Gas Chromatographic FID Evaluation of roots of *Manniophytum fulvum*

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Abstracts

The phytochemical composition of the root extract of *Manniophyton fulvum* was screened by gas chromatography coupled to flame ionization and pulse flame photometric detectors. Among the twenty four known flavonoids were detected, only quercetin (107.44mg/100g), kaemoferol (66.19mg/100g), quercetrin (49.87mg/100g), rutin (28.52mg/100g) and, apigenin (28.67mg/100g) representing (37.99%,23.41%, 10.14%, 17.64%, and 10.09%) respectively were prominent. The rest were in insignificant amount. The roots extracts contain nine glycoside compound consisting mainly of digoxin (27.166mg/100mg 58.5%), digitoxin (17.9 mg/100g (38.51%) and 0.8mg/100g (2.99%) of kampferol-3-rhamnoside.The rest were of a very little amount. For saponin, the FID gas chromatography analysis revealed that euphol, saponine, sapogenin had the highest concentration of 20.268490mg/100g, 0.3369mg/100g, and 0.065978mg/100g making up of

97.9%,1.62% and 0.3% percentage composition respectively . The rest were in insignificant amount. Seven benzoic acid were detected by the GC- FID consisting mainly of p-hydroxyl benzoic 6.288160mg/100g (65.4%), gallic acid 2.96280 mg/100g (30.8%) and gentisic acid 0.355mg/100g (3.71%). Out of the ten carotenoid detected, the highest concentration were violaxanthine 47.80526 (49.95%), astraxanthine 26.62538, (27.82%), lutein 8.24911 (8.93%) and neoxanthine 6.54195mg/100g. (6.84%). For phytosterol, the bioactive compound include sitosterol 14.2007mg/100g(71.53%), campesterol 3.54mg/100g (17.85%) and stigmasterol 2.1093mg/100g (10.6%) making up highest concentration. Three allicin compound were detected which include diallylthiosulphinate (95.92%), methyl allyl thiosulphinate (3.50%) and allyl methyl thiosulphinate (0.58%). These results shows that *Manniophyton fulfum* has many bioactive molecules suggesting strong therapeutic and nutraceutical potential suggesting their likely uses in the management and prevention of diseases.

Keyword: Gas chromatographic FID, phytochemical, *Maniophyton fulvum*, nutraceutical.

Introduction

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been derived from natural sources, with

many of these isolations based on the uses of the agents in traditional medicine (Cragg and Newman, 2001). Phytomedicine also known as botanical medicine or herbal medicine is a branch of science in which plant-based formulations are used to alleviate diseases. Recently there has been a shift in universal trend from synthetic to herbal medicine, which can be termed ‘Return to Nature". Medicinal plants have been known for millennia and highly esteemed all over the world as a rich source of therapeutic agents for the prevention of diseases and ailments (Sharma *et al.,* 2008). The search for eternal health and longevity and for remedies to relieve pain and discomfort drove an early man to explore his immediate natural surroundings and led to the use of many plants, animal products, minerals, etc and the development of a variety of therapeutic agents (Nair *et al.*, 2007).

The importance of medicinal plants and traditional health system in solving health care problems of the world is gaining increasing attention. Because of this resurgence of interest, the research on plants of medicinal importance is growing phenomenally at the international level, often to the detriment of natural habitats and mother populations in the countries of origin. Most of the developing countries have adopted traditional medicine as an integral part of their culture. Historically, most medicinal preparations are derived from plants, whether in the simple form of raw plant materials or in the refined form of crude extracts, mixers, etc. (Krishnaraju *et al.,* 2005).

