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

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Received: 03 November 2018, Revised and Accepted: 09 January 2019

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 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 well as 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.

INTRODUCTION

Herbal medicines are being gaining 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 mund 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, phycobilin, and sugar [5]. The bioactive compounds of A. serpyllifolia are related to have important biological applications such as antibacterial, antilulcer, 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,
Antimicrobial 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, 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 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 disc). 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, steroids, terpenoids, and tannin while saponin was absent in methanol and aqueous leaf extracts (Table 1) [22] also showed the presence of phenolics, flavonoids, tannin, steroids, terpenoids, and sterones 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, quinins, oils, and resins present in the methanol extracts of A. serpyllifolia. The major phytochemical constituents 3,7,11,15-Tetramethyl-2-

**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

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**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 | Benzoquinone, 2,2-dihydro | C₈H₆O             | 118.1                   |
| 12.761| 3.52 | 1-hexadecanol          | C₁₃H₂₆O₂           | 242.44                  |
| 16.908| 3.79 | n-Pentadecanol         | C₁₅H₂₆O₂           | 228.42                  |
| 17.44 | 19.94| Pentadecanal           | C₁₅H₂₆O₂           | 226.40                  |
| 17.877| 2.71 | 3,7,11,15-Tetramethyl-2-hexadecen-1-ol | C₃₁H₄₀O₂ | 296.53                  |
| 18.967| 8.11 | 3,7,11,15-Tetramethyl-2-hexadecen-1-ol | C₃₁H₄₀O₂ | 296.53                  |
| 22.442| 4.35 | n-Pentadecanol         | C₁₅H₂₆O₂           | 228.42                  |
| 25.46 | 1.59 | Cyclodecane            | C₁₀H₁₆O₂           | 224.43                  |
| 26.925| 14.12| Methyl stearate        | C₁₉H₃₈O₂           | 298.51                  |
| 29.392| 3.83 | n-Pentadecanol         | C₁₅H₂₆O₂           | 228.42                  |
| 30.003| 10.96| Phytol acetate         | C₁₅H₂₆O₂           | 338.57                  |
| 38.248| 29.95| Squalene               | C₃₀H₄₂O₃           | 410.81                  |
| 39.083| 2.65 | trans-Geranylgeraniol  | C₁₉H₃₈O₃           | 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 |
|----------------------------|-----------|-----------------------|
| Benzoquinone, 2,2-dihydro   | ![Image](197x395 to 290x412) | Liver targets         |
| 1-Hexadecanol               | ![Image](197x422 to 239x431) | Decreased hyperoxic in rats |
| n-Pentadecanol              | ![Image](197x434 to 248x445) | Inherited human peroxisomal disorders |
| Pentadecanal                | ![Image](197x448 to 270x461) | Inherited human peroxisomal disorders |
| 3,7,11,15-Tetramethyl-2-hexadecen-1-ol | ![Image](197x464 to 222x475) | Antimicrobial         |
| Cyclodecane                 | ![Image](197x492 to 250x499) | Chemical and physical properties |
| Methyl stearate             | ![Image](197x513 to 292x524) | Antifoaming agent and fermentation nutrient. |
| Phytol, acetate             | ![Image](197x527 to 211x535) | Antimicrobial, Anticancer, Cancer preventive, diuretic antiinflammatory |
| Squalene                    | ![Image](197x562 to 231x568) | Antibacterial, Antioxidant, Antitumor, Cancer preventive, immuno stimulant, Chemo preventive, Lipoxygenase-inhibitor |
| Trans-Geranylgeraniol       | ![Image](197x584 to 270x592) | 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          | 53.2±1.05            | 44.0±1.05        |
| GPX          | 261.1±0.02           | 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

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 44.0±1.05 µ mole of H₂O₂ 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 H₂O₂ by transforming the H₂O₂ and O₂. 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 µ mole of H₂O₂ 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 µ moles of CDBN – 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 typh") [30]. 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
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 high zone bacteria (11.5±0.24) *S. aureus,* *Pseudomonas* floure and to the Gc-ms analysis for the natural compound in the *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.

**ACKNOWLEDGMENT**

The authors are thankful to the Department of Botany, Periyar University, for providing necessary facility to carry out this study.

**AUTHOR’S CONTRIBUTION**

All the authors have contributed equally

**CONFLICTS OF INTEREST**

There are no conflicts of interest to declare

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

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