	2.04
8	EXO-2-HYDROXY-9,9DIEUTERIO-BICYCLO(3.3.1)-NONANE	18.68	C9H14D2O	140	1.36
9	1,3-di-iso-propylnaphthalene	19.20	C16H20	212	3.58
10	1,2,3,4,5,6-Hexahydro-1,5-imino-7bromo-10-hydroxy-9-methoxy-3,8,11-trimethyl-3-benzazocin-4-0ne	20.06	C15H19BrN2O3	354	4.22
11	5à,14á-Pregn-16-en-20-one,3á,14,15à-trihydroxy-3-(5-Cyano-4-methoxycarbonylmethyl-4,5-dimethyl-2-thioxo-pyrrolidin-3-yl)-propionic acid,methyl ester	20.98	C14H20N2O4S	312	2.95
12	Pentadecanoic acid,14-methyl-,methyl ester(CAS)	21.72	C17H34O2	270	5.86
13	Apoatropine[endo-à-Methylenebenzeneacetic acid 8-methyl-8-azabicyclo[3.2.1]oct-3-yl ester	22.71	C17H21NO2	271	1.48
14	3-Amino-2,4-dicyano-5-methylbiphenyl	23.90	C15H11N3	233	2.43
15	17-Octadecynoic acid,methyl ester	25.54	C19H34O2	294	5.83
16	1-Propyl-2-methyl-7-methoxy-5H,6H,pyrido[3,4,-b]indole	26.30	C16H20N2O	256	2.01
17	(4aà,8à,8aà)-(+-)Octohydro-8-hydroxy-4a,8-dimethyl-2-(1H)-naphthalenone Tetrahydroactinidiolide	26.81	C12H2OO2	196	4.O8
18	(R)-(-)-METHYLHEXADEC-8-ENAL1,12-octadecandiol	28.94	C17H32HO	252	3.35
19	2-Bezyloxy-10-bromo-3-methoxy-5,6,8,12-tetrahydro-13aH-thieno[2′,3′:5,6][1,3]oxazepinol[2,3-a]isoq uinoline	29.84	C18H22BrNO3S	471	2.59
20	5-Heptenoic acid,7[2-[3(methoxyimino)butyl]-3,5-bis[(trimethylsilyl)oxy]cyclopentyl]-methyl ester,[1R-(1à,2á,3à,5à]-(CAS)	30.19	C24H47NO5Si2	485	1.45
21	3,7-Dibenzyl-1,7 dihydro-8-phenyl-2H-purin-2-one 1,2,5,6-tetrahydrocoronene	30.49	C25H20N4O	392	1.98
22	4-FLUORO-2,′3′,5′,6′-TETRAMETHYLBIPHENYL(2-Hydroxy-2-methyl-3-tosyl)propyl Thiocyanate	30.82	C16H17F	228	2.79
23	Pyranthrene	31.43	C30H16	376	3.49
24	1-(2-Hydroxymethoxy-6-methoxyphenyl)ethanol	33.20	C10H14O3	182	7.64
25	03027205002 FLAVONE	34.22	C27H30O15	594	2.29
26	1,3,4,7-Tetraphenylthieno[3,4-c]pyridine	34.81	C31H21NS	439	1.24
27	(5-n-Pentadecyl-2,4-dinitro-1-hydroxy)benzene	35.95	C21H34N2O5	394	2.89
28	4-(1,2,2,2,-Tetraffluro-1-trifluromethylethyl)-2-bromo-3-methoxy-5-fluoro-6-pent-1-ynylpyridine	37.05	C14C14H10BrF8NO	439	14.34
29	1,3,8-trimehthyl-4-propyl-5-ethyl-2-(1-hydroxyethyl)-7-methoxycarbonylethyl-6,ç-methylenecarbonyl-porphine	38.03	C36H42N4O4	594	1.28
30	1-2-Dimethyl-3-trimethylsilyl-9H-cabazole	39.48	C17H21NSi	267	1.47

3.2 ANTIMICROBIAL ACTIVITY

Antimicrobial activity is used to test whether the leaf extract has capability to control the growth of the microorganism. The present approach is the microbiological study involving antibiotic sensitivity test employed to the microorganisms.

Antimicrobial activity of methanol and chloroform extracts of *Andrographis paniculata* against selected bacterial and fungal pathogens were tested. The result obtained, the zone of inhibition was recorded at three concentrations of 100,150,200 µg/ml in **Table.3&4** respectively. The disk diffusion assay on the methanol and chloroform showed that methanol extract have increasing inhibitory effect than chloroform extracts on microbial growth with increasing concentration of an extraction. In these tested microorganisms against methanol and choloform extracts all selected pathogenic strains are sensitive to leaf extract except *Candida albicans.* This antimicrobial property may be due to the presence of bioactive molecules and utilization of these potent compounds could be helpful for the production of new antimicrobial agent.

