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Phytochemical and chromatographic analysis of chloroform extract of *Marsdenia latifelia*

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ABSTRACT

Marsdenia latifolia also known as Gongronema latifolium Benth is a green leafty vegetable that originated from sub-saharan Africa. The ground leaves was extracted using soxhlet apparatus and concentrated in a rotator evaporator to yield 8.0g of chloroform extract. Phytochemical screening carried out on the extract revealed the presence of alkaloids, steroids, flavonoids , saponins and tannins. The presence of alkaloids was confirmed using analytical TLC and preparative TLC with the aid of different solvent system and spraying with freshly prepared Dragendoff's reagent. Tannins were confirmed to be absent through a standard test in a precoated plate and spraying with ferric chloride. Steroidal compounds were confirmed present using a precoated plate and sprayed with a mixture of 10% H_2SO_4 and ethanol in the ratio of 3:1.

Keywords: Marsdenia latifelia, Phytochemical, chromatography

INTRODUCTION

Over the years, some plants have been known to produce reserve foods in the form of fats and oils rather than starch found in others. Some oils from plants are consumed together with other nutrients such as carbohydrates, proteins, vitamins and water for the maintenance of good health.

Medicinal herbs have been cultivated and are abundant in many parts of Nigeria depending on climatic factors. These plants contain bioactive components used in the treatment of diseases or as starting material for the partial synthesis of some useful drugs (Swaminathen and Kochhar, 1988). The knowledge of the usefulness of herbs man is as old as man himself. Man's selection of specific herb material for the treatment of his diseases was based more on his ability to rationalize rather than on the knowledge of the plant constituents (Sofowara, 1982). An herbal remedy is one which main therapeutic activity depends upon the plant or fungal metabolites it contains and is not definable in terms of the particular system of medicine for which it is employed (Shellard, 1979).

In Nigeria, several herbal preparations from leafy vegetables are employed for the treatment of a variety of diseases, such as diabetes and hypertension. These two diseases have been reported to be on the increase in the country (Odetola, 1997). The large scale isolation of these useful constituents becomes necessary and is achieved chemically through physiochemical separation techniques such as solvent partition, Chromatography, ion exchange resins and acid-base shake out (Harborne, 1973).

Research in the field of medicinal herbs must be multidisciplinary in approach. Pharmacology and related fields have unveiled a wide range of leafy vegetables that are useful as Antitumour, antimalaria, antifertility and anti-inflammatory, hypoglycemic and antimicrobial drugs. Pecae a drug produced from a shrub-like plant *Cephalis ipecaunha* is diaphoretic, emetic and expectorant for amoebic dysentery and diarrhea (Sofowara, 1982). Evan *et al.,*

(1989) have reported on the use of *Enanta Chloranta* in the treatment of jaundice and Malaria. Sofowara (1982) also reported that *Enanta chloranta* cures Liver damage.

Marsdenia latifolia also known as *Gongronema latifolium* Benth Hook, is an herbaceous shrub, with yellow flowers and the stem yields characteristic milky exudates when cut. It is commonly grown in gardens in southern part of Nigeria. It is locally called "utasi" by the Efiks/Ibibios and Quas; "utazi" by the Igbos and "arokeke" by the Yorubas in Nigeria. The Efiks and Quas in Calabar use *M. latifolium* crude leaf extract in the treatment of malaria, diabetes, hypertension, and as laxative. Also it is used as a spice and vegetable (Morebise, 2002). Scientific studies have established the hypoglycaemic, hypolipidaemic and antioxidative effects of aqueous and ethanol extracts of *M. latifolium* leaf (Ugochukwu *et al*., 2003; Ogundipe *et al*., 2003). Morebise *et al*. (2002) reported that the leaf extract has anti-inflammatory properties.

The aim of this research study is to assess the bioactive components present in the chloroform extract of *Marsdenia latifolia* leaves using phytochemical screening and chromatographic analysis.

MATERIALS AND METHODS

Collection and Treatment of Samples

Marsdenia latifolia were collected from a local farm in Ikot Udoro of Oruk Anam Local Government Area in Akwa Ibom State. The leaves were taken to a taxonomist Dr. Mrs. Bassey of the Botany Department, University of Uyo for identification. The leaves were plucked from the stem and washed. The leaves were cut into sizeable length with a knife and sun dried for three days. The dried leaves were ground into powder using mortar and pestle in the laboratory. The ground sample was sieved through a mesh size of 0.5mm, it was then stored in a dry airtight containers for subsequent analysis.

