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Identification of Active Principles of *M. balsamina* (Balsam Apple) Leaf Extract

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Momordica balsamina leaf was investigated for it's chemical and traces elemental content. The air-dried samples were subjected to soxlet extraction using the solvents petroleum ether, methanol and water of different polarities. The extract fractions were concentrated and subjected to phytochemical screening to identify which component was high in concentration. The aqueous fraction, which is commonly used by traditional herbal healers, was further, subjected to column chromatographic separation and the recombined fraction of the column was further subjected to thin layer chromatography for identification. The air-dried sample was digested and analyzed for trace elements using atomic absorption spectroscopy. The phytochemical analysis revealed the presence of alkaloid in high concentration followed by saponins, tannins and reducing compound, respectively. Sterols and triteperes were absent in all the solvents used. The analysis of the recombined column fraction of the aqueous extract indicated the presence of the tannins in two of ten fractions, alkaloids in four, saponins in two and the last fraction indicated the presence of reducing compounds. The thin layer chromatography identified the presence of alkaloids, saponins and tannins in the different fractions of the column. In conclusion therefore, *M. balsamina* as shown here do posses some active chemical constitutes like alkaloids, tannins, saponins, Fe, Zn and Mn.

Key words: *M. balsamina,* identification, chemical constituents

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INTRODUCTION

Plants are an indispensable source of chemical compounds and plant physiologists in collaboration with chemists and biochemists have been able to isolate and characterize a myriad of chemical compound from plants[1,2]. Therefore, research on natural substances has remained essential both in the developed and developing countries. In fact, about 60% of medicine sold in pharmacies came directly from natural sources[3] and these natural resources provide us with model of active molecules which serve as the "keys" for the locks" to enable copying or modification of the substance and to study their chemical as well as pharmacological activities[1].

Momordica balsamina linn contains a bitter principle, momordocin. The young leaf has been found to contained 3.6 μg 100 g^{-1} of vitamin C and yields two resin acids and momordocin[4]. The plant contains highly aromatic volatile oil, a fixed oil, carotene, a resin, two alkaloids one of which is momordocin and a saponin. Momordocin is an amaroid and is obtained as a crystalline powder. It also contains 0.038% of an unnamed alkaloid. The seed yield 32% of purgative oil[4]. The total carotenoid pigment is estimated to be 8.53 μg 100 g^{-1} and the vitamin potency is 2.4 to 5.6 IU g^{-1}[4]. Clear reddish-brown oil from the seed assays 46.7% alpha-eleostearic acid, 7.7% linoleic, 15.8% of oleic acid and 29.8% of stearic acid. The dried root yields 12.84% of ash and the dried fruit 11.7% and both ashes contain iron, phosphorus and calcium[4].

The seed oil of *M. balsamina* has been shown to have effects on fertilization[5]. Biological studies on the effect of saponins of *M. balsamina* and other cucurbitaceous plants on biophalaria Alexandria snails have been carried out[6]. The water soluble saponins isolated from the seed of *M. balsamina* and *M. charantia* were screened for their molluscidal activity against alexandra snails. The saponins were found to have effect on egg production, hatchability and growth of the newly hatched snails[5].

This study was therefore carried out to identify the active chemical principles in the aqueous leaf extract of *M. balsamina,* which may provide validation for some of its reported pharmacological activities.

MATERIALS AND METHODS

Sample collection and identification: Fresh sample of the leaves without the stalk were collected in the month of September 2003 along the bank of River Ngada in Shekwari village, Jere local government area, Northern Borno state, Nigeria.

A taxonomist in the Department of Biological Sciences, University of Maiduguri, Maiduguri Nigeria, identified the plant. A voucher specimens deposited in the Department of Biochemistry Research Laboratory, University of Maiduguri. The sample was air dried in laboratory and ground to fine powder and stored in a plastic container at 4°C.

Extraction techniques and chemical analysis: About 5 g of the air dried powdered sample was sequentially extracted in a soxlet extractor with the following solvents: petroleum ether, methanol and distilled water. The crude extract were concentrated *in vacuo*, properly labeled and stored in the refrigerator at 4°C until used[7].

The extracts were subjected to various phytochemical analyses. The different chemical constituent tested for included saponins, tannins, alkaloids, polyuronides, flavones aglycones, reducing compounds, sterols and triterpenes as highlighted below.

Phytochemical analysis: The crude extract of petroleum ether, methanol and water of the plant were subjected to qualitative chemical screening for the identification of the various classes of active chemical constituents using the method described by Trease and Evans[7].

