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Research Article

PRELIMINARY PHYTOCHEMICAL SCREENING AND GC- MS ANALYSIS OF ETHANOLIC STEM EXTRACT OF *ANDROGRAPHIS PANICULATA*

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ABSTRACT

Andrographis paniculata is a widely distributed plant in the tropical and sub tropical area. Traditionally the plant has been used for various ailments like, including gastrointestinal complains, throat infections dispel toxins, and for increasing biliary flow. In the present study qualitative analysis, stem extracts of *Andrographis paniculata* were analyzed for the presence of alkaloids, carbohydrates, saponin, protein, phytosterol, phenolic compounds, flavonoids and glycosides were screened in five solvent extracts. Among the five different extracts of *Andrographis paniculata*, ethanolic extract of the stem showed maximum amount phytochemicals was screened in *Andrographis paniculata*. Quantitative estimation of *Andrographis paniculata* showed the presence alkaloids, saponins, total phenols and total flavonoids determined by using Standard methods. Further it is subjected to identify the number of phytoconstituents present in the *Andrographis paniculata* by GC-MS analysis. In total there are 13 compounds were identified in GC-MS analysis of ethanolic stem extracts of *Andrographis paniculata* stem which are medicinal important to become a potential drug in future perspective.

INTRODUCTION

Medicinal plants are rich source of novel drugs that forms the ingredients in traditional system of medicine, modern medicines, pharmaceutical intermediates and lead compounds in synthetic drugs [1]. The reason for using them as medicine lies in the fact that they contain chemical components of therapeutic value [2]. These compounds are naturally occurring in the medicinal plants, leaves, vegetables and roots that have defense mechanism and protect from various diseases. The medicinal value of plants lies in some chemical substances (usually secondary metabolites) that produce a definite physiological action as the human body. In recent times focus on plant research has increased all over the world and a large body of evidence has collected to show immense potential of medicinal plants used in various traditional systems [3] including treatment against hepatocellular carcinoma [4].Herbal medicines are being used by nearly about 80% of the world population, primarily in developing countries for primary health care [5]. Assessing the current status of health care system, inadequacies of synthetic drugs are likely to be more glaring in the coming years.

Andrographis paniculata, commonly known as Siriyanangai in Tamil, belongs to the family *Acanthaceae* is widely used in the Indian traditional system of medicine. *Andrographis paniculata* grows erect to a height of 30– 110 cm in moist, shady places. The slender stem is dark green, squared in cross-section with longitudinal furrows and wings along the angles. The lance shaped leaves have hairless blades mea ruing up to 8 centimeters long by 2.5 wide. The small flowers are borne in spreading racemes. The fruit is a capsule around 2 centimeters long and a few millimeters wide. It contains many Yellow brown seeds. It is also known as *Bhui-neem*, meaning "neem of the ground", since the plant, though being a small annual herb, has a similar strong bitter taste as that of the large Neem tree. It is an annual herbaceous plant which is commonly cultivated in Southern Asia, in China, and in some parts of South East Asia. The major component of *Andrographis paniculata* is andrographolide is a bitter, colorless, and crystalline in appearance, is called diterpene lactone [6]. It is used to treat poisonous bites, diabetes and respiratory tract infection. The plant possesses anti-inflammatory, anti-pyretic, anti-viral, immune stimulatory, anti-cancer, anti-hyperglycemic and anti-oxidant properties [7]. Many of the plants are rich in secondary metabolites and are potent source of drugs. In the present study, we have concentrated on the preliminary screening, quantitative determination, and the qualitative separation of secondary metabolites from stem of selected medicinal plants.

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MATERIALS AND METHODS

Sample Collection

Andrographis Paniculata stem were collected from Kolli hills and fresh leaves were collected and authenticated with the help of Rapinat herbarium.

Extraction

Fifty grams of the shade-dried and stem powdered of *Andrographis Paniculata* were packed in a wide-mouthed bottle. The powdered material is soaked in different solvent namely Ethanol, Chloroform, Petroleum ether, Benzene and Dichloromethane in a bottle. The bottle is closed in air-tight and allowed to stand for three days, undisturbed. After three days, the different extracts were collected and were concentrated by distillation process and the concentrated extracts were taken for the phytochemical screening and ethanolic extracts were analyzed by GC-MS analysis.