Extraction Method

Preliminary test was carried out with the fresh leaf and on the ground leaf samples. The ground leaf samples were extracted using sohxlet apparatus. Petroleum ether, Chloroform and ethanol were used as extracting solvents.

200g each of the ground leaf sample were weighed out and packed into three sohxlets. The thimbles of the sohxlet apparatus were tapped for uniform packing and elimination of air space.

Each sample was extracted with 400cm^3 of petroleum ether for eight hours at the temperature of 60-70^{o}C. The marc (already extracted plant material) was re-extracted with 400cm^3 of chloroform. Concentration of the extracts was carried out using a rotator evaporator to yield 9.3g of the petroleum ether extract, 8.0g of the chloroform extract. Phytochemical and chromatographic analysis was carried out only on the chroform extract.

Phytochemical Analysis

Phytochemical analysis was carried out to detect the presence or otherwise of some secondary metabolites (Udo *et al*., 2012). The standard methods laid down by the Association of Official Analytical Chemistry (A.O.A.C. 1975) and Trease, and Evans, 2001, were used to analyse the bioactive components in the plant extract. The phytochemical tests carried out in this work include alkaloids, saponins, glycosides, tannins, steroid, terpenes, flavonoids and deoxy-sugar.

Chromatographic Analysis

- ***Preparation of the Plates***

Several plates measuring 20cm x 20cm were used for the thin layer chromatography (TLC) plates and kiesel gel 60G was used as the adsorbent. The adsorbent was mixed with distilled water in a conical flash in the ratio of 1:2 (that is 1g of the silica gel to 2ml of distilled water) and shaken vigorously for 90 seconds till slurry was formed. Time taken for the formation of slurry was determined by a stop watch.

The plates were washed with distilled water, wiped with ethanol followed by acetone. The wiped plates were mounted horizontally on a metal template and the slurry poured on the upper end of the central glass plate and spread evenly over the plates with a commercial spreader moved in one direction. The spreading lasted for about 15 seconds.

Plates coated to 0.25mm thick were used for analytical TLC. While those coated to 0.5mm thick were used for the preparative TLC. The slurry was allowed to solidify on the plates and after solidification, the plates were activated in a scientific oven at 110^{o}C for 30 minutes. Excess adsorbent on the back and edges of the plates were wiped before developing the plates.

Several commercially prepared plates measured 10cm x 5cm were also used for the analysis. The precoated plates were prepared on Aluminium Sheets Art 5554 De-Alufolein Kiesel gel 60 F_{254}.

- ***Preparation of Solvent System***

Three transparent glass tanks containing different solvent systems were prepared according to the ratio given below. The solvent systems and their ratios were chosen based on the result from photochemical screening.
Tank I: Contains Chloroform ($CHCl_3$) and Methanol (CH_3OH) in the ratio of 3:1.
Tank II: Contains Methanol (CH_3OH) and Ammonia (NH_3) in the ratio of 5:1
Tank III:Contains Chloroform, Acetic acid and distilled water in the ratio of 10: 7:1.

- ***Spotting, Development and Detection of Spots by Analytical TLC***

The crude extract was dissolved in Chloroform (solvent of extraction) and with the aid of capillary pipettes, spots of about 2mm in diameter and 5cm apart were made on a base line measured 2cm from the edge of the plate. The spots were allowed to dry in air for five minutes before developing in the solvent tanks which were shaken for five minutes and kept for thirty minutes for saturation to occur.

Two analytical chromoplates, each was placed in tank I and II with the spotted end made to face downward. The depth of the developing solvent was less than the spotted height. The tanks were sealed and allowed to stand undisturbed until the solvent front traversed the length of the plate to 1cm of the top of the coated portion of the plates. The plates were removed and the solvent front marked with a pencil. The plates were allowed to stand for three minutes before detecting the spots.

Spots on the developed plates were detected using UV light type A 409 (wavelength 250nm) and spraying with freshly prepared Dragendorffs spray reagent. The colours and retentions factors obtained were noted.

- ***Spotting, Development and Detection of Spots by Preparative TLC***

The chloroform extract was applied as a band 2.0cm from the base of each of the three preparative TLC plates used with the aid of a Pasteur pipepette. The plates were developed using ascending technique to a height of 16cm using tank II. After development, the plates were brought out, allowed to dry and viewed with a UV light of the type mentioned above.

