Identification of Medicinal Organic Components of *Andrographis paniculata* Leaf Extracts

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**Abstract.** Active metabolites of methanol, ethanol, ethyl acetate and aqueous extracts of the leaves of *Andrographis paniculata* were identified using thin layer chromatography and gas chromatography-mass spectrometry (GC-MS). Results from this study identified some of active metabolites as andrographolide, 14-deoxyandrographolide, neoandrographolide, 14-deoxy-11,12-di-dehydroandrographolide, andrographinin, isoandrographolide, 14-acetylandrographolide, 19-O-andrographolide, beta-sitosterol and 14-deoxy-14,15-dihydroandrographolide. Ethanol was a more effective solvent for the extraction as most of the phytochemicals were identified in this extract. Research had shown that extracts from the leaves of the plant possess a lot of therapeutic properties such as anti-microbial, anti-inflammatory, anti-diabetic, immune modulatory and antimalarial activities which are of immense benefit to man.

**Keywords:** andrographolide, ethanol extract, therapy, antimalarial, chromatography, *Andrographis paniculata* leaf

**Introduction**

The use of herbal products from medicinal plants has gained global acceptance in recent times. This is due to the effectiveness of some plants in the management and cure of numerous illnesses. Some of the plants are readily available within our environment and can be obtained with little or no cost. Some of the medicinal plants include Aloe vera, neem, *Ocimum gratissimum* (scent leaf), ginger, soyabean, root of curcuma, red wine, green tea and *Andrographis paniculata*. Chemical compositions and the active metabolites of these plants still under investigation (Okhuaroobo et al., 2014; Niranjan et al., 2005).

The use of traditional medicines in recent times have gained global attention and acceptance. This is because most traditional drugs from medicinal plants have proved to be very effective in the treatment of ailments, readily available and less expensive. The efficacy and effectiveness of these plants are dependent on the chemical constituents.

*Andrographis paniculata* known as King of Bitters is a unique medicinal plant that has gained global attention. It belongs the family Acanthaceae usually cultivated and distributed in China, India, Pakistan, Srilanka, America, West Indies, Africa and some parts of Europe (Joselin and Jeeva, 2014). It is an annual herbaceous, branched, erect and attractive plant of about half to one meter in height. It is grown due to its famous therapeutic values. It breeds and thrives in nearly all types of soils, in which it is found all over the World (Lattoo et al., 2006; Wait et al., 2005). Some parts of the plant which includes the leaves and roots have been widely used in traditional treatment of ailments and many disorders in most Asian and African continents. Many of such disorders and diseases include body high temperature, influenza, respiratory tract infections as well as remedy against poisons, snakes and insects bites (Samy et al., 2008; Gabrielian et al., 2002). However, the use of *Andrographis paniculata* in traditional cure of diseases is principally based on the undeniable assertions to their healing properties and mythology. Therefore, to substantiate the claim of the therapeutic properties and commercial values of *Andrographis paniculata*, scientists have increased investigation on quantification and identification of active metabolites in the extracts of the plant.

The aim of this study is the quantitative identification of the organic metabolites of methanol, ethanol, ethyl acetate and aqueous extracts of the leaves of *Andro-
Andrographis paniculata and a review some of its medicinal values for the benefit of man.

