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Research Article

PHYTOCHEMICAL, GAS CHROMATOGRAPHY WITH MASS SPECTROMETRY ANALYSIS OF *ANDROGRAPHIS SERPYLLIFOLIA* METHANOL LEAF EXTRACT AND ITS ANTIOXIDANT AND ANTIBACTERIAL ACTIVITIES

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ABSTRACT

Objective: The present study is to evaluate the preliminary study of phytochemical screening and biological applications of *Andrographis serpyllifolia* methanol leaf extracts.

Methods: The methanol leaf extracts of *A. serpyllifolia* was prepared using Soxhlet apparatus and the extract was analyzed using gas chromatography with mass spectrometry (GC-MS). *In vitro* antioxidant activity was determined by superoxide dismutase, catalase, glutathione peroxidase, and glutathione S-transferase. Further, the antibacterial activity of methanolic leaf extract of *A. serpyllifolia* was tested against various human pathogens by using agar disc diffusion method.

Results: Preliminary phytochemical screening and GC-MS results revealed phenols, aromatic carboxylic acids, and esters in the chloroform extract to be the molecules responsible for the antioxidant and antibacterial activity of *A. serpyllifolia* methanol extract and fractions showed the presence of various secondary metabolites present.

Conclusion: The present study strongly recommended that the methanolic extract of *A. serpyllifolia* leaves possesses compounds that inhibit the growth of microbes as wells excellent antioxidant activities. The study further suggested the potential therapeutic use of these extract in cancer study.

Keywords: *A. serpyllifolia*, Methanol extracts, Phytochemical, Gas chromatography with mass spectrometry, Antimicrobial, Antioxidant.

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INTRODUCTION

Herbal medicines are being gained a lot of acceptance in recent years because they are a natively higher therapeutic window, less side effects, and scientific essential of therapeutic activities [1]. Nowadays, medicinal plants have been found to possess antimicrobial properties [2]. Many plants having several phytoconstituents, the phytochemicals play a vital role against various diseases such as asthma, arthritis and cancer. The phytochemicals are cured many diseases without causing any harm to human beings these can also be considered as "man-friendly medicines" [3]. The World Health Organization (WHO) indicated that 70–80% of the world's population depends on herbs as a primary health care source [4].

A. serpyllifolia belongs to the family *Acanthaceae* and is commonly known as round leaf Kariyat, Aaku chandrika. It is an irregular herb auxiliary plant species and mostly found in southern India (Tamil Nadu, Kerala, Andhra Pradesh, and Karnataka). The entire plant comprises phenols, alkaloids, steroids, saponins, flavonoids, terpenoids, tannins, anthraquinones, glycosides, phycobalamin, and sugar [5]. The bioactive compounds of *A. serpyllifolia* are related to have important biological applications such as antibacterial, antiulcer, anti-diabetic, anticancer, and anti-inflammatory activities [6]. *A. serpyllifolia* plant is used as traditional Indian herbal medicine for the treatment of dysentery and malaria. The plant extract is used to treat wounds and also effective in jaundice. Andrographolide has been reported as one of the potential bioactive constituents of *A. serpyllifolia* which is found to be liable for numerous clinical and pharmacological activities [7]. In many countries, medicinal plants are the most potent antimicrobial natural source, used as ethnomedicine [8]. The medicinal property of these plants lies in the presence of bioactive components. Plants are rich in a variety of phytochemical secondary metabolites, such as alkaloids, phenolics, terpenoids, and flavonoids which have been found in many studies to have significant antimicrobial activities [9,10]. Oxidative stress-induced by free oxygen radicals is the main reason for various degenerative diseases such as gastric ulcers, cancer, atherosclerosis, and other conditions. Medicinal plants are the source of various antioxidants acting as oxygen scavengers. Recently, attention has been focused on antioxidants from natural sources to avoid drawbacks of human-made antioxidants [11]. In recent study, the potent antioxidant activity is attributed to active compounds present large amounts in the plants [12].

