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Phytochemical evaluation of *Andrographis paniculata* L. (L.)

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Abstract
Andrographis paniculata L. (L.). belonging to Acanthaceae family is a widely used medicinal plant. This plant is known to possess anti-microbial, cytotoxicity, anti-protozoan, anti-inflammatory, anti-oxidant, immunostimulant, anti-diabetic, anti-infective, anti-angiogenic, hepato-renal protective, sex hormone/sexual function modulation, liver enzymes modulation and insecticidal properties. The present study is aimed to find out the phytochemical evaluation of *Andrographis paniculata* whole plant using qualitative tests, FTIR and GC-MS. The phytochemical tests for different active principles revealed the presence of alkaloids, tannins, flavonoids and triterpenoids. Phytol was detected as a major ingredient in GC-MS analysis.

Keywords: acanthaceae, *Andrographis paniculata*, FTIR, GCMS, phytochemical qualitative tests

Introduction
Andrographis paniculata is an annual herb belonging to family Acanthaceae grows abundantly throughout tropical and sub-tropical regions. It is commonly known as Kalmegh, Green chireyta, King of Bitter and Bhunimba. It is a widely used medicinal plant in various systems of medicine. This plant is proved to have anti-microbial, cytotoxicity, anti-protozoan, anti-inflammatory, anti-oxidant, immunostimulant, anti-diabetic, anti-infective, anti-angiogenic, hepato-renal protective, sex hormone/sexual function modulation, liver enzymes modulation, insecticidal and toxicity activities (Abhishek *et al*,. 2010) [1]. Medicinal property of *A.paniculata* is attributed due to the presence of biologically active compounds in the plant. The present experiment was conducted to identify various active components and their classes in the locally available *A. paniculata* plant.

Materials & Methods
Collection of plants and authentication
A.paniculata L. (L.). whole plants were collected during the month of January 2018 from mannuthy area of Thrissur district and authenticated at Department of Botany, St Thomas College, Thrissur (Plate 1). The whole plant was shade dried and pulverised using an electrical pulveriser. About 50g powder was taken and extracted with methanol using accelerated solvent extractor (Thermoscientific, model: Di1x ASE 150). The methanolic extract was then concentrated using a rotary vacuum evaporator under reduced pressure and temperature and stored under refrigeration (4 ºC) until further use

Plate 1: *Andrographis paniculata* whole plant

Qualitative tests

The whole plant was tested for the presence of various active chemical constituents namely steroids, alkaloids, tannins, phenolic compounds, flavonoids, glycosides, diterpenes, triterpenoids and saponins (Harborne, 1998) [4].

Fourier transform infrared (FTIR) spectroscopy

Functional groups present in *A. paniculata* powder was identified using Fourier transform infrared (FTIR) spectroscopy (Perkin Elmer, FTIR spectrophotometer, Singapore). Weighed 2 mg of *A. paniculata* powder and 298 mg of dry fine powder of potassium bromide (KBr) and were transferred into a mortar and mixed well. The KBr-sample mixture was transferred to an evacuable die that has a barrel diameter of 13 mm and the die was pressed at around 8 to 10 tons for 1 to 2 minutes in a hydraulic hand press. Re-crystallization of the KBr results in a clear transparent disk about 1 mm thick and the infrared spectrum was recorded in the scan range from 4000 cm^{-1} to 400 cm^{-1}on FTIR spectrophotometer with a resolution of 0.5 cm^{-1}. The structurally related compounds were identified through Fluka library supplied by Perkin-Elmer (Swapna *et al*., 2012) [16].

Gas chromatograph–mass spectrometry analysis

The GC-MS analysis of the methanolic extract was carried out at Kerala Forest Research Institute, Peechi, Thrissur using Shimadzu GCMS Model Number: QP2010S. The compounds were separated on Rxi-5Sil MS capillary column (30 m × 0.25 mm; i.d. 0.25 µm film). The sample, dissolved in methanol and filtered through 0.22µ syringe filter was used for analysis. The column oven temperature was programmed from an initial temperature of 80 ºC (4 min), then temperature raised to 280 ºC at the rate of 5 ºC min^{-1}, finally 280 ºC was maintained isothermally with a final time of 6 min. The injection temperature and ion source temperature were 260 and 200 ºC, respectively. Helium (99.999%) was used as the carrier gas with a flow rate of 1 ml min^{-1}. The ionizing energy was 70 eV. All the data were obtained by collecting the full-scan mass spectra within the scan range 50–500 amu. Compounds were identified using the National Institute of Standards and Technology (NIST 11) & Wiley 8 library (Victoria *et al*., 2014) [17].