Four bands were detected from each of the three plates. The bands were scraped out with a razor blade and dissolved in methanol; the mixture was allowed to stand for thirty minutes. The mixture was filtered and four different filtrate fractions labeled 1, 2, 3 and 4 were obtained. These fractions were spotted on an analytical plate and developed in tank II using the same procedure as described above the developed plate was allowed to dry and viewed with the UV light. The number of components obtained from each fraction, colour and their retention factor were noted.

To obtain homogenous components, another preparative TLC was carried out with the fraction labeled 3. Two bands were obtained when viewed with UV light. The bands were scrapped out, dissolve in methanol and filtered. Fractions laballed 3_1 and 3_2 were obtained and were concentrated to obtain smaller volumes.

Finally, analytical TLC was carried out using tank II and all the fractions (1,2, 3_1, 3_2 and 4) were spotted on it and developed. The developed plates were viewed with a UV light and after being sprayed with freshly prepared Dragendorff's reagent. The results were obtained and noted.

- ***Spotting, Development and Detection of Spots Using Precoated Plate***

Several precoated plates were used for the analysis. The fractions were spotted on them and developed in tank II and tank III. Development was to 8cm from the base line which was 1cm from the edge of the plate. After development, the solvent front was marked with a pencil and allowed to dry. The plates were viewed with UV and several spray reagents were used to spray them. The colour of spots and the retention factor were noted. In all cases, developments of the plates after spraying were carried out in the oven at 100 to 110^{o}C.

RESULTS AND DISCUSSION

Phytochemical Analysis

The results of phytochemical analysis on the chloroform extract of *Marsdenia latifelia* are presented in Table 1.

Table 1: Results for Phytochemical Analysis

TESTS	OBSERVATION	INFERENCE
ALKALOIDS		
(i) Dragendorff's test	A pink precipitate was obtained	Alkaloids present
(ii) Picric Acid Test	A yellow precipitate was observed	Alkaloids present
(iii) Mayers Reagent Test	A milky Colouration was observed	Alkaloids present
TANNINS		
(i) Bromine water Test	The Bromine water was decolourized	Tannins Present
(ii) Ferric Chloride Test	A blue black precipitate was not observed, instead a black colouration	Tannins Present
PHLOBATANNINS		
(i) Formaldehyde, HCl Test	A black solution was obtained	Phlobatannins absent
SAPONNINS		
(i) Frothing Test	Frothing obtained was not stable and did not persist on boiling	Saponins absent
(ii) Fehlines Solution Test	A blue black precipitate was observed	Saponins absent
CARDIAC GLYCOSIDES		
(i) Salkowski Test	A black colouration was observed	Cardiac glycosides absent
(ii) Keller Kiliani Test	A black colouration with no interphase was observed	Cardiac glycosides was absent
STEROID TEST		
(i) Acetic Anhydride Test	A bluish green interphase was obtained	Steroids was present
DEOXY SUGAR TEST		
(i) Glacial acetic acid Test	A green ring was obtained instead of a violent ring	Deoxy sugar confirmed absent
FLAVONOIDS TEST		
(i) Magnesium metal Test	Effervescence occurs followed by the formation of an orange colour	Flavonoids present
TERPENES TEST		
(i) Acetic Anhydride Test	A bluish green interphase was obtained instead of a pink colour interphase	Terpenes confirmed absent

The result revealed the presence of some classes of chemical compounds such as alkaloids, steroids, saponins and flavonoids. Cardiac glycosides, Deoxy Sugar, Phlobatannins, Terpenes and Tannins were however absent in the leaf extract. The medicinal properties of these components have been reported (Gill, 1992, Banso and Adeyemo 2007, Kubmarawa *et al*., 2007).

Chromatographic analysis

Separation of components by analytical thin layer chloromatography, laboratory preparative thin layer chromatography and precoated plates were carried out on the chloroform extract. The results are presented in table 2 to 8.

TLC profiling of the extracts gives an impressive result that directing towards the presence of number of phytochemicals. Various phytochemicals gives different Rf values in different solvent system. This variation in Rf values of the phytochemicals provides a very important clue in understanding of their polarity and also helps in selection of appropriate solvent system for separation of pure compounds by Column Chromatography.

Compound showing high Rf value in less polar solvent system have low polarity and with less Rf value have high polarity. Mixture of solvents with variable polarity in different ratio can be used for separation of pure compound from plant extract. The selection of appropriate solvent system for a particular plant extracts can only be achieved by analyzing the Rf values of compounds in different solvent system. In the present state of affairs, TLC profile of all the plant extract of the leaves in different solvent system indicate the presence of diverse type of phytochemicals in this plant. Different Rf values of the compound also reflects an idea about their polarity. This information will help in selection of appropriate solvent system for further separation of compound from this plant extracts.