Nowadays, millions of people in the world suffer from chronic wound burns without effective solutions. Burn followed by microbial infection is a very serious complication that often results in the patients' death [13]. About 45% of mortality is recorded in burned patients as a consequence of microbial infections [14]. On the other hand, the WHO regarding drug resistance has encouraged and promoted screening and utilization of medicinal plants as a new alternative therapy against multi-drug resistant pathogens that cause severe infections and difficult-to-treat diseases [15]. Therefore, the present study reports the evaluation of phytochemical and GC-MS analysis of methanol extracts of *A. serpyllifolia*. Further, to assess the antibacterial and antioxidant activities of methanolic leaf extracts of *A. serpyllifolia*.

MATERIALS AND METHODS

Collection of plants

Plants of *A. serpyllifolia* were collected from Yercaud (11.7753°N, 78.2093°E), Salem, Tamil Nadu, India, during September. The taxonomic identification of plant was confirmed by the Botanical Survey of India,

Coimbatore (No. BSI/SRC/5/23/2017/Tech/1672), the voucher specimen of *A. serpyllifolia* (Vahl) Wight was preserved in institution herbarium.

Preparations of methanol extracts
Freshly collected plant leaves were first washed with running tap water and then thoroughly with distilled sterile water. Then, it was shed dried for 5–7 days. Dried plant materials were crushed and ground into fine powder using blender. Powdered plant material was extracted using methanol solvents (100 ml) at 40–60°C in a Soxhlet apparatus. Concentrated plant extract was prepared by removing the excess of solvent and used for further experiments [16].

Phytochemical analysis
The qualitative preliminary detection of alkaloids, flavonoids, phenols, tannins, and saponins was carried out following the procedure of Harborne [17]. The preliminary phytochemicals were analyzed to detect the presence or absence of the specific phytochemical groups.

Gas chromatography with mass spectrometry analysis
The GC-MS analysis of the methanolic leaf extract of *A. serphyllifolia* was performed using a Perkin Elmer GC-MS (Model Clarus 680, MS-clarus 600 EI) equipped with an Agilent column (30 m × 250 µm × 0.25 µm). The oven temperature was programmed at 60°C for 2 min and then increased to 30°C for 6 min, at 10°C/min. Helium was used as the carrier gas at flow rate of 3.0 ml/min. The 1 µl of the methanol extract of *A. serphyllifolia* was injected with split ratio10:1 at injector temperature was 260°C. The characterization of compounds was determined based on the retention time. The spectrums of the components were compared with the database of the spectrum of known components stored in the GC-MS National Institute of Standard Technology library using Turbo mass software (5.4.0).

Antioxidant activity
The catalytic activities of antioxidant enzymes catalase (CAT) [18], superoxide dismutase (SOD) [19], glutathione S-transferase (GST) [20], and glutathione peroxidase (GPx) [19] were evaluated in methanolic leaf extracts of *A. serphyllifolia*.

Table 1: Phytochemicals screening from *Andrographis serpyllifolia* leaf phytochemicals methanol extract

Phytochemical test	Methanol extract
Alkaloids	+
Flavonoids	+
Phenols	+
Carbohydrates	-
Saponins	+
Oil and resin	+
Tannins	+
Amino acids	+

+: Present, -: Absent

Antibacterial activity
The antimicrobial activity of silver nanoparticles was evaluated against Gram-positive (*Staphylococcus aureus*, *Bacillus subtilis*, *Corynebacterium diphtheria*) and Gram-negative (*Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*) bacterial by agar well diffusion method. All the microorganisms were obtained from Microbial Type Culture Collection, Institute of Microbial Technology, and Chandigarh, India. All the bacterial strains were maintained in the nutrient agar medium and subcultured frequently and used for the studies. A single colony of bacterial cultures was prepared by transferring into a tube containing 10 ml nutrient broth and grown overnight at 37°C. The individual microorganisms were prepared by spreading 100 µl of culture on the nutrient agar plate with the help of spreader. Sterile discs were prepared by using Whatmann No.1 filter paper. The discs were placed on agar plates and different concentrations of methanolic leaf extracts (25, 50 and 75 µg/L) were added on the disc with the help of micropipette. The sterile distilled water was used as a control. The plates were incubated at 37°C for overnight in a bacteriological incubator. After 12 h incubation, the plates were removed and observed for the zone of growth inhibition, which will appear as clear (around the discs). The diameter of such zone of growth inhibition was measured using a meter ruler and the mean value for each pathogen was recorded and expressed in millimeter.

