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PHYTOCHEMICAL SCREENING OF EXTRACTS FROM THE LEAVES OF *Andrographis paniculata, Tithonia diversifolia* and *Macrosphyra Longistyla*

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ABSTRACT

Alkaloids, Saponins, Flavonoids, Tannins, Glycosides, Terpenoids and Steroids distribution in *Andrographis paniculata*, *Tithonia diversifolia* and *Macrosphyra longistyla* were assessed in a non-polar solvent. All the plants were found to contain these phytochemicals except for the absence of flavonoids in *A. paniculata* and alkaloids in *M. longistyla*. The presence of these phytochemicals showing potential disease-preventive properties indicates that the leaves of *A. paniculata, T. diversifolia* and *M. longistyla* could serve as basic ingredients in drug making for use in treatment of various ailments.

INTRODUCTION

The emergence of drugs derived from medicinal plants have been an encouragement to the scientist to constantly search for natural products as a source of new drugs that can act as an alternative to the already existing drugs.[1] The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids, and phenolic compounds. [2,3]

Andrographis paniculata belongs to the *Acanthaceae* family and is commonly known as "King of Bitters" or "*Kalmegh*". It is native to India and Srilanka and is also distributed in different regions of Southeast Asia, China, America, West Indies and Christmas Island. It is cultivated because of its well-known medicinal value and it grows well in most soil types. [4-8] The aerial parts, roots and whole plant of *A. paniculata* have been used for centuries in Asia traditionally as powder, infusion, or decoction form either alone or in combination with other medicinal plants for the treatment of various ailments.[9]

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Table 1: Medicinal uses of *A. paniculata*,[10] T. diversifolia [1] and M. longistyla [21]

Plant	Part	Medicinal uses	References
Andrographis paniculata	**Whole plant**	Snakebite and insect sting treatment, dyspepsia, influenza, dysentery, malaria and respiratory infections.	[11, 12]
	Leaf	Fever, colic pain, loss of appetite, irregular stools and diarrhoea, common cold, cough, fever, hepatitis, tuberculosis, mouth ulcers, bronchitis gastro-intestinal disorder and sores.	[13-15]
	Aerial part	Common cold, hypertension, diabetes, cancer, malaria and snakebite, urinary tract infection.	[13, 15, 16]
	Root	Febrifuge, tonic, stomachic and anthelmintic	[11]
Tithonia diversifolia	**Leaf**	Stomach aches, sore throats, indigestion	[17]
	Aerial part	Skin diseases, menstrual pain, hepatitis, treatment of diabetes and diarrhoea	[1]
	Root	Inflammatory and heart related diseases	[18]
Macrosphyra longistyla	**Leaf**	Boiled and eaten as vegetables, cutaneous, subcutaneous parasitic infections, abortifacients, embolic, leprosy, diabetes and infant malnutrition	[21-24]
	Root	Kidney problems	[25]

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Tithonia diversifolia belongs to the plant family known as *Asteraceae* and is commonly known as the Mexican Sunflower. The plant is about 3 m high and 10 cm wide and can withstand heat and drought.[1] *T. diversifolia* is an important medicinal plant with its leaf considered as the major organs used solely or in combination with other plants for treatment of different ailments (Table 1).

Macrosphyra longistyla is a medicinal plant found in several tropical countries. It has long, arching stems that are about 4 m long. [19,20] It belongs to the family Rubiaceae. The plant parts used include the leaf, flower and root. The flower is generally used for healing. *M. longistyla* has been used traditionally as an antihemorragic in Benin,[26] as an antidiabetic in Nigeria and Cote d'Ivoire, [27,28] as a contraceptive and for restoration of fertility,[29] for ulcers,[30] and for diarrhoea. [31]

The aim of this study is to investigate the various phytochemical properties using *N*-hexane extracts of the leaves of *A. paniculata*, *T. diversifolia* and *M. longistyla* that are available for medicinal, pharmaceutical and agricultural use.

MATERIALS AND METHODS

Collection of plant materials

The leaves of the plants were collected and authenticated at the Forest herbarium section of the Forestry Research Institute of Nigeria (FRIN), Ibadan, Oyo. The plant samples were air-dried at room temperature for three weeks in the laboratory and then pulverised with an electric blending machine to obtain a representative sample weighing 100 g.