Table 2: Results of analytical TLC, using chloroform: methanol (3:1), as solvent system, developed 17.1cm

Fraction	Colour when viewed	Colour when sprayed	Solute front	R_f values
Chloroform	Red	Brown	11.8	0.69
Extract	Red	Pink	12.2	0.71

Table 3: Results for analytical TLC, using methanol: ammonia (100ml: 1.5ml) as solvent system, developed to 16cm

Fraction	UV colour	Colour sprayed	Solute front	R_f values
Chloroform	Red	Brown	13.9	0.869
Extract	Red	Pink	12.5	0.781

In the result above, four bands were obtained when viewed in the UV. The bands were scraped out dissolved in Methanol and filtered. The filtrate fractions were labeled 1, 2, ,3 and 4. The filtrates were spotted on an analytical plate and developed using the methods earlier described. The result obtained is as shown by Table 4.

Table 4: Results for preparative TLC solvent system: methanol : ammonia (100ml: 1.5ml) developed to 16cm

Fraction	Number of components and green indicated by brown spots	Solute front	R_f values
Fraction labelled (1)	One	12.5	0.781
Fraction labelled (2)	Two spots were obtained	13.9	0.869
		14.9	0.931
Fraction labelled (3)	Three spots were obtained	13.0	0.813
		14.4	0.900
		15.3	0.956
Fraction labelled (4)	One spot was obtained	13.4	0.838

Preparative TLC was carried out again with the fraction labeled 3. Two bands were further obtained. The bands were scraped out dissolved in methanol and filtered. The filtrate fractions obtained were labeled 3 and 3_2. 3_1 and 3_2 and all other fractions earlier obtained were spotted on analytical plate and developed. The result is as shown in Table 5.

Table 5: Results for preparative TLC using methanol: ammonia (100ml: 1.5ml) as Solvent system, developed to16cm

Fraction	Number of components	UV colour	Colour after spraying	Solute front	R_f values
1	One	Blue	Pink	13.8	0.863
2	Two	Green	Pink	13.9	0.869
		Light green	Pink	14.5	0.910
3_1	One	Blue	Pink	14.2	0.888
3_2	One	Light blue	Purple	14.0	0.875
4	Two	Green	Pink	13.9	0.869
		Green	Pink	14.4	0.900

Table 6: Results using precoated plate for spotting, solvent system : methanol: ammonia (100ml: 1.5ml), developed 8cm

Fraction	Number of components	UV colour	Colour after spraying	Solute front	R_f values
1	One	Green	Pink	6.7	0.836
2	One	Blue	Pink	7.2	0.900
3_1	One	Green	Pink	4.8	0.600
3_2	One	Green	Pink	3.5	0.4375
4	One	Blue	Pink	2.1	0.2625

Table 7: Results using precoated plate with solvent system : methanol: ammonia (100ml: 1.5ml) , spray reagent: 15ml 10% H_2SO_4 + 5ml C_2H_5OH + 1ml distilled water development: 8cm

Fraction	Number of components	UV colour	Colour after spraying	Solute front	R_f values
1	One	Bluish Green	Brown	5.4	0.800
2	Two	Light blue	Brown	6.3	0.788
		Blue	Brown	3.5	0.44
3_1	No spot		-	-	-
3_2	No spot		-	-	-
4	Two	Bluish Green	Brown	6.5	0.813
		Bluish Green	Brown	2.3	0.288

Table 8: Results using precoated plate for spotting, spray reagent : ferric chloride, solvent system : chloroform: acetic acid : water (10 :7 :1) development : 8cm

Fraction	Number of components	UV Colour	Colour after spraying	Solute front	R_f values
1	One	Green	No colour Changes was observed	7.5	0.9373
2	One	Green		7.3	0.9125
3_1	One	Green		6.5	0.8125
3_2	One	Green		6.5	0.8125
4	One	Green		6.6	0.825
5	One	Light Brown	Blue black	5.8	0.725

CONCLUSION

The phytochemical screening and chromatographic analysis of chloroform extract of leaves of *Marsdenia latifolia* have shown the presence of pharmacologically active substances such as alkaloids, steroids and flavoniods. Tritepenes, deoxy sugar, cardial glycosides, Phlobatannins, tannins, were confirmed absent. Hence chloroform should be used in the extraction where the paramount interest is in the extraction of alkaloids, steroids and flavoniods.

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