PHYTOCHEMICAL SCREENING AND CHEMICAL COMPOSITION OF FIXED OIL FROM STEMS OF *ANDROGRAPHIS PANICULATA*

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ABSTRACT

*Andrographis paniculata* is one of the medicinally important plant belonging to the family of *Acanthaceae* which is widely used for the traditional medicine of diseases. The present study attempts to evaluate the phytochemicals and fatty acids composition from stems of *Andrographis paniculata*. The presence of some phytochemicals like alkaloids, saponins and flavonoids explained the medicinal action of the plant encountered in its therapeutic uses. The fatty acid compositions of the petroleum ether extract of stems of this plant were determined by Gas Chromatography-Mass Spectrophotometer. Ten compounds were identified and the major constituent was methyl palmitate (54.22%).

KEYWORDS: *Andrographis paniculata*, phytochemicals, fatty acids, Gas Chromatography-Mass Spectrophotometer.

INTRODUCTION

A large number of medicinal plants are being exploited from the natural flora and meet increasing demand for plant-based drugs.[¹] These can be derived from any parts of the plant like leaves, flowers, barks, roots, fruits and seeds etc.[²] Traditionally, natural plant products have been the source in the search for new drugs by pharmaceutical companies.[³,⁴] Plant medicines are 100% natural and very effective against serious diseases and widely used because of its therapeutic characteristics.[⁵-⁷] Certainly, the public demands and the markets
have been increased but the extinction or less genetic diversity of many medicinal are also in a great risk.\cite{8} For the synthesis of complex chemical substances, the knowledge of the chemical constituents is so valuable.\cite{9}

*Andrographis paniculata* (Burm.F) Ness (A. paniculata), belonging to *Acanthaceae* family is an annual herb commonly known as “Kalmegh” or “King of bitters” cultivated in many regions of South Asian countries reason of its well-known medicinal value.\cite{10,11} A. *paniculata* is the traditional medicines of India, China, Hongkong, Pakistan, Bangladesh, Malaysia, Philippines, Indonesia and Thailand are broadly used this plant.\cite{12,13} It grows erect to a height of 30-110 cm (12-43 in) in moist, shady places. The slender stem is dark green, squared in cross-section with longitudinal furrows and wings along the angles. It can be found in a variety of habitats, such as plains, hillsides, coastlines, and disturbed and cultivated areas such as roadsides, farms, and wastelands. In India *A. paniculata* is consumed for the hepatic disorders above 50% of herbal compositions materialistic.\cite{14}

*A. paniculata* has been used as medicinal plant for its several pharmacological properties especially anti-microbial, anti-cancer, anti-inflammatory, anti-oxidant, immunostimulant, anti-diabetic, anti-infective, hepato-renal protective, anti-angiogenic, anti-allergic, anti-diarrheal, anti-hepatitis, anti-HIV, anti-hyperglycemic, anti-malarial, cardiovascular, cytotoxic, hepatoprotective and sexual dysfunctions.\cite{15-18}

In addition, a great number of researches have been accomplished on *A. paniculata* but a very few systematic researches on fatty acid composition of the plant by GC-MS analysis has been reported. Consequently, the present study was assumed with an aspiration to bring off a complete investigation of the composition of fatty acids from petroleum ether extract of *A. paniculata* with GC-MS analysis.

**MATERIALS AND METHODS**

**Collection of plant material**

Fully matured fresh stems of *A. paniculata* were collected from the area of Jahangirnagar University, Dhaka, Bangladesh in the month of April 2017 and identified by the taxonomist of Bangladesh National Herbarium, Dhaka, where a voucher specimen (No. 45932) has been deposited.
Preparation of sample
The matured stems of *A. paniculata* were washed to remove dirt. Then it was oven-dried at reduced temperature less than 45°C to make it suitable for grinding purpose. The screened (20 mesh) powder was then stored in air-tight container with marking for future experiments.

Solvents
Petroleum ether (b.p 40-60°C, Merck, Germany) of analytical grade was used for extraction of plant material. Solvent from extract were recovered under distillation and the dried extracts were stored in a sealed vial at 4°C until further analysis.