Medicinal values of Andrographis paniculata. The whole plant of Andrographis paniculata has been used traditionally for many decades in the treatment and management of many ailments. Leaves have been used as a traditional therapy for the cure of contagious and fever initiating diseases, indigestion, loss of appetite, frequent stools and diarrhea, colic pain, loss of appetite, influenza, cough, hepatitis, tuberculosis, mouth ulcers, bronchitis gastro-intestinal disorder and sores (Poolsup et al., 2004; Panossian et al., 2002; Saxena et al., 1998). Countries like Malaysia use the aerial parts in the treatment of flu, high blood pressure, high blood sugar level, malignancy, malaria related ailment and snake bite (Perry and Metzger, 1980). The Chinese traditional healers used it to clear sick individuals that are having temperature and also disperse poisons from their systems (Li et al., 2007; Deng, 1978). The plant also helps in treating disorders like dysmenorrhea, leucorrhoea, pre-natal and post-natal care, intricate illnesses such as jaundice, gonorrhoea and general ailments like injuries, cuts, abscess and skin related diseases (Abubakar et al., 2011; Poolsup et al., 2004; Panossian 2002; Bensky et al., 1993). The entire plant of Andrographis paniculata was recommended for treatment of jaundice alongside with other plants and was effective as immune system booster, used as an anti-inflammatory and antipyretic drug for the management of fever, laryngitis, and diarrhoea (Chao et al., 2010; Chao and Lin, 2010). Fresh leaves extract which have andrographolide (Fig. 1), is used as a local remedy in the treatment of stomach-ache pain, sore throat, malaria, fever and many chronic contagious illnesses (Roy et al., 2010; Calabrese et al., 2000).

Nasir et al., (2013) found that extracts of Andrographis paniculata may possibly possess the potentials of human immune deficiency virus (HIV) and recommended that Andrographis paniculata could combined with contemporary medicines against acquired immune deficiency syndrome (AIDS) (Li et al., 2007).

Niranjan et al. (2005) obtained seven metabolites from the n-hexane and methanol extracts of Andrographis paniculata. Notable among them are Andrographolide and 14-deoxy-11, 12-di-dehydroandrographolide (Fig. 2) which exhibited anti-HIV action with 50% effective concentration (EC 50) of 49 and 57 μg/mL, respectively. The first stage of the clinical test of andrographolide in HIV positive individuals showed a substantial increase in the mean CD4+ lymphocyte level of HIV infected individuals (Calabrese et al., 2000).

Materials and Methods

Collection and preparation of sample. Fresh leaves of Andrographis paniculata plant was obtained from a botanical garden in Eleme, Port Harcourt, rivers state, Nigeria and taken to the Plant Science and Biotechnology laboratory of the University of Port Harcourt for proper identification. The fresh healthy leaves of Andrographis paniculata were air dried for three weeks and then grounded to form a fine powder.

Extraction with methanol and ethanol. Hundred grams (100 g) powder of Andrographis paniculata was macerated with 600 mL of methanol for three days and washed with 400 mL of methanol during filtration.

The extract was concentrated by fractional distillation to remove the methanol. Thin layer chromatography (TLC) was carried out on the extracts using ethyl acetate as eluent, while chloroform (about 2 drops) was used to make solution of the extract and the various spots were viewed using UV lamp.

Similarly, 20 g powder Andrographis paniculata was macerated with 200 mL of ethanol for three days and washed with 200 mL during filtration. The extract was concentrated by evaporation. Thin layer chromatography was carried out in the same manner as the methanol extract. Thereafter, both methanol and ethanol extracts were spotted separately on the same plate at the same time.

Gas chromatographic -mass spectrometric analysis of extracts. Sample solution was prepared by soaking
three portions of 5 g of the dried granulated samples in ethanol and ethyl acetate for 48 h, after which the samples were filtered to obtain a clear solution. 1 mL solution was transferred into a 2 mL vial and analysed with GC-MS.

Aqueous extract was obtained by soaking 10 g of the dried sample in 25 mL hot distilled water and kept in a still position for 48 h. Sample was filtered and the filtrate evaporated over warm water bath. The concentrate was reconstituted with 2 mL methanol and introduced into a 2 mL vial prior to analysis.

GC-MS analysis on the ethanol, ethyl acetate and aqueous extracts were carried out using Agilent 7890A-5975C GC-MS system employing the following conditions: HP5-column (30 m × 0.25 mm × 0.25 μm), operating in electron impact mode at 70 eV; carrier gas flow (a constant) was 1 mL/min, injection volume was 0.5 μL with split ratio of 10:1, and injector temperature was 250 °C. Ion source temperature was 280 °C and oven initial temperature was 110 °C (hold 2 mins) 110 to 200 °C at 10 °C/min.