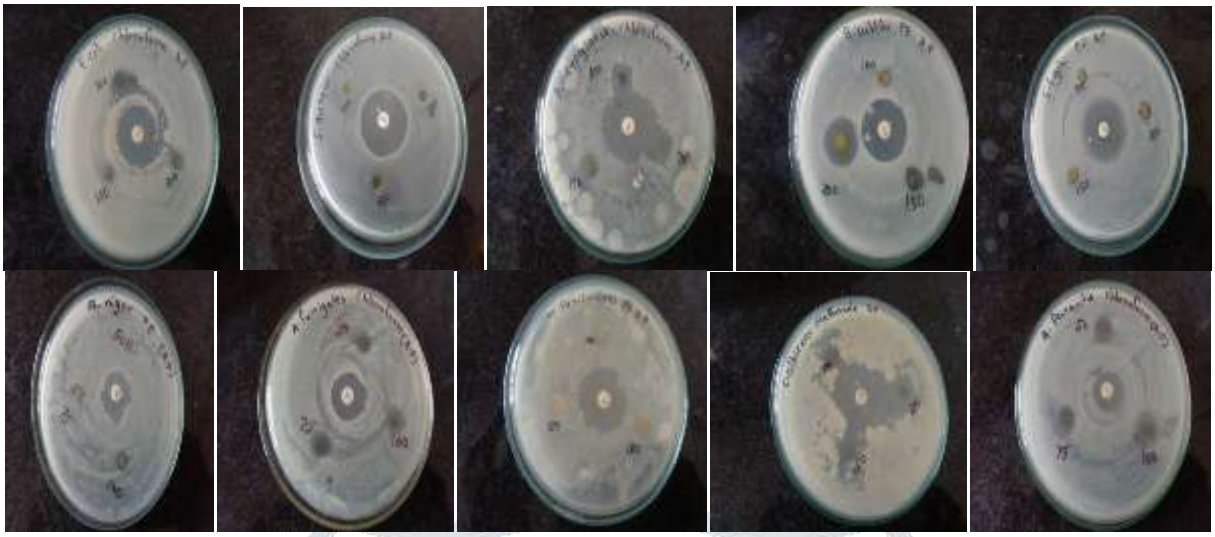
Table.3 : Antibacterial activity of *Andographis paniculata* leaf extracts

Micro organisms (Bacterial strains)	Methanol extract (Zone of inhibition in mm)				Chloroform extract (Zone of inhibition in mm)		
	Concentration in µg/ml				Concentration in µg/ml		
	C	100	150	200	100	150	200
E.coli	21	11	12	13	12	13	15
S.aureus	20	13	14	11	11	13	14
P.aeroginosa	16	11	12	14	9	11	4
B.subtilis	20	7	8	12	13	14	15
S.typhi	23	10	12	13	11	13	14

Table.4 : Antifungal activity of *Andographis paniculata* leaf extracts

Micro organisms (Fungal strains)	Methanol extract (Zone of inhibition in mm)				Chloroform extract (Zone of inhibition in mm)		
	Concentration in µg/ml				Concentration in µg/ml		
	C	100	150	200	100	150	200
A.niger	22	12	13	14	14	15	16
A.fumigates	24	19	20	21	18	19	20
M.puricurus	26	12	14	15	17	18	19
C.albicans	-	-	-	-	-	-	-
A.parasitis	20	11	12	13	15	16	18

Figure.1 Antimicrobial activity of *Andrographis paniculata* leaf extracts



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4. SUMMARY

Medicinal plants play a vital role in preventing various diseases. The antimalarial, anti-bacterial and anti-fungal activities of the medicinal plants are due to the presence of the secondary metabolites i.e., saponins, phenols, tannins and steroids.Medicinal plants are used for discovering and screening of the phytochemical constituents which are very helpful for the manufacturing of new drugs. The antibacterial efficacy of this plant confirms the claims of traditional healers who use to locate the active principles from various extract of the plants. The results of present study suggest that leaf can be used as a source of phytochemicals for pharmacological preparations.

Thus, we hope that the important phytochemical properties, antimicrobial, identified by our study in the local plant of *Andrographis paniculata* will be helpful in the commercial interest in both research institutes and pharmaceuticals companies in the manufacturing of the new drugs for treatment of various diseases.

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