Qualitative analysis of primary and secondary metabolites

The stem extracts of *Andrographis Paniculata* were analysed for the presence of alkaloids, carbohydrates, saponin, protein, phytosterol, phenolic compounds, flavonoids and glycoside according to the common phytochemical methods described by Harborne (1998) [8].

Quantitative Determination of Andrographis Paniculata

Determination of Alkaloid by the method of Harborne (1973)

5 gs of the *Andrographis paniculata* stem powdered was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 h. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitated was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed.

Determination of Saponin by the method of Obadoni and Ochuko (2001)

5 gs of the *Andrographis paniculata* stem powdered put into a conical flask and 100 ml of 20% aqueous ethanol were added. The samples were heated over a hot water bath for 4 h with continuous stirring at about 55^0C. The mixture was filtered and the residue re-extracted with another 200 ml of 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90^0C. The concentrate was transferred into a 250 ml separation funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the samples were dried in the oven to a constant.

Determination of total phenols by spectrophotometric method of Kim et al., (2003)

A diluted *Andrographis paniculata* Stem extract (1 ml) or Gallic acid standard phenolic compound was added to a 25 ml volumetric flask, containing 9 ml of distilled water. 1 ml of Folin-Ciocalteu's phenol reagent was added to the mixture and shaken. After 5 min, 10 ml of 7% Na_2CO_3 solution was mixed in to the test sample solution was diluted to 25 ml distilled water and mixed thoroughly. The mixture was kept in the dark for 90 min at 23°C, after which the absorbance was read at 750 nm. Total phenol content was determined from extrapolation of calibration curve which was made by preparing Gallic acid solution (Fig. 1). The estimation of the phenolic compounds was carried out in triplicate. The Total Phenolic content was expressed as milligrams of Gallic acid (GAE) equivalents per gram dried sample. (Table 2).

Determination of total Flavonoids by the method of Katasani (2001)

Andrographis paniculata Stem extract (0.5 ml) were mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1M potassium acetate and 2.8 ml of distilled water. It remained at room temperature for 30 minutes. The absorbance of the reaction mixture was measured at 510 nm using UV-Visible spectrophotometer. The calibration curve was prepared by preparing quercetin solutions at concentrations 20 to 80 µg/ ml in methanol (Fig 2). The Total flavonoids content was expressed as milligrams of quercetin equivalents per gram of dried sample. (Table 3).

GC-MS analysis

The ethanolic leaves extract of *Andrographis paniculata* was analyzed for its chemical constituents by GC-MS analysis. The GC-MS analysis was performed on a combined GC-MS instrument (ITQ 900 Model of Thermo Fisher Scientific make) using a HP-5 fused silica gel capillary column. The method to perform the analysis was designed for both GC and MS. 1 µL aliquot of sample was injected into the column using a PTV injector whose temperature was set at 275°C. The GC program was initiated by a column temperature set at 60°C for 5 min, increased to 300°C at a rate of 8 C/min, held for 10 min. Helium was used as the carrier gas (1.5 mL/min). The mass spectrometer was operated in EI mode with mass source was set at 200°C. The chromatogram and spectrum of the peaks were visualized. The particular compounds present in the ethanolic extract of the leaves were identified by matching their mass spectral fragmentation patterns of the respective peaks in the chromatogram with those stored in the National Institute of Standards and Technology Mass Spectral database (NIST-MS, 1998) library.

RESULTS AND DISCUSSION

Qualitative phytochemical analysis of primary and secondary metabolites from Andrographis paniculata

Phytochemical analysis is the techniques to identify the phytochemical constituent of the medicinal plants. In this phytochemical screening we find the potential of phytochemicals act as an effective drug. The present study carried out the *Andrographis paniculata* stem extract revealed the presence of medicinal active phytoconstituent were qualitatively analyzed for stem separately and the results are presented in Table 1.