Results and Discussion

Phytochemical screening-qualitative tests

Presence of alkaloids, tannins, flavonoids and triterpenes were detected during the phytochemical screening of whole plant *A. paniculata* (Table 1) (Plate 2, 3, 4 and 5). Sithara *et al*. (2016) [15] indicated availability of phenolic compounds, alkaloids, flavonoids, steroids and tannins in petroleum ether and chloroform extracts of *A. paniculata* leaves. Dwivedi *et al*. (2015) [3] reported the presence of steroids, flavonoids, quinones and proteins in the methanolic extract of leaves of *A. paniculata*. Lalitha *et al*. (2015) [7] detected presence of flavonoids, glycosides, saponins, phenols, tannins and steroids in methanolic extract of *A. paniculata* leaves. They could not detect the presence of alkaloids in methanolic extract of *A.paniculata* leaves, but presence of alkaloids was detected in the chloroform extracts of same plant by same authors.

Table 1: Phytochemical screening *Andrographis paniculata* whole plant powder

Active principle	Result
Steroids	Absent
Alkaloids	Present
Phenolic compounds	Absent
Tannins	Present
Flavonoids	Present
Glycosides	Absent
Diterpenes	Absent
Triterpenoids	present
Saponins	Absent

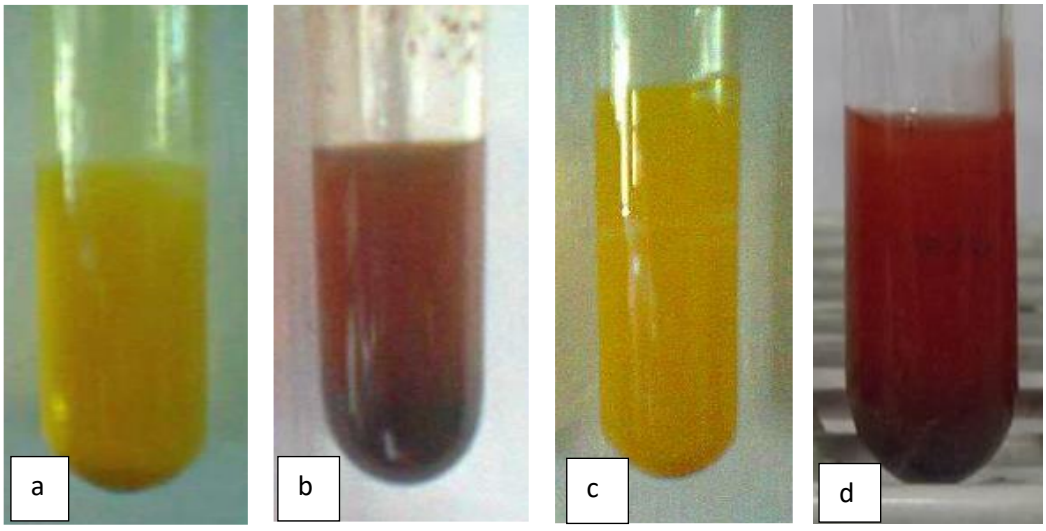

Plate 2: Test for alkaloids - Mayer's test (a), Wagner's test (b), Hager's test (c), and Dragendroff's Test (d)

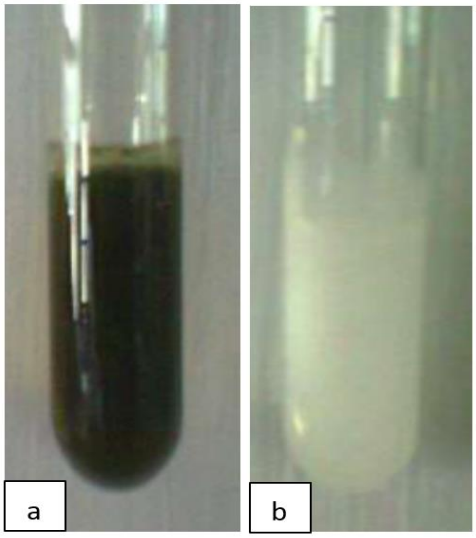

Plate 3: Test for Tannins –Ferric chloride test (a) and Gelatin test (b)