Identification of bioactive compounds. Elucidation of GC mass spectrum was achieved using the database. The spectra of the unknown components were compared with the known components stored in the National Institute of Standards and Technology (NIST) library. The molecular weights and structures of bioactive compounds in the extracts were established. Fig. 2 and Table 2.

Results and Discussion

The bioactive compounds of the leaf extract of Andrographis paniculata are shown in Table 2. Ten different compounds were identified. The retention time, molecular weights and their relative percentages are also listed. Most of the structures of compound on the internet have been slightly modified.

Thin layer chromatographic analysis (TLC). Thin layer chromatographic analysis (TLC) of methanol and ethanol extracts showed five spots each, representing the occurrence of five different compounds in the sample extracts. Both extracts were spotted separately on the same plate at the same time, it was observed that the ethanol extract showed the same fractions as methanol extract and the Rf values were calculated using the equation below:

\[ R_f = \frac{D_1}{D_2} \]

where:

- \( D_1 \) is distance travelled by the component
- \( D_2 \) is distance travelled by the eluent
- \( R_f \) value is a useful index for comparing various compounds.

It was observed that the methanol extract and ethanol extract have similar \( R_f \) values (Table 1) indicating similarity in the compounds present in the test sample.

Results of the gas chromatography mass spectrometry (GC-MS) analysis. Ethanol, aqueous and ethyl acetate extracts from the leaves of Andrographis paniculata were used for the GC-MS analysis. This technique gave detailed information about the plant and its components. Results of the ethanol extract identified nine bioactive compounds, while aqueous and ethyl acetate extracts showed seven and five compounds, respectively. Most of the bioactive metabolites were common in all the three extracts with the same peaks but at different retention times. The representative total ion chromatogram (TIC) is shown in Fig. 3.

Both ethyl acetate and ethanol extracts showed the presence of 14-acetylandrographolide which was not seen in aqueous extract. Various organic metabolites identified from the leaf extracts of Andrographis paniculata, the retention time (RT), molecular weights and relative percentages are shown in Table 2.

The compounds present in the ethanolic leaf extract of Andrographis paniculata identified by GC-MS analysis are shown in Fig. 1 and Table 2. The retention time (RT), molecular weight (MW), molecular formula (MF) and peak areas (%) are also shown. A total of ten bioactive compounds were identified in the extracts using different solvents. Notable among them are Andrographolide (3.23%), Neandrographolide (6.88%), 14-Acetyl-andrographlode (6.06%) and β-sitosterol (7.68%). Compounds like β-sitosterol which are widely

| Table 1. Results of TLC for methanol and ethanol extracts |
|-----------------|-----------------|-----------------|----------------|
| \( D_2 \) & \( D_1 \) & \( R_f \) values & \( D_1 \) & \( R_f \) values |
| 6.8 & 2.1 & 0.3 |
| 6.8 & 2.5 & 0.4 |
| 6.8 & 3.2 & 0.5 |
| 6.8 & 3.8 & 0.6 |
| 6.8 & 4.8 & 0.7 |
Fig. 2. Structures of the Identified bioactive compounds of *Andrographis paniculata*. 
distributed in plant kingdom and vegetable oil and 19-
-O-andrographolide were isolated from the leaf extract of *Andrographis paniculata*.

Ethnobotanical uses of the leaves and roots of *Andrographis paniculata* in Asia and Europe includes the cure of wide spectrum to be used of health ailments. It is recommended to be used to get rid of body heat in fevers and to dispel toxins from body (Hossain *et al.*, 2014). Some authors also recommend them it for use in cases of leprosy, gonorrhea, scabies, skin eruptions, chronic and seasonal fever for its high “blood purifying” properties (Kabir *et al.*, 2014).