In the bid to maximise the potentials of phytomedicine, there is need to investigate the chemical composition and biological activities of medicinal plants. The product of the various investigations carried out in the past two centuries has yielded compounds for the development of modern synthetic organic chemistry as a major route for a discovery of novel and more effective therapeutic agents (Nair *et al.,* 2007). Despite all efforts, out of an estimate of about 250,000 flowering plants occurring on the planet earth, 60% have not been screened for biological activity (Turker and Usta 2008). Nigeria is blessed with the abundance of medicinal plants with vast therapeutic potentials which phytochemistry has not been elucidated. Such medicinal plant includes *Manniophyton fulvum.*

Manniophyton fulvum which belong to the family of *Euphorbiaceae.*remains one of the popular herbs amongst local traditional medicine practitioners in the south-south region of Nigeria. It is also geographically distributed widely in tropical Africa, from Sierra Leone to Sudan, and South-ward to Angola (Brown *et al.,* 1913). In African traditional medicine the root, stem, bark and leaf are credited with analgesic properties and are used to treat diarrhoea, stomach ache, cough and bronchitis (Burkil, 1985). According to unconfirmed folklore in the south-south region of Nigeria, traditional herbalists have used the root extract of *Manniophyton fulvum* to treat erectile dysfunction. The red stem sap is credited with haemostatic properties, while the leaf sap is used against ear problems, caries (Harbone, 1973).

In Congo (Brazzaville), it is considered a cicatrisant on wounds, and also good for treating dysentery, piles, haemophthysis and dysmenorrhea (Bouquet and Debray, 1974; Bouquet, 1969). The red stem-sap is used typically in Ivory Coast on herpes and other dermal infections. Furthermore, a decoction of the young shoots, bark and stem, the husk of the nut and the sap are used as a remedy for cough in Congo (Brazzaville), Ivory coast and Sierra Leone (Bouquet and Debray, 1974; Bouquet, 1969).

In recent researches, the antioxidant and antidiarrheal property of the plant have been determined and the result was positive (Ezeigbo *et al.,* 2010). The leaf extract of the plant was found less toxic than the root extract, being that the dosage required to cause death in 50% of the animals using the root extract is about 850 mg/kg whereas the leaf extract produced toxicity at a dose of 1050 mg/kg (Agbaire *et al.,* 2013). This research therefore is aimed at evaluating the qualitative and quantitative phytochemistry of the root of *Manniophyton fulvum* which so as to ascertain the level of bioactive agents in it.

MATERIALS AND METHODS

Chemical used.
All chemical and drugs were obtained commercially and were of analytical grade.

Collection of plant samples

Roots of *Manniophyton fulvum* were obtained from Umuada Ngodo Isuochi, Umunneochi LGA, Abia State, Nigeria. For easy identification, the fresh leaves and fruits of the plant was also harvested. The plant was identified, confirmed and authenticated at the Taxonomy Unit, Department of Plant Science and Biotechnology both at Abia State University and University of Port Harcourt, Nigeria and sample specimens where deposited at the University Herbarium.Voucher number (UPH No): V – 1,035 and carpological number (UPH No): C – 056 were assigned to the leaves and fruits respectively.

Analysis of the phytochemical profile

Calibration, identification and quantification

Regression analysis verified the linearity of the dependence of response on concentration. Identification was based on a comparison of retention times and spectral data with standards. Quantification was performed by establishing calibration curves for each compound determined, using the standards.

DETERMINATION OF FLAVONIODS

The dried extract of ethanolic and aqueous extraction, was sampled and made to be free of water by ensuring constant weight for a period of time in the laboratory. 1.09g of the sample was weighed into the 250ml conical flack capacity with addition of 100ml of distilled water and boiled for 10minutes. The flavoniods extract was obtained by pouring a mixture of 100ml of the boiling methanol and

water (70:30) v. v into the materials. The mixture was allowed to macerate for about 6 hours and then concentrated to 5ml for gas chromatography analysis.

Determination of the sterol composition

Extraction

Sterol analysis was carried out by following the modified AOAC 970.51 official methods. (AOAC International, 2000) The aliquot of the extracted oil was added to the screw-capped test tubes. The sample was saponified at 95 °C for 30 min, by using 3 mL of 10% KOH in ethanol, to which 0.20 mL of benzene had been added to ensure miscibility. Deionised water (3 mL) was added and 2 mL of hexane was used in extracting the non-saponifiable materials (sterol, etc.). Three extractions, each with 2 mL of hexane, were carried out for 1 h, 30 min and 30 min respectively, to achieve complete extraction of the sterols. The hexane was concentrated to 1 mL in the vial for gas chromatography analysis and 1 µL was injection into the injection port of GC.