In these screening process alkaloids, tannins, saponins, flavonoids and carbohydrate, glycosides, phenols shows different types of results in different solvents. The petroleum ether being highly non-polar in nature was able to extract very less phytochemical compound characterized like carbohydrate and reducing sugars. Ethanolic extract was found to have a wide range of bioactive compounds like alkaloids, flavonoids, carbohydrates, reducing sugar, saponins, protein, phytosterols and glycoside. Chloroform extract was positive for reducing sugars, carbohydrate, tannins, saponin, protein, phytosterols and alkaloids. Dichloromethane extract was positive for alkaloids, reducing sugar, phytosterol, phenolic compounds and tannin. Benzene extract was found to have alkaloids, carbohydrate, reducing sugar, phytosterols and phenolic compounds. Among the five different extracts of *Andrographis paniculata,* the Ethanolic extract of the stem showed maximum amount phytochemicals was screened in *Andrographis paniculata.* The presence of bioactive constituents indicates that the *Andrographis paniculata* can be used in a multitude of ways for the beneficiary of population.

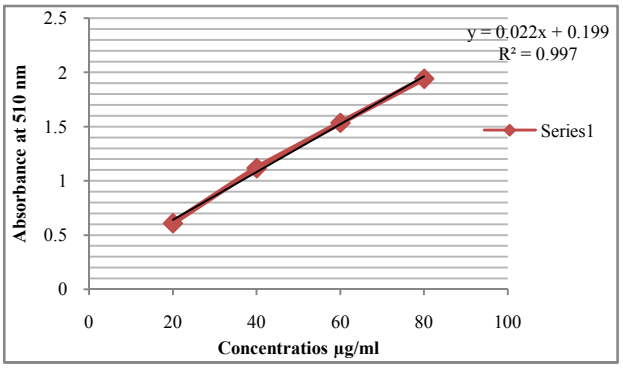

Fig 1 Standard Curve for Total Flavonoids using Quercetin

Table 1 Qualitative Phytochemical analysis of primary and secondary metabolites from stem of *Andrographis paniculata*

S.No	Phytochemical Tests	Ethanol	chloroform	Dichloromethane	Petroleum ether	Benzene
1.	**Alkaloids** Mayer's test Wagner's test	+	+	+	–	+
2.	**Carbohydrates** Molisch's test Fehling's test	+	+	–	+	+
3.	**Sugar** Benedict's test	+	–	+	+	+
4.	**Saponin** Foam test	+	+	–	–	–
5.	**Protein** Millon's test Ninhydrin test	+	+	–	–	–
6.	**Phytosterol** Libermann -Burchard's test	+	+	+	–	+
7.	**Phenolic compounds** Ferric chloride test	–	+	+	–	+
8.	**Tannin**	–	–	+	–	+
9.	**Flavonoids** Alkaline reagent test	+	–	–	–	–
10.	**Glycoside** Legal's test	+	–	–	–	–

(Presence of phytoconstituent = +) (Absence of phytoconstituent = -)

Quantitative determination of secondary metabolites from Andrographis paniculata stem

The amount of alkaloids, Saponin, total phenols and flavonoids was determined by using standard methods (Table- 2).while Table 2 revealed the amount of flavonoids and standard curves of Quercetin shown on figure- 1. Total phenolic compound and standard curves of Gallic acid Shown on figure 2. In Quantitative estimation *Andrographis paniculata* stem contained higher content of phenols and flavonoids with lesser amount of saponins and alkaloids.

Table 2 Quantitative phytochemical analysis of *Andrographis paniculata* stem

S.No	Name of the phytochemical constituents	Results (mg/gm)
1.	Alkaloids	30±0.12
2.	Saponin	32±0.10
3.	Total Phenols	269.04±0.83
4.	Total Flavonoids	237.016±0.59

Values were expressed as Mean ± SD for triplicate.

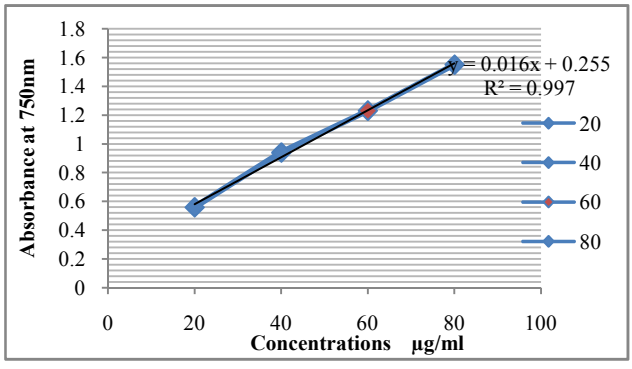

Fig 2 Standard Curve for Total Phenol using Gallic acid

GC-MS analysis of ethanolic stem extract of the Andrographis paniculata

The components present in the ethanolic extract of the stem from *Andrographis paniculata* was identified by GC-MS chromatogram are shown in Figure-3.