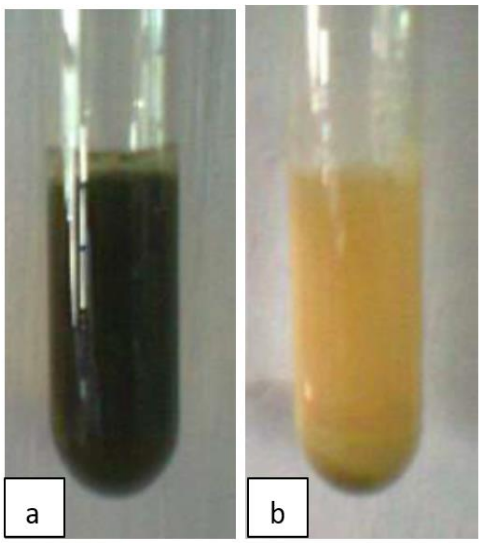

Plate 4: Test for flavonoids - Ferric chloride test (a) and lead acetate test (b)

Plate 5: Test for triterpenoids- Salkowski test

Matsuura and Fett-Neto (2015) [10] observed that alkaloids act against pathogens and predators due to their toxicity. Tannins are capable of inhibiting cell wall synthesis by forming irreversible complexes with prolene rich proteins (Mamtha et *al*., 2004) [9]. Panche *et al*. (2016) [12] reported that flavonoids possess anti-oxidative, anti-inflammatory, anti-mutagenic and anti-carcinogenic properties coupled with their capacity to modulate key cellular enzyme function. Lee *et al*. (2014) [8] reported that triterpenoid compounds have anti-hypertensive effects, with capacity to inhibit platelet aggregation and increase endothelial-dependent vasodilation. Medicinal properties of this plant could be due to the presence of these active ingredients.

Fourier transform infrared spectroscopy

The structurally similar compounds identified in the powder of *A. paniculata* were l (-)-glyceraldehyde unnatural form, heptyl-beta-d-glucopyranoside, tomatine, b-cyclodextrin, octyl-beta-d-glucopyranoside, digitonin, chitin, dodecyl-b-d-glucopyranoside, quabain, pectin exapples (Table 2, Fig.1). Dwivedi *et al*, (2015) [3] reported the presence of alkynes, aromatics, alkenes, alcohols, alkanes and carbonyl group in the compound andrographolide isolated from *A. paniculata*.

Table 2: The result of FTIR analysis of *A. paniculata* powder

Search Score	Structurally related compound	Type of compound
0.689992	L(-)-GLYCERALDEHYDE UNNATURAL FORM	Aldehyde
0.619338	HEPTYL-BETA-D-GLUCOPYRANOSIDE	Protein
0.602312	TOMATINE	Glycoalkaloid
0.586652	B-CYCLODEXTRIN	Polysaccharide
0.580801	OCTYL-BETA-D-GLUCOPYRANOSIDE	Protein
0.51538	DIGITONIN	glycoside
0.496701	CHITIN	Carbohydrate
0.496136	DODECYL-B-D-GLUCOPYRANOSIDE	Protein
0.492868	QUABAIN	glycoside
0.487604	PECTIN EX APPLES	Polysaccharide