Determination of the saponin composition

Extraction

The extraction was carried out by following the modified method of Guo et al. (2009). The sample was pulverized and the saponin was extracted three times with

redistilled methanol. The saponins were removed with 20 mL of the solvent for 20 min by sonication. The combined extracts were concentrated to syrup under reduced pressure, and then suspended in water. The suspension was extracted with petroleum ether, chloroform and 1- butanol saturated with water, successively, to give the respective extract after removal of the solvent. The combined extract was filtered and concentrated to 1 mL in the vial for gas chromatography analysis and 1 µL was injected into the injection port of GC.

Determination of the allicin composition

Extraction

The allicin extraction was carried out by following the modified method Chehregani et al. (2007). The fresh samples (5.0 g) were washed, mined and added to an adequate amount of water to a concentration of 12.5% (w/v). The sample was ground was thoroughly blended. The products were squeezed through gauze cloth to remove the larger particles and the extracts were passed through a 0.2 µm filter (Millipore). The process was carried out at room temperature and the filtrate was sterilised and stored at 4 °C before analysis.

The extract was re-extracted with petroleum ether and later concentrated by pouring the petroleum ether extract into the round bottom flask of the rotator evaporator arrangement. Evaporation concentrated it. Then the concentrated extract

was dried of water by using the hydrous sodium sulphate before gas chromatography analysis.

Determination of the carotenoids composition

Extraction

The carotenoids extraction was carried out by following the modified method Takagi (1985). The pulverised sample (5.0 g) was homogenised in 75 mL acetone and kept at room temperature for 1 h in the dark. The homogenate was filtered through filter paper by suction. Extraction was repeated three times with the same volume of acetone. The extracts were combined and evaporated under reduced pressure and the residue was re-extracted by a mixture of diethyl ether and petroleum ether in equal ratio. The extract was poured into the round bottom flask of the rotator evaporator arrangement. Evaporation concentrated it. Then the concentrated extract was dried of water by using the anhydrous sodium sulphate before gas chromatography analysis.

Determination of the glycoside composition

Extraction

The extraction was carried out by following the modified method of Oluwaniyi and Ibiyemi (2007). The pulverised sample (10 g) was extracted by soaking for 2 h

with 10 mL of 70% alcohol and then filtered, concentrated. The redistilled hexane was used to replace the initial solvent and the hexane was concentrated to 1 mL in the vial for gas chromatography analysis.

Determination of the benzoic acid derivatives composition

Extraction

BDA content was extracted following modified method of Andary et al. (2013). Two-stage extraction procedures followed for the effective removal of BAD as described below.

Stage 1:

The sample (50.0 mg) was extracted with 5 mL of 1 M NaOH for 16 h on a shaker at ambient temperature. After extraction, the sample was centrifuged (5000 x g), rinsed with water, centrifuged again, and the supernatants were combined and placed in a disposable glass test tube and heated at 90 °C for 2 h to release the conjugated phenolic compounds. The heated extract was cooled, titrated with 4 M HCl to pH <2.0, diluted to 10 mL, with deionized water, and centrifuged to remove the precipitate. The supernatant was saved for subsequent purification and the residue was extracted further in stage 2.

Stage 2:

The residue from stage 1 above was extracted with 5 mL of 4 M NaOH, heated to 160 °C in Teflon. After cooling, the mixture was filtered. The supernatant was collected and the residue washed with water (deionised). The supernatants were combined and adjusted to pH <2.0 with 4 M HCl. The filtrates were combined for further purification.

Preparation for GC-FID

The concentrated extract (2.0 mL) was transferred to 5.0 mL glass vial. The extract was saturated with sodium chloride slat before the addition to 250.0 µL of ethyl acetate. The mixture was agitated manually for 10.0 min at room temperature and later centrifuged for 15 min at 2500 rpm. The organic phase was removed to a 1 mL vial. The extraction was repeated twice and the organic phases were mixed together. 50.0 µL of the N, O-Bis (trimethylsilyl) trifluoroacetamide were added and the mixture was manually agitated for 2 min at room temperature for derivatization.