Test for saponins: Five milliliter of ether, methanol or water extract was vigorously shaken with 10 mL of distilled water for 2 min. The appearance of foam that persists for at least 15 min or the forming of an emulsion when olive oil was added confirmed the presence of saponins.

Test for tannins: One milliliter of ether, methanol or water extract was mixed with 10 mL of distilled water and filtered. Ferric chloride ($FeCl_3$) reagent (3 drops) was added to the filtrate. A blue-black or green precipitate confirmed the presence of gallic tannins or catechol tannins, respectively.

Identification of polyuronides: Ten milliliter of ethanol placed in a test tube, the ether, methanol or the aqueous extract of *M. balsamina* (2 mL) was added drop wise. The occurrence of violet or blue precipitate denoted the presence of mucilage.

Test for alkaloids: A small portion (0.2 mL) of the extract was stirred and placed in 1% aqueous hydrochloric acid (5 mL) on a steam bath. Then 1 mL of the filtrate was treated with Mayer's reagent (3 drops) while another portion was similarly treated with Dragedorff's reagent. Turbidity or precipitation with these reagents was considered as evidence for the presence of alkaloids.

Test for reducing compounds: Two milliliter of the extract were placed in test tube and 5 mL mixture of equal

volumes of fehling's solution A and B were added and boiled in a water bath for 2 min. The test tube was observed for brick red precipitate.

Identification of sterols and triterpenes: The aqueous extract (*M. balsamina*) 10 mL was placed in a small beaker and evaporated to dryness. The residue was dissolved in acetic anhydride (0.5 mL) and chloroform (0.5 mL). The solution was transferred into a dry test tube and concentrated sulphuric acid (2 mL) was added. Brownish red or violet rings at the zone of the contact with the supernatant and green or violet coloration denoted the presence of sterols and triterpenes.

Identification of flavone aglyones: A portion of the extract of *M. balsamina* (2 mL) was heated, metallic magnesium and concentrated hydrochloric acid; (5 drops) were added. A red or orange coloration indicate the presence of flavones aglyones.

Column chromatography: Aqueous fraction of the soxlet extract concentrates was separated to different components with chromatographic column packed with silica gel (60-120 mash). The solvent combination used, as the mobile phase was ethyl acetate- formic acid–water at the ratio of 4:5:1. About 2 mL of eluetes were collected in different test tubes until all the coloured substances were completely eluted. This solvent combination was used for only tannin and alkaloid separation. For separation of saponins the solvent combination of methanol – water at the ratio of 8:2 was used.

At the end of elution every 3 test tube of the same colour were recombined and concentrated. The concentrates were further subjected to thin layer chromatography (TLC) as indicated below:

Thin layer chromatography (TLC): Thin layer chromatographic separation was carried out using precoated plates of gel 60G (0.5 mm thick and 7.20 cm). The plates were washed with the solvent system to be used, prior to chromatographic process. Heating on a hot plate dried these plates. The runs were by ascending mode. Spot movements were noted and the various RF (relative frequency) values calculated.

Determination of elemental content of *M. balsamina* leaf: One g of the powdered leaf was digested with 5 mL of nitric acid and hydrochloric acid mixture (6:1), as digestion mixture and heated on plate in a fume cupboard this was done severally until a clear digest was obtained. The white precipitate was obtained indicating that the carbon has been burnt off. The precipitate was now dissolved in deionized water and made to 50 mL in a volumetric flask for elemental analysis using sp-9-single beam atomic absorption spectrophotometer (Philip/Pye Unicam Ltd England)[8]. Diluted samples were aspirated into the atomic absorption flame. The elemental concentrations were determined by comparing the signals from the sample with the signals from the aqueous standard.

RESULTS

Extraction and chemical analyses: The aqueous extract of *M. balsamina* leaf gave a yield of 11.9% w/w, had a dark green colour with a pungent smell. The methanolic extract gave a yield of 11.2% w/w and petroleum ether extract produced a yield of 11.3% w/w.

The number of positive signs indicated the intensity of reactions that reflects the quantity present (Table 1). The result indicated that the most abundant compound in the leaf extract was alkaloid, followed by saponins, tannins, reducing compounds and flavones. Aqueous extraction produced the highest concentration for all the constituents determined.

Chromatographic separation of the aqueous fraction of the extract of *M. balsamina*: The extract was separated into four clear bands of different colours in the column (Fig. 1). The analysis of the recombined column fraction using thin layer chromatography and phytochemical test indicated the presence of tannins in 2 of 10 fractions, alkaloids in 4, saponins in 3 and the last fraction indicated the presence of reducing compounds (Table 2).