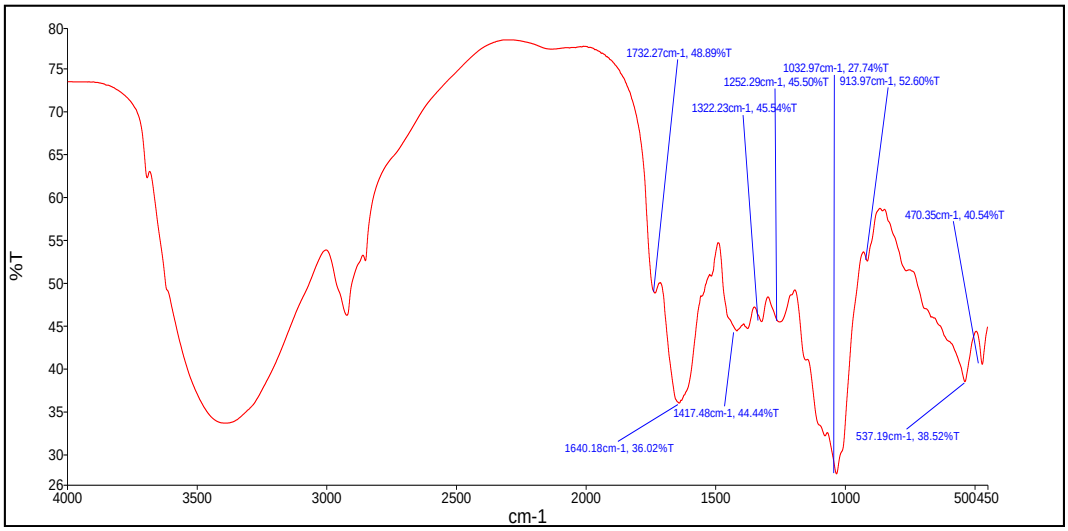

Fig 1: Peak obtained on FTIR analysis

Gas chromatography–mass spectrometry

Whole plant methanolic extract of *A. paniculata* on GC-MS analysis revealed 17 different types of compounds. Wide variety of chemicals namely Neophytadiene (20.84%), 1H-pyrazole, 3-methyl-(0.77%), Citronellyl propionate (3.90%), Heptadecanoic acid, methyl ester (8.30%), 3,5-diethyl-3,5-dihydro-3,5-dimethyl-4-methylene-4H-pyrazole (0.78%), 3,6-dodecadien-1-OL, (E,E)-(2.12%), Phytol (26.01%), Pentanoic acid, 2-methyl-(0.43%), Benzene, 1-methyl-4-[[3-methyl-2-[[4-methyl-3-(1-methylethyl)-2-pentenyl]oxy]-2-butenyl]sulfonyl]-(0.67%), 1H-imidazo[1,5-A]azepine-3,8(2H,5H)-dione, tetrahydro-1,1-dimethyl-(3.81%), 5,7-dihydroxy-6-methoxy-2,8-dimethylchromone (2.10%), 9-phenanthrenol,1,2,3,4,4a,9,10,10a-octahydro-7-methoxy-1,1,4a-trimethyl-8-1-methylethyl)-,(5.26%), Squalene (17.96%), (-)-.Beta.-caryophyllene epoxide (3.07%), (-)-(2S,4R)-1,2,4'-tri-o-benzyl-4-(hydroxymethyl)-1,2,4-hexanetriol (0.58%), Trans-2-[(1e,5e)-2,6,10-trimethyl-1,5,9-undecatrienyl]cyclopropanecarboxylic acid (0.72%), Tert-Butylhydroquinone, bis(trifluoroacetate) (2.68%) were detected (Table 3, Fig 2). Phytol was the major characterised component followed by Neophytadiene and Squalene in sizeable concentrations. Phytol is an antimicrobial, anticancer, anti-inflammatory and diuretic agent (Praveen kumar *et al*., 2010) [13]. Phytol was observed to have antibacterial activities against *Staphylococcous aureus* by causing damage to cell membranes as a result there is a leakage of potassium ions from bacterial cells. Phytol is a key acyclic diterpene alcohol that is a precursor for vitamins E and K1. It is also having carminative and antiviral properties. Neophytadiene belongs to class of organic compounds known as sesquiterpenoids and known to have antipyretic, analgesic, and anti-inflammatory, antimicrobial and antioxidant (Raman *et al.*, 2012) [5]. Karaket *et al*, (2018) [6] also observed the presence of Neophytadiene in dichloromethane extract of *A. paniculata.* Squalene is a natural 30-carbon isoprenoid compound and intermediate metabolite in the synthesis of cholesterol. It is not susceptible to lipid peroxidation and provides skin protection (NCBI, 2018) [11]. Presence of squalene was also observed by Kalaivani *et al*. (2012) [5] in ethanolic extract of *A. paniculata* leaves. Squalene is known to neutralize different xenobiotics, anti-inflammatory, anti-atherosclerotic and antineoplastic, role in skin aging and pathology, and Adjuvant activities (Raman *et al.*, 2012) [5]. All these compounds plays an important role in the medicinal properties of *A. paniculata.*