Extraction of fatty acids and preparation of methyl ester (FAMEs)
The stem of *A. paniculata* were collected and washed individually from running tap water to remove soil particles and other dust. Then they were dried at room temperature and powdered by Fritsch mortar grinder, Germany. The natural fatty acids were extracted separately from the stem powder (100 gm) of the plant with petroleum ether (b.p 40°C-60°C) in a Soxhlet apparatus for 72 h. The extracts were concentrated under reduced pressure in a rotary evaporator. The extracts were filtered using Whatman No.1 filter paper and then vacuum distilled to remove solvent completely. The extracts from the stem of *A. paniculata* was 4.46 gm (4.46% w/w). Petroleum ether extracts for stems of *A. paniculata* were kept in a nitrogen atmosphere in a refrigerator. The fatty acids present in the extracts were converted to fatty acid methyl esters (FAMEs) first and analyzed according to the method reported by Griffin\(^{[19]}\) for GC-MS analysis.

The fatty acid composition was determined by analysis of their methyl esters. The fatty acid methyl esters (FAMEs) were prepared by esterification reaction by using BF\(_3\)-MeOH complex according to AOAC method.\(^{[20]}\) 10 mg of extract was taken in a screw capped glass tube. 1 ml of BF\(_3\)-MeOH complex were added and then heated at 100 °C for 1 hour in a water bath. After that it was cooled at room temperature and 1 ml of deionized water and 2 ml of hexane was added. The glass tube was vortexed and centrifuged at low RPM for two minutes. The upper layer was collected by means of syringe and kept in closely tight glass vial in refrigerator. Then the prepared FAMEs were ready to analyze.

Gas Chromatograph-Mass Spectrum analysis
GC-MS analysis of stems of *A. paniculata* from petroleum ether extracts were carried out on an Agilent 7890A system equipped with mass Spectrophotometer detector and split less
injection system. The GC was fitted with a HP-5MS capillary column (30 m × 0.25mm: film thickness: 0.25μm). The temperature program was as follows: injector temperature 260°C, initial oven temperature at 70°C, then increased at 10°C/min to 150°C for 5 min. then 12°C/min to 200°C for 15 min. and then 12°C/min to 220°C for 15 min. Helium was used as the carrier gas at 17.69 psi pressure with flow 0.6ml/min. Samples were dissolved in methanol and 1μl aliquot was injected automatically. MS was set in scan mode. The ionization was electron ionization. The mass range was set in the range of 50-550 m/z. MS spectra of separated components were identified on NIST libraries for fatty acid compositions.

**Alkaloid Determination**\(^{[21]}\)

5 gms of powdered sample were taken into a 250 ml conical flasks and 200 ml of 10% acetic acid in ethanol were added, covered the contains by aluminum foil and allowed to stand for 2 days then filter. After filtration, the extract was reduced to one fourth of its original volume on a water bath. To the reduced volume of the extract, concentrate ammonium hydroxide was added in drops until the precipitation was completed. The whole solution was allowed to settle and the precipitate was collected by filtration, dried and weighed.

**Saponin Determination**\(^{[22]}\)

5 gms of powdered sample were taken into a 250 ml conical flask, 250 ml of 25% ethanol was added, the suspension was heated with continuous stirring on a water bath at about 60°C for 4 hrs. The mixture was filtered and the residue re-extracted with another 200 ml of 20% ethanol and then filtered. The combined extracts were reduced to 40 ml over water bath at 90°C. The concentrate mixture was transferred into a 250 ml separating funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered into 250 ml conical flask while the ether layer was discarded. The purification process was repeated thrice; 60 ml of n-butanol was added. The combined n-butanol extracts were washed with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the sample was dried in the oven to a constant weight. The saponin content was calculated in percentage.

**Flavonoid Determination**\(^{[23]}\)

5 gms of powdered sample were taken into 250 ml conical flasks with 150 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatmann
filter paper 42 (125 mm). The filtrate was then transferred into a crucible and evaporated to dryness over a water bath and weighed.

RESULTS AND DISCUSSION

Fatty acid analysis

GC-MS analysis of fatty acids of stems of *A. paniculata* from petroleum ether extracts showed the presence of 10 compounds. GC-MS analyzed results which include the active principles with their retention time, molecular formula, molecular weight and composition of the fatty acids of stems of *A. paniculata* from petroleum ether extracts are presented in Table 1 and Figure-1, respectively.