Chromatographic conditions

The gas chromatograph was an HP 6890 (Hewlett Packard, wellington, DE,USA), GC apparatus, fitted with flame ionization detector (FID), powered with HP Chemstation Rev.A 09.01(1206) software, to identify and quantify compounds. The column was a capillary DB-5MS (30mx0.25mmx0.25µm thickness). The inlet

and detection temperatures were 250◦C and 320^{o}C. Split injection was adopted with a split ratio of 20:1. The Carrier gas was nitrogen gas .The compressed air and hydrogen pressures were 38 psi and 28psi. The oven programmed was; initial temperature at 60◦C for 5 mins. First ramping at 10◦C/min for 20 min was followed by a second ramping at 15◦C /min for 4 min.

Result

Table 1 Flavonoid composition of *Manniopyton fulvum* roots determined by gas chromatography

Compounds	**Retention time (min)**	**Composition × 10^{-4} (mg/100g)**
Catechin	13.535	0.020
Resveratrol	14.915	0.178
Apigenin	16.034	286679.900
Diadzein	16.243	1296.100
Butein	16.449	1.738
Naringenin	16.668	4.460
Baiochanin	17.358	1.803
Luteolin	17.768	18588.600
Kaemferol	18.050	661903.900
(-) – Epicatechin	19.396	5.157
Salvagenin	20.466	12.478
(-) – Epicatechin–3–gallate	21.668	0.040
Gallocatechine	22.065	2.062
Quercetin	22.600	1074141.300
Isorhamnetin	23.738	24.762
Myricetin	24.609	3.454
Sinensetin	24.998	14.740
Kaempferol–3–arabinoside	25.359	0.913

Naringenin	26.059	2.413
Quercitrin	27.287	498662.700
Isoquercetin	27.426	791.555
Orientin	28.094	0.199
Rutin	28.092	285225.200
Isoorientin	28.524	96.121
Total composition		**2827460.700**

Table 2 Glycoside composition of *Manniopyton fulvum* roots determined by gas chromatography

Compounds	**Retention time (min)**	**Composition × 10^{-3} (mg/100g)**
Kampferol-3-O-rhamnoside	16.038	635.858
Arbutin	17.469	353.984
Salicin	18.765	57.267
Amyygdalin	19.521	0.300
Ouabain	20.473	2.259
Digitoxin	21.824	17897.140
Vitexicarpin	22.242	241.466
Digoxin	22.606	27166.900
Costugenin	23.970	120.116
Total composition		**46475.290**

Table 3 Saponin composition of *Manniopyton fulvum* roots determined by gas chromatography

Compounds	**Retention time (min)**	**Composition × 10^{-4} (mg/100g)**
Gitogenin	17.899	36.183
Solagenin	18.607	7.388
Diaosgenin	19.501	229.581
Tigogenin	19.935	20.223
Neohecogenin	21.020	3.071
Hecogenin	21.793	2.467
Sapogenin	23.239	659.776
Euphol	25.067	202684.910
Saponine	25.559	3369.490
Total composition		**207013.190**

Table 4 Benzoic acid composition of *Manniopyton fulvum* roots determined by gas chromatography

Compounds	**Retention time (min)**	**Composition × 10^{-3} (mg/100g)**
Gentisic acid	4.931	354.843
Protocatechuic acid	8.760	0.351
p–Hydroxybenzoic acid	10.940	6288.160
Gallic acid	13.776	2962.840
2,3 – Dihydroxybenzoic acid	16.555	0.229
2,3,4 – Trihydroxybenzoic acid	18.697	3.408
2,4,6 – Trihydroxybenzoic acid	19.793	6.256
Total composition		**9616.090**

Table 5 Carotenoid composition of *Manniopyton fulvum* roots determined by gas chromatography

Compounds	**Retention time (min)**	**Composition × 10^{-2} (mg/100g)**
Malvidin	19.808	20.099
Carotene	21.229	38.545
Lycopene	21.547	0.190
Beta – cryptoxanthin	22.183	28.893
Lutein	22.586	824.911
Zeaxanthin	24.161	269.864
Antheraxanthin	24.733	1.145
Astraxanthin	25.780	2662.538
Violaxanthin	26.400	4780.526
Neoxanthin	27.873	654.195
Total composition		**9570.908**

Table 6 Phytosterols composition of *Manniopyton fulvum* roots determined by gas chromatography