N-hexane extracts

500 mL of *N*-hexane was measured into the round bottom flask of a Soxhlet apparatus. The grounded leaves were packed in small bags of muslin cloth and placed in the sample container (thimble) of the Soxhlet. The sample was continuously extracted for 24 h.

Phytochemical screening of crude extracts

The preliminary phytochemical screening of the crude extract was carried out using standard procedures. These include tests for alkaloids using Dragendorff and Wagner reagents, Salkowski test for steroids, test for flavonoids, test for saponins and a ferric chloride test for phenolic compounds.

Test for alkaloids: 0.5 mL of each plant extract was stirred with 5.0 mL of 1% aqueous HCl solution on a steam bath. 1.0 mL of the solution was treated with few drops of Meyer's reagent and another 1.0 mL portion was treated with Dragendorff's reagent. Turbidity or precipitation with either of this reagent was taken as preliminary evidence for the presence of alkaloids in the extracted sample.

Test for flavonoids (Shinoda's test): A portion of the extract was dissolved in water and then filtered. 2.0 mL of conc. HCl and magnesium metal was added to 1.0 mL of the filtrate. A pink/orange colouration was indicative of flavonoids.

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Test for saponins: 0.1 g of the extract was boiled in 5 mL of distilled water in a water bath and filtered. The filtrate was shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of emulsion.

Test for terpenoids: 0.5 g of the extract was mixed in 2 mL of chloroform, and 2 mL of conc. H_2SO_4 was carefully added to form a layer. A reddish-brown colouration of the interface was formed to show positive results for the presence of terpenoids.

Test for tannins: 0.5 g of the extract was boiled in 20 mL of water in a test tube and then filtered. A few drops of 0.1% ferric chloride were added and observed for bluish-green or blue-black colouration.

Test for steroids: 0.5 g of the extract was mixed in 2 mL of chloroform, and 2 mL of conc. H_2SO_4 carefully so that the acid formed a lower layer and the interface was observed for a reddish-brown colour indicative of steroid ring.

Test for phenols: The extract was dissolved in ethanol, and a few drops of ferric chloride solution was added. The formation of a purple colouration indicated the presence of phenols.

RESULTS AND DISCUSSION

Table 2: Qualitative analysis of the phytochemicals of the medicinal plants

Plants	Alkaloids	Tannin	Saponin	Terpenoid	Flavonoid	Steroid	Phenols
T. diversifolia	+	+	+	+	+	+	+
M. longistyla	-	+	-	+	-	+	+
A. paniculata	-	-	+	+	-	-	+

+ = presence of constituent

- = absence of constituent

The present study carried out on the plant samples revealed the presence of medicinally active constituents. The phytochemical characters of the three medicinal plants investigated are summarised in Table 2.

The phytochemical screening of the plants studied showed that all the leaves were rich in terpenoids and phenolic compounds. *T. diversifolia* contained all the phytochemical characters investigated. Alkaloids and Flavonoids were absent in *M. longistyla* and *A. paniculata.* Tannins and Steroids were absent in *A. paniculata* only while Saponin was absent in *M. longistyla* only. Saponins are found in most plants as nitrogen-free glycosides, each consisting of a sapogenin and a sugar molecule.

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The absence of alkaloids in *M. longistyla* supports the observation of Durugbo E. U. et al. [21] The absence of flavonoids in *A. paniculata* however contradicts the observation of Okhuarobo et al. [10] The presence of terpenoids in *A. paniculata*, *T. diversifolia* and *M. longistyla* has also been reported by other researchers. [1,10,21]

Steroids were found to be present in *T. diversifolia and M. longistyla.* It should be noted that steroidal compounds are of importance and interest in pharmacy due to their relationship with such compounds as sex hormones.

These phytochemical compounds have pharmacological effects and have been the basis of chemical synthesis of drugs used in modern medicine and are responsible for their use in traditional medicine. [32] Glycosides are large and varied groups of naturally occurring plant products, characterized, on hydrolysis, by the formation of sugar and non-sugar moiety.

CONCLUSION

The plants studies here can be seen as a potential source of useful drugs. Further studies on the isolation, characterization and elucidation of the bioactive compounds as well as the antimicrobial activities of these plants are being investigated.

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