RESULTS

Phytochemical screening
The qualitative phytochemical screening of *A. serpyllifolia* methanolic leaf extracts revealed the presence of bioactive compounds such as alkaloids, flavonoids, phenols, saponins, tannins, amino acids, oils, and resins while carbohydrates was absent in the methanolic extracts (Table 1) [21] showed the phytosterols, flavonoids, phenols, alkaloids, carbohydrate, glycoside, sterols, steroids, terpenoids, and tannin while saponin was absent in methanol and aqueous leaf extracts of *Rhododendron arboreum* [22] also showed the presence of phenolics, flavonoids, tannin, steroids, diterpenes, and triterpenes in the methanolic extract revealed the presence of hydrocarbon alkane, steroids, ester, fatty acids, flavonoids, terpenes. The plant of *Borassus flabellifer* plant extract The phytochemical screening showed the presence of alkaloids, flavonoids, glycosides, saponins, tannins, phytosterols, triterpenoids, and phenols in the immature palmyra palm fruits extract [23].

Gas chromatography with mass spectrometry analysis
The GC-MS chromatogram is shown in Fig. 1 highlighted the 13 Phyto-compounds present in the methanolic leaf extract of *A. serpyllifolia*. The composition of each compound was represented based on a peak area percentage. This analysis also provides the information regarding the molecular weight of each compound was in (Table 2). From the GC-MS analysis was highlight many secondary metabolites such as alkaloids, phenols, terpenoids, saponins, tannins, carbohydrate, amino acids, quanins, oils, and resins present in the methanolic extracts of *A. serpyllifolia*. The major phytochemical constituents 3,7,11,15-Tetramethyl-2-

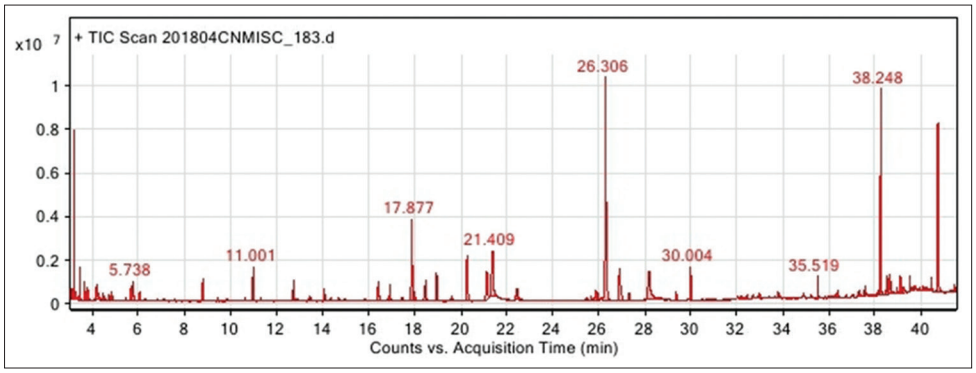


Fig. 1: Gas chromatography with mass spectrometry analysis of methanolic leaf extract of *Andrographis serpyllifolia*

Table 2: Gas chromatography-mass spectrometry analysis of chemical composition identified in the methanolic leaf extract of *Andrographis serpyllifolia*