Table 1: GC-MS analysis of fatty acids from petroleum ether extract of *A. paniculata* stems.

<table>
<thead>
<tr>
<th>SL</th>
<th>Retention time (min)</th>
<th>Name of the compound</th>
<th>Molecular Formula</th>
<th>Conc. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>9.96</td>
<td>Methyl Myristate</td>
<td>C₁₅H₃₀O₂</td>
<td>1.13</td>
</tr>
<tr>
<td>2.</td>
<td>10.71</td>
<td>Methyl Pentadecanoate</td>
<td>C₁₆H₃₂O₂</td>
<td>0.50</td>
</tr>
<tr>
<td>3.</td>
<td>11.58</td>
<td>Methyl Palmitate</td>
<td>C₁₇H₃₄O₂</td>
<td>54.22</td>
</tr>
<tr>
<td>4.</td>
<td>12.56</td>
<td>Methyl Heptadecanoate</td>
<td>C₁₈H₃₆O₂</td>
<td>1.22</td>
</tr>
<tr>
<td>5.</td>
<td>13.65</td>
<td>Methyl Stearate</td>
<td>C₁₉H₃₈O₂</td>
<td>6.66</td>
</tr>
<tr>
<td>6.</td>
<td>13.81</td>
<td>Trans-9-Elaidic acid methyl ester</td>
<td>C₁₉H₃₆O₂</td>
<td>9.00</td>
</tr>
<tr>
<td>7.</td>
<td>13.96</td>
<td>Cis-9-Oleic acid Methyl ester</td>
<td>C₁₉H₃₆O₂</td>
<td>8.15</td>
</tr>
<tr>
<td>8.</td>
<td>14.57</td>
<td>Methyl Linoleate</td>
<td>C₁₉H₃₆O₂</td>
<td>16.54</td>
</tr>
<tr>
<td>9.</td>
<td>15.35</td>
<td>Methyl Arachidate</td>
<td>C₂₁H₄₂O₂</td>
<td>1.40</td>
</tr>
<tr>
<td>10.</td>
<td>16.07</td>
<td>Methyl Eicosanoate</td>
<td>C₂₂H₄₄O₂</td>
<td>1.22</td>
</tr>
</tbody>
</table>

Total 10 fatty acids were identified as their methyl esters in the case of stem *A. paniculata*. The major constituent was Methyl Palmitate (54.217%) with retention time 11.58 min.
Phytochemical Content

The present investigation showed that stems of *A. paniculata* contain phytochemicals such as flavonoids, alkaloids and saponins in significant quantities (Fig.-2). It was found that stems of this medicinal plant exposed higher flavonoids content (64.31%), saponins and alkaloids present as 26.79% and 1.71%, respectively. Flavonoids has been used a wide range of pharmacological activities including anti-allergic, anti-microbial, anti-inflammatory, anti-oxidant, anti-viral and anti-diarrheal.\[^{24}\] The amphipathic nature of saponins gives the activity as surfactants, possibly making saponins useful for development of cosmetics and drugs, vaccines, dietary supplements. Saponins also used in anti-oxidant, anti-cancer, anti-inflammatory and weight loss etc.\[^{25}\] Alkaloids have been reported to possess analgesic, antispasmodic and bactericidal, antimalarial and analgesic activities.\[^{26}\] The presence of these secondary metabolites suggests that the plant might be of medicinal and industrial importance.

![Fig. 1: Structure of the identified fatty acid ester from petroleum ether extracts of *A. paniculata* stems.](image)

![Fig. 2: Phytochemical contents from stems of *A. paniculata*.](image)
CONCLUSION
The present study found 10 constituents from stems of *A. paniculata* from petroleum ether extracts by Gas Chromatography-Mass Spectroscopy (GC-MS) analysis. The presence of these chemical compounds justified the extensive uses of stems of the plant by traditional physician to treat various disorders. It could be concluded that contains various chemical constituents that can be bioactive compounds of medical importance. However, further studies are needed to evaluate its bioactivity and toxicity profile.

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REFERENCES