Compounds	**Retention time (min)**	**Composition × 10^{-4} (mg/100g)**
Cholesterol	19.151	0.009
Cholestanol	20.533	0.933
Ergosterol	21.442	0.995
Campesterol	21.820	35436.600
Stigmasterol	23.114	21092.500
5-avenasterol	23.968	0.618
Sitosterol	25.090	142006.700
Total composition		**198538.355**

Table 7 Allicin composition of *Manniopyton fulvum* roots determined by gas chromatography

Compounds	**Retention time (min)**	**Composition × 10^{-8} (mg/100g)**
Diallyl thiosulphinate	16.203	1232.810
Methyl allyl thiosulphinate	17.206	45.027
Allyl methyl thiosulphinate	18.208	7.478
Total composition		**1285.310**

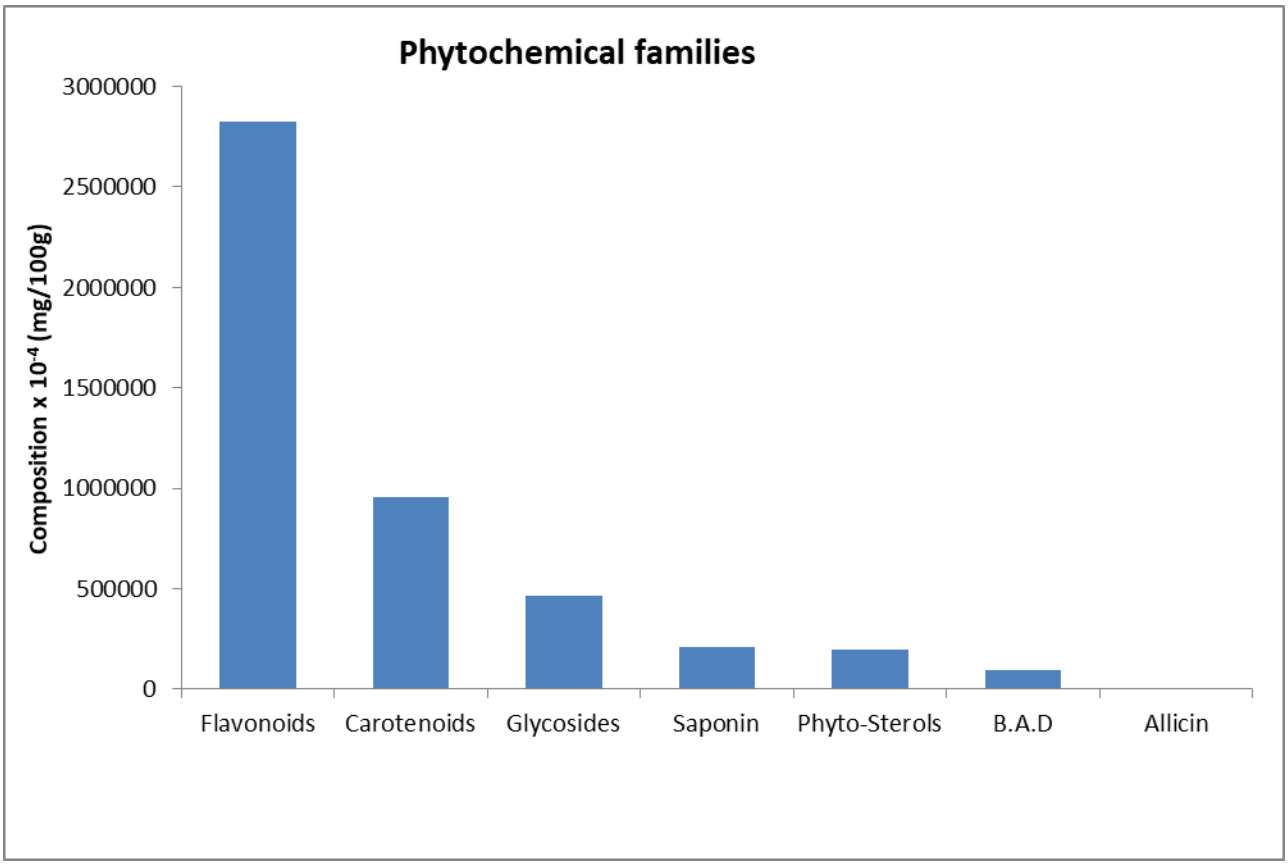

Figure 4.1 Summary of the composition of phytochemicals in *Manniophytun fulvun* roots