Table 3: The results of GC-MS analysis of methanolic extract of *A. paniculata*

Peak	Retention time	Area %	Name	Base m/z
1	26.701	20.84	Neophytadiene	68.05
2	27.200	0.77	1h-pyrazole, 3-methyl-	82.10
3	27.577	3.90	Citronellyl propionate	82.10
4	28.503	8.30	Heptadecanoic Acid, Methyl Ester	74.05
5	31.704	0.78	3,5-diethyl-3,5-dihydro-3,5-dimethyl-4-methylene-4h-pyrazole	67.05
6	31.811	2.12	3,6-dodecadien-1-ol, (e,e)-	79.05
7	32.061	26.01	Phytol	71.05
8	32.334	0.43	Pentanoic Acid, 2-Methyl-	74.05
9	35.110	0.67	Benzene, 1-methyl-4-[[3-methyl-2-[[4-methyl-3-(1-methylethyl)-2-pentenyl]oxy]-2-butenyl]sulfonyl]-	58.05
10	39.058	3.81	1h-Imidazo[1,5-A]Azepine-3,8(2h,5h)-Dione, Tetrahydro-1,1-Dimethyl-	181.00
11	40.106	2.10	5,7-dihydroxy-6-methoxy-2,8-dimethylchromone	181.00
12	41.996	5.26	9-phenanthrenol,1,2,3,4,4a,9,10,10a-octahydro-7-methoxy-1,1,4a-trimethyl-8-1-methylethyl)-,	283.05
13	43.279	17.96	Squalene	69.10
14	43.629	3.07	(-)-.beta.-caryophyllene epoxide	55.05
15	44.904	0.58	(-)-(2s,4r)-1,2,4'-tri-o-benzyl-4-(hydroxymethyl)-1,2,4-hexanetriol	313.05
16	45.830	0.72	Trans-2-[(1e,5e)-2,6,10-trimethyl-1,5,9-undecatrienyl]cyclopropanecarboxylic acid	69.10
17	46.781	2.68	Tert-Butylhydroquinone, bis(trifluoroacetate)	343.10

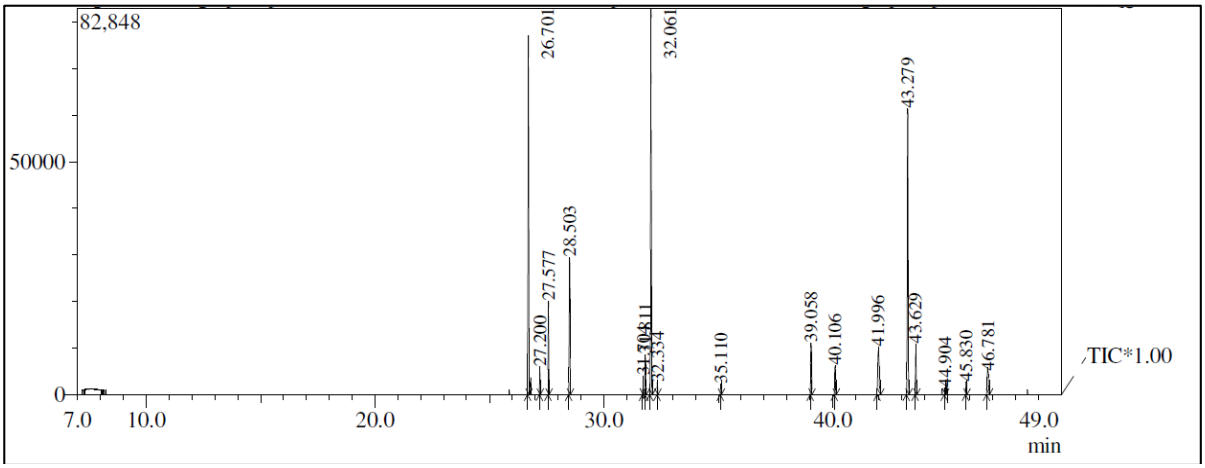

Fig 2: Chromatogram obtained at the GC-MS analysis of methanolic extract of whole plant of *A. paniculata*

The differences in the compounds reported in previous studies with this results might be due to variation in the geographical areas from which plants were collected, which might influence the growth and chemical constituents of the plants. The phytoconstituents identified from this study could be utilised scientifically.

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