RT	Area	Compound name	Molecular formula	Molecular weight (g/mol)
6.042	2.38	Benzofuran, 2,2,-dihydro	C_8H_8O	118.1
12.761	3.52	1-hexadecanol	$C_{16}H_{34}O$	242.44
16.908	3.79	n-Pentadecanol	$C_{15}H_{32}O$	228.42
17.44	19.94	Pentadecanal	$C_{15}H_{30}O$	226.40
17.877	2.71	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296.53
18.967	8.11	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296.53
22.442	4.35	n-Pentadecanol	$C_{15}H_{32}O$	228.42
25.46	1.59	Cyclodexadecane	$C_{16}H_{32}$	224.43
26.925	14.12	Methyl stearate	$C_{19}H_{38}O_2$	298.51
29.392	3.83	n-Pentadecanol	$C_{15}H_{32}O$	228.42
30.003	10.96	Phytol acetate	$C_{22}H_{42}O_2$	338.57
38.248	29.95	Squalene	$C_{30}H_{50}$	410.81
39.083	2.65	trans-Geranylgeraniol	$C_{20}H_{34}O$	290.49

RT: Retention time

Table 3: Chemical structure of identified phytocompounds in the methanolic leaf extract of *Andrographis serpyllifolia* by gas chromatography-mass spectrometry

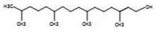
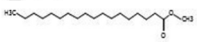
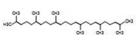
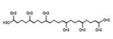
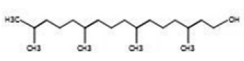
Name of the phytocompounds	Structure	Biological properties
Benzofuran, 2,2,-dihydro-		Liver targets
1-Hexadecanol		Decreased hyperoxic in rats
n-Pentadecanol		Inherited human peroxisomal disorders
Pentadecanal-		Inherited human peroxisomal disorders
3,7,11,15-Tetramethyl-2-hexadecen-1-ol		Antimicrobial
Cyclodexadecane		Chemical and physical properties
Methyl stearate		Antifoaming agent and fermentation nutrient.
Phytol, acetate		Antimicrobial, Anticancer, cancer preventive, diuretic antiinflammatory
Squalene		Antibacterial, antioxidant, pesticide, antitumor, cancer preventive, immunostimulant, chemo preventive, lipoxygenase-inhibitor
Trans-Geranylgeraniol		The biological activity of farnesol

Table 4: Antioxidant activity of methanol extract of *Andrographis serpyllifolia*

Antioxidants	Antioxidant activity	
	Standard control	Methanol extract
SOD	31.1±1.05	26.4±0.25
CAT	53.23±1.27	44.58±2.36
GPX	261.1±0.52	231.4±1.46
GST	172.3±2.23	153.1±1.43

SOD: Superoxide dismutase, CAT: Catalase, GPX: Glutathione peroxidase, GST: Glutathione-s-transferase

hexadecen-1-ol (8.11%), Pentadecanal (19.94%), Phytol acetate (10.96%) and Squalene (29.95%) were observed in methanolic leaf extract of *A. serpyllifolia* (Fig. 1). These important bioactive compounds molecular structure and biological properties are indicated in (Table 3). Phytol is an acyclic diterpene alcohol and chlorophyll constituents. It is used as a precursor for the production of synthetic forms of Vitamin E and Vitamin k1. Vitamin E is one of the active natural antioxidants; it is the most effective chain-breaking antioxidant within the cell membrane. Vitamin E also acts in the prevention of free radical formation [24] and also phytol was most potent antitubercular activity against *Mycobacterium tuberculosis* H37Rv strain. Squalene is ubiquitously distributed in human tissues where it is transported in serum generally in association with very low-density lipoproteins. Squalene is also investigated as an adjunctive cancer therapy [25]. The *Ruta graveolens* GC-MS analysis of methanolic leaf extract revealed the presence of approximately 26 phytochemical constituents [26].