Elemental content of plant: Elemental analyses of *M. balsamina* showed high concentrations of Fe, Zn and Mn ($28+0.52\times10^4$, $15.91\pm13\times10^4$ and $30.27\pm22\times10^4$ $\mu g\ g^{-1}$,

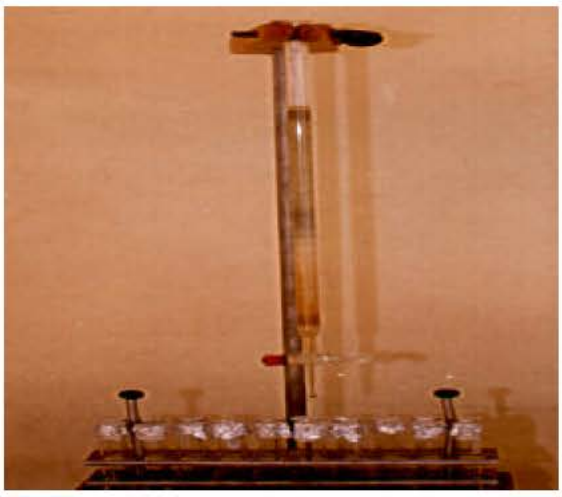

Fig 1: Column chromatographic separation of aqueous fraction of balsam apple

Table 1: Chemical constituents of the petroleum ether, methanol and distilled water extract of *M. balsamina*

	Extracts		
Chemical constituents	petroleum ether	Methanol	Distilled water
Saponins	+	-	+++
Tannins	+	-	++
Polyuronides	-	+	-
Alkaloid	++	+	+++
Reducing compounds	+	+	++
Sterols and triterpene	-	-	-
Flavone aglyones	+	-	+

- = Negative result, + = Positive result, ++ = Present in high concentration +++ = Present in very high concentration

Table 2: Column chromatographic separation of the aqueous fraction of the extract of *M. balsamina*

Chemical constituents	Fractions of the extract/10 test tubes
Tannins	2
Alkaloids	4
Saponin	3
Reducing compounds	1

Table 3: Thin layer chromatographic identification of the recombined column fraction of aqueous extract of *M. balsamina*

Extract	Number of spots	Rf
Saponins	4	0.79bg
		0.7lg
		0.70p
		0.72b
Tannins	2	0.67bg
		0.68lg
Ameroidal	4	0.50db
Alkaloid		0.42db
		0.51db
		0.50db
Alkaloid	2	0.82db
		0.83db

Bg= Bluish gray, lg= Light green, p= Pink, b= Brown, lb= Light brown and db= Dark blue.

Table 4: Elemental analysis of *M. balsamina* leaf

Elements	Concentration ($\mu g\ g^{-1}\ x10^4$)
Zinc (Zn)	15.91±0.13
Copper (Cu)	6.85±0.22
Cadmium (Cd)	N.D
Manganese (Mn)	30.27±0.32
Chromium (Cr)	N.D
Ferric (Fe)	28.00±0.52
Calcium (Ca)	2.45±0.44
Potassium (K)	0.98±0.11
Lead (pb)	N.D

N.D. denotes not detectable, n=5

respectively), while copper, potassium and calcium occurred in low concentrations ($6.85x10^4$, $0.98x10^4$ and $2.45x10^4$ $\mu g\ g^{-1}$, respectively) and cadmium, chromium and lead were not detected (Table 4).

DISCUSSION

The phytochemical studies, chromatographic analyses and elemental analyses of leaf of *M. balsamina* provided useful classes of chemical compounds and trace elements such as saponins, tannins, alkaloids, reducing compounds, flavones, polyuronides, Zn, Fe and Mn. The qualitative analysis revealed the presence of alkaloids in high concentration followed by saponins, tannins and reducing compounds, respectively. Sterols and triterpenes were absent in all the solvents used. Again the phytochemical analyses of the recombined column elutes indicated the presence of tannins in two of the ten fractions, alkaloids in four, saponins in three and the last fraction indicated the presence of reducing compound. Then using thin layer chromatography the presence of these chemical compounds were confirmed (Table 2 and 3). According to Zweig and Sherma[9] a substance can be directly identified from its Rf value. These classes of chemical compounds and elements have been known to exert pharmacological and antagonistic effects and still some are capable of protecting the active ingredients in the herb from decomposing either chemically or physiologically[1]. These observations are in agreement with the report in Kekanah hemme page[4] which indicated that the plant contains momordocin (amaroidal alkaloid), saponins vitamins C and A, Fe, P and Ca.

In conclusion therefore, the result of the present study provided supportive scientific evidence that the leaf extract of *M. balsamina* possesses some active chemical principles, which are pharmacologically active in treatment of some ailments such as anemia, diabetes, among others.

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