The inhibition of the growth of *Mycobacterium tuberculosis* by the extracts of *Andrographis paniculata* has been studied using intracellular and axenic hypoxic conditions. The inhibition increase with “double stimuli” or higher concentration of the extract. Organic solvent extracts were found to inhibit bacterial growth more than the aqueous extracts (Bhatter *et al.*, 2015). The methanolic leaves extract of *Andrographis paniculata* showed a potential destructive effect on *Staphylococcus aureus* where total cell killing was attained in 24 h based on the time-kill assay. So the plant leave extract is considered to have potential for development as an alternative treatment for bacterial infections (Hussain *et al.*, 2017)

**Conclusion**

*Andrographis paniculata* is a beneficial therapeutic plant used for the management and cure of many ailments. Thin layer chromatographic analysis of both ethanol and methanol extracts spotted five different compounds which were confirmed with the Rf values that same compounds were present in both extracts. A total of ten bioactive compounds were identified from the ethanol, aqueous and ethyl acetate of the leaves extract of the plant. The chemical structures, molecular formulae, molecular weight, m/z and signal intensities were all elucidated in the mass spectrum of the identified compounds. Ethanol was more efficient in the extraction. Among the compounds andrographolide and 14-deoxy-11,12-didehydroandrographolide appeared in the three extracts, while ethyl acetate and ethanol extracts indicated the occurrence of 14-deoxy-14,15-didehydroandrographolide. Compounds like, 19-O-andrographolide and beta-sitosterol were isolated from the plant but more investigation should be carried out to determine the exact nature of these new isolates. Extracts from the leaves of the plant possess a lot of therapeutic properties which is of immense benefit to man. However, we suggest that more research should be carried out on the phytochemistry, toxicity and mode of action of the bioactive compounds isolated to guarantee its use in modern medicines.

![Fig. 3. Representative GC-MS chromatogram of ethanolic leaf extract of *Andrographis paniculata*.](image)

<table>
<thead>
<tr>
<th>RT</th>
<th>Bioactive compound</th>
<th>Molecular formula</th>
<th>Formula weight</th>
<th>Peak area (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.69</td>
<td>Andrographolide</td>
<td>C₂₀H₂₆O₅</td>
<td>350.45</td>
<td>3.23</td>
</tr>
<tr>
<td>7.32</td>
<td>14-deoxyandrographolide</td>
<td>C₂₀H₂₆O₄</td>
<td>334.45</td>
<td>4.69</td>
</tr>
<tr>
<td>15.73</td>
<td>Neoandrographolide</td>
<td>C₂₀H₂₂O₇</td>
<td>478.62</td>
<td>6.88</td>
</tr>
<tr>
<td>10.19</td>
<td>14-deoxy-11,12-didehydroandrographolide</td>
<td>C₁₀H₁₉O₄</td>
<td>318.41</td>
<td>3.67</td>
</tr>
<tr>
<td>13.00</td>
<td>14-deoxy-14,15-didehydroandrographolide</td>
<td>C₂₀H₂₃O₅</td>
<td>348.43</td>
<td>9.76</td>
</tr>
<tr>
<td>14.70</td>
<td>Andrographanin</td>
<td>C₂₀H₂₆O₃</td>
<td>318.45</td>
<td>6.50</td>
</tr>
<tr>
<td>16.96</td>
<td>Isoandrographolide</td>
<td>C₁₀H₁₉O₃</td>
<td>336.42</td>
<td>5.03</td>
</tr>
<tr>
<td>17.98</td>
<td>14-acetylandrographolide</td>
<td>C₂₂H₂₇O₆</td>
<td>392.49</td>
<td>6.06</td>
</tr>
<tr>
<td>4.66</td>
<td>19-O-andrographolide</td>
<td>C₂₀H₂₆O₅</td>
<td>350.45</td>
<td>2.43</td>
</tr>
<tr>
<td>18.60</td>
<td>β-sitosterol</td>
<td>C₂₀H₃₆O</td>
<td>414.72</td>
<td>7.68</td>
</tr>
</tbody>
</table>
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Conflict of Interest. The authors declare that there is no conflict of interest.

References


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