Table 3 GC-MS spectrum identified in ethanolic extract of stem from *Andrographis paniculata*

S.No	RT	Name of a compound	Molecular Formula	MW	Peak Area %	Uses
1.	14.66	Benzoic acid, 4-diethylamino-ethyl ester	$C_{13}H_{19}NO_2$	221	1.58	Anti tumour agents.
2.	18.21	9-Nonadecyne	$C_{19}H_{36}$	264	7.90	Nano technology, pheromonal activity.
3.	18.67	11-Hexade cyn-1-ol	$C_{16}H_{30}O$	238	0.37	pheromonal activity.
4.	19.87	3-Formyl-2,2,4,5-tetramethyloxazolidine-4-carboxylic acid ,methyl ester	$C_{10}H_{17}NO_4$	215	0.17	Anti bacterial and cancer.
5.	21.00	9-Hexadecenal	$C_{16}H_{30}o$	238	3.36	pheromonal activity.
6.	23.59	2-Cyclohexyl-4a,7-dimethyl-3,4,4a,5,6,8a-hexahydro-2H-1,2-benzoxazine-3-carbonitrile	$C_{17}H_{26}N_2O$	274	0.96	Not reported
7.	24.54	4-nitro-1-methylethyl ester	$C_{10}H_{11}NO_4$	209	8.58	Not reported
8.	26.42	Normelicopine	$C_{16}H_{13}NO_5$	299	0.03	Anti inflammatory, anti fungal and anti plasmodial neuro protective effects.
9.	27.61	Cyclic methaneboronate, pregnane-3,20-dione	$C_{22}H_{33}BO_4$	372	3.42	Not reported
10.	27.92	Cholestan-26-oic acid	$C_{27}H_{46}O_5$	450	13.2	Neurological disease treatment
11.	29.75	Acetamide,2-chloro-N-(2,6-diethylphenyl)-N-(methoxy methyl)	$C_{14}H_{20}ClNO_2$	269	1.03	Not reported
12.	30.38	2,3-Dicyano-2-(2-oxo-cyclododecyl)-succinonitrile	$C_{18}H_{22}N_4O$	310	0.65	Organometallic catalyst, polymer
13.	33.64	25-Hydroxycholesterol	$C_{27}H_{46}O_2$	402	0.56	Inflammatroy treatment

The active principles with their retention time (RT), molecular formula, molecular weight (MW) and peak area as a percentage are presented in Table 2. The major phytocompounds and their biological activities obtained through the GC-MS analysis of *Andrographis paniculata* have been tabulated Table-3.

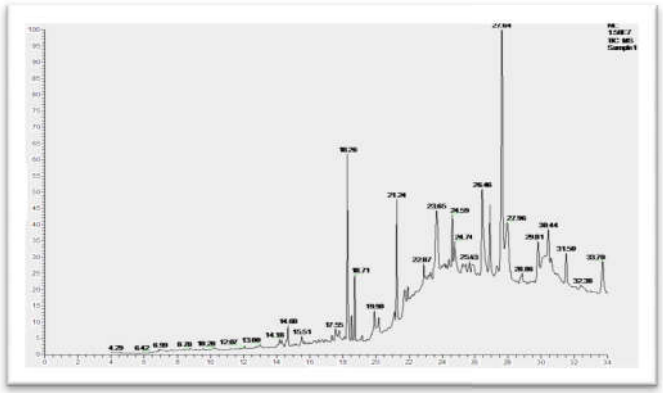

Figure 3 GC-MS chromatogram of ethanolic extracts of stem of *Andrographis paniculata*

CONCLUSIONS

The qualitative and quantitative analysis of the five different stem extract of *Andrographis paniculata* reveals the presence of medicinally valued bio active components like, flavonoids, tannins, alkaloids, steroids, and glycosides. Medicinal plants play a vital role in preventing various diseases. The anti - diuretic, anti-inflammatory, anti-analgesic, anti-viral, anti-malarial, anti-bacterial and anti-fungal activities of the medicinal plants are due to the presence of the above mentioned secondary metabolites.*Andrographis paniculata* will also have wonderful medicinal and diseases curing ability.

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