Antioxidant activity

The antioxidant activity of *A. serpyllifolia* was evaluated and compared with methanolic extract and standard control of ascorbic acid. The level of enzymatic antioxidants such as SOD, CAT, GPx, and GST values showed in (Table 4). The activities of SOD and CAT levels were found to be 26.4±0.25 units/mg protein and 44.58±2.36 µmole of H_2O_2 consumed/min/mg proteins. SOD is one of the antioxidant enzymes that play a key role in cellular defense against ROS Bowler [27]. Similarly, CAT is also one of the major antioxidant enzymes it eliminates H_2O_2 by transforming the H_2O and O_2. The stimulation of SOD activity along with CAT seemed to play a protective role against membrane damage as Cu is particularly toxic to membranes [28]. SOD and CAT in *Tylophora pauciflora* were found to be 29.78±0.57 units/mg protein and 39.87±0.51 µmole of H_2O_2 consumed/min/mg proteins, respectively [29]. GPx and GST levels in methanolic leaf extracts shows the high level of GPx (231.4 ± 1.46 µg of glutathione oxidized/min/mg protein) and 153.1 ± 1.43 µmoles of CDNB – GSH conjugate formed/min/mg protein.

Antibacterial activity

Biomolecule coated AgNPs showed strong antibacterial activity against various microbial pathogens such as two positive bacteria (*B. subtilis* and *Staphylococcus epidermidis*) and two negative bacteria (*Escherichia coli* and *Salmonella typhi*). A methanolic extracts was showed notable antibacterial activity against all the bacterial strains compared to controls. Methanolic extracts (50 µg) exhibited the maximum zone of growth inhibition (12 mm) was obtained in *B. subtilis* followed by *S. aureus* (Table 5). Antibacterial activity results showed that the maximum zone of inhibition was observed in methanolic extracts compared to control. These results strongly suggested the

Table 5: Antibacterial activity of methanol extract of *Andrographis serpyllifolia* leaf against human pathogens

Bacteria	Control (distilled water)	Antibiotic (streptomycin)	Methanolic extract (µg)	
			25	50
Klebsiella pneumonia	0.0±0	12.5±0.17	6±0.18	9.5±0.15
Corynebacterium diphtheria	0.0±0	8±0.24	5±0.21	7.5±0.12
Staphylococcus aureus	0.0±0	14.3±0.25	6.5±0.21	11.5±0.24
Pseudomonas aeruginosa	0.0±0	9.5±0.21	5.5±0.15	8.5±0.17
Pseudomonas fluorescens	0.0±0	11±0.12	5.5±0.12	9.5±0.16
Bacillus subtilis	0.0±0	16±0.17	8±0.23	12±0.21

biosynthesized AgNPs using aqueous extract of *E. acaulis* showed effective antibacterial activity against human pathogens this may be possible bioactive compounds present in the plant extracts (Table 1). The ethanolic extract of the leaves of *A. serpyllifolia* at a concentration of 1.50 mg/disc showed excellent antimicrobial activity against *S. Typhi* [30].

DISCUSSION

This study was work phytochemical screening the present of alkaloids, flavonoids, phenols saponins oil and resin, tannins, amino acids plant leaf methanol extract (*A. serpyllifolia*), and antimicrobial activity for antioxidant[] the antimicrobial activities for zone inhibition for hight zone bacteria (11.5±0.24) *S. aureus*, Pseudomonas floure and to the Gc-ms analysis for the natural the compound in *A. serpyllifolia* plant leaves for this current reported in this plant medicine properties or in traditional medicine for well know inflammation and pain centipede using this plant *A. serpyllifolia.*

CONCLUSION

Phytochemical and GC-MS analysis of *A. serpyllifolia* confirmed that the methanolic extracts were rich in phenolics, flavonoids, alkaloids, and various bioactive compounds were detected. Therefore, methanol leaf extracts of *A. serpyllifolia* have potential bioactive compounds which are responsible for antimicrobial activity against *B. subtilis*, *Staphylococcus epidermidis*, *E. coli* and *S. typhi*. The methanol extract also showed significant antioxidant properties, indicative of its potential as a source. Further research is also required for isolation and identification of active biomolecules and principles present in this extract, and hence that they could be exploited for pharmaceutical use at the industrial scale.

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AUTHOR'S CONTRIBUTION

All the authors have contributed equally

CONFLICTS OF INTEREST

There are no conflicts of interest to declare

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