DISCUSSION

The results obtained from the phytochemical profile of this study revealed that the root of *Manniophyton fulvum* contains bioactive agents as shown in the tables above. Some of the primary compounds of the plant have been shown in various studies (Guardia *et al.,* 2001; Hou *et al.,* 2005; Giuliani *et al.,* 2008; Mackraj *et al.,*

2008; Nogueira *et al.,* 2011; Ather *et al*; 2007;) These compounds may be credited for the medicinal properties of the aqueous root extract of *Manniphyton fulvum.*

The roots of *manniphyton fulfum* has high Flavonoids, such as quercetins. Flavoniods had previously been reported to have multiple biological properties including anti-microbial, cytotoxicity, anti-inflammatory as well as anti-tumour activities. But the best described properties of almost all the group of flavonoid is ability to act as a powerful anti-oxidants which can protect the body against free radicals reactive oxygen species. (Tapas et al., 2008; Atmani et al., 2009) .For instance querctins are antioxidants (Bando *et al.,* 2010), they scavenge damaging particles in the body known as free radicals, which damage cell membranes, tamper with DNA, and even cause cell death. Antioxidants can neutralize free radicals and may reduce or even help prevent some of the damage they cause (Boota *et al.,* 2008).

Quercetin acts like an antihistamine and an anti-inflammatory (Guardia *et al.,* 2001), and helps to protect against heart disease (Egert *et al.,* 2009) and cancer. Quercetin can also help to stabilize the cells that release histamine in the body and thereby may help reduce symptoms of allergies and anti-inflammatory effect. Studies show that quercetin supplementation reduces blood pressure in people who have hypertension (Mackraj *et al.,* 2008). Kaempferol is known for it strong anti-

oxidant and anti-inflammatory properties(Lau, 2008). It also has antibacterial, anti-cancer, anti-fugal,cardioproctective,hepatoprotective,hypocholesterolemic, hypoglycemic, hypotensive and immunomodulatory activities (Ahmad et al.,1993; song et al;2003, Desousa et al 2004) This results suggest that the root extracts has both anti-inflammatory and anti-microbial activities

The roots of *Manniophytum fulfum* has moderate glycoside, prominent which is digoxin. Digoxin is a structurally related and more lipophilic cardiac glycoside that largely replaced quabain for therapy because of its superior oral bioavailability. The most common indications for digoxin are atrial fibrillation and atrial flutter with rapid ventricular response; though beta blockers and/or calcium channel blockers are a better first choice. Digoxin is used widely in the treatment of various arrhythmias. (Hallberg *et al.,* 2007).

The leaves have moderate saponin, low phytosterol and benzoic acid but a very insignicant amount of allicin. Saponins had been reported to have broad range of pharmcological properties (Soetan 2008). Allicin has been reported to have an anti-inflammatory, antimicrobial, anti oxidation, anti-thrombotic, ` anti-ulcer, cardioprotective, hypolipidemic, hypotenisve and insecticidal properties (Elilat et al,1995; Elkayam et al 2003). Phytosterol reduces cholesterol levels by competing with cholesterol absorption in the gut of humans (Tilvis and Miethinen 1986)

Carotenoid is another bioactive compound found in *Manniphytum fulvum.* In humans, three carotenoids (beta-carotene, alpha-carotene, and beta-cryptoxanthin) have vitamin A activity (meaning they can be converted to retinal), and these and other carotenoids can also act as antioxidants. In the eye, certain other carotenoids (lutein, astaxanthin and zeaxanthin) apparently act directly to absorb damaging blue and near-ultraviolet light, in order to protect the macula of the retina, the part of the eye with the sharpest vision (Kidd *et al.,* 2011).

CONCLUSION

These results suggest strong nutraceutical potential of this plant and recommend further research in its therapeutic uses in the management and prevention of disease as a result of its rich phytochemical composition.

It is a potential pharmaceutical which will help to alleviate some certain kind of diseases and infections such as cancer, cardiovascular diseases, type 2 diabetics, cough, hypertension, piles, asthma, malaria etc.

Ethical approval and consent: NA

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