Int J Pharm Bio Sci 2015 July; 6(3): (P) 377 – 382

Research Article **Medicinal chemistry**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

PHYTOCHEMICAL ANALYSIS OF THE CRUDE EXTRACTS OF MONODORA MYRISTICA SEED

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ABSTRACT

Monodora myristica is one of the plants used as antimalarial remedies in Ibo-Nigeria folkloric medicine which is claimed to possess antimicrobial properties. It has been reported to possess high antifungal properties which have been confirmed by the authors in preceding preliminary studies. The seed of the plant yields a colorless volatile oil which has a pleasant taste and aroma. As such it is used as a condiment for soup. It is added into snuff as a flavouring agent. The seeds are also used to treat migraine by external application to the fore-head. It is used as a stomachic and by mixing it with palm-oil and also as a stimulant. Pomade made from the pulverized seeds fried in oil as well as the powder is used to treat Guinea worm and other sores. This study was designed to ascertain the phytochemical constituents of Monodora myristica seed. Monodora myristica seeds were sun-dried, milled and extracted by cold maceration with 95% methanol. Aqueous solutions of this methanolic extract were then used to conduct various phytochemical tests. The result showed the presence of glycosides, sterols, triterpenoids, aldehydes and unsaturated compounds. The study concluded that the constituents of Monodora myristica may be triterpenoids existing as steroidal aglycones or glycosides.

KEYWORDS: **Monodora myristica seeds, Preliminary analysis, Phytochemistry, Ibo-Nigeria folkloric medicine.**

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1. INTRODUCTION

The Monodora plant is an ornamental tree with a height of up to 30m high, dense foliage and spreading crown. It flowers from September to April at which time the new leaves appear. The fruits are produced between April and September. They are about 15cm in diameter, green, round, woody and are suspended in a long stalk. The pulp is white and contains numerous seeds of about 2.5cm long. The pharmacognostic profile of Monodora myristica shows that it belongs to the phylum - angiospermae, subphylum – dicotyledon, class – magnolidae, order – magnoliales (annonale), family – annonaceae, genus – monodora and specie – monodora myristica. It grows widely in Cameroon, Nigeria and other West and sub - saharan African countries. It is called 'Efuru' or 'Ehuru' in Ibo - Nigeria, 'Gujiya dan miya' in Hausa - Nigeria and 'Abo lakoshe', 'arigbo' or 'eyi naghose' in Yoruba - Nigeria.[1] Monodora myristica is one of the antimalarial plants in Ibo-Nigeria folkloric medicine with claims of antimicrobial properties. It has been reported to possess high antifungal properties which have been confirmed by the authors in preceding preliminary studies.[2] A related specie M. tenuifolia is used as an anthelmintic and in Yoruba – Nigeria traditional medicine for the treatment of toothache. Some other family members are also used in the treatment of malaria. The pulverized seeds have also been employed as pesticides in the preservation of some agricultural products. It exhibits significant antimicrobial properties especially against fungi and thus can be employed for the treatment of fungal infections. It is also used as a flavouring agent (local spice) in the preparation of the pepper source (ose orji) used for eating Garden egg by Ibo – Nigerians.[3] It is also of value in the prevention of biodegradation of plant agricultural products e.g Okra (Abelmoschus spp) where it has shown significant ability to inhibit the growth of fungi isolated from a deteriorating Okra sample. It has also been associated with anti – protozoal and anti – parasitic activities. The seed oil gave a high yield of saturated and unsaturated fatty acids on saponification, gas – liquid chromatography revealed large amounts of C-16 and C – 18 fatty acids with traces of other fatty acids while the iodine values indicated that both ethanol and hexane extraction produced excellent yields of fatty acids with hexane showing more efficiency as a crystallization solvent at a solvent – oil ratio of 3:1 at 50^{o}C. The essential oil of the seed has also been found to be mainly monoterpenic, the major component being alpha – phellandrene (Sabinene) and Myrcene while the oils from the leaves was mainly sesquiterpenic with the major constituent being beta – caryophylene.

2. EXPERIMENTAL

2.1. MATERIALS

2.1.1. Plant Material

This consisted of the seeds of Monodora myristica. They were collected in September at Nsukka in Enugu state of Nigeria by Mr Paulinus Ugwu and Mr J.E Ekekwe both of Botany department of University of Nigeria, Nsukka. They were then prepared by cutting, sun - drying and milling. The powdered forms were then used in the experiments.

2.1.2. Reagents

Sulphuric acid, Chloroform, Ammonia solution, Ferric chloride, Fehling's solution 1 and 11, Ethylacetate, Hydrochloric acid, Glacial acetic acid, Aluminium chloride, Ethanol, Bromine water, Mayer's reagent, Distilled water, Sodium hydroxide, Tollen's reagent, 2,4 – dinitrophenylhydrazine, Acetic anhydride, acetic acid, silica gel GF_{254}.

2.1.3. Solvents

Methanol, Ethylacetate, Methylethylketone (MEK), MEK / Hexane, Dimethyl sulphoxide (DMSO), Chloroform and Ethanol.

2.1.4. Instrumentation

Uniplan TLC spreader, Chromatographic tank, Aluminium plates, Silica plates, Seperating funnel, Evaporating dish, Rotary evaporator, Water bath, Capillary tubes, Test tubes, Conical

flasks, Measuring cylinders, Beakers, Pipettes, Funnels, Filter papers, Weighing balances, Glass chromatoplates, UV lamp, Bunsen burner and Spatula.

3. METHODS

The extraction was carried out by cold maceration using 95% methanol. The powdered drug was cold macerated with the solvent for 24 hours. The extract was filtered off. The process repeated several times until the constitutions were completely extracted, indicated by the colorlessness of the extraction solvent. The crude extract was then used to carry out the various phytochemical tests.

3.1. PRELIMINARY PHYTOCHEMICAL TESTS ON CRUDE MONODORA MYRISTICA EXTRACT:

3.1.1. TEST FOR ALKALOIDS

0.1g 2ml aqueous solution extract of the methanolic extract was boiled for 2 minutes with 5ml of 2% HCl on a steam bath and the mixture was filtered. 1 ml portions of the filtrate were treated with drops of the following reagents and observed for precipitates.

(a) Wagner's reagent (iodine ion potassium iodide solution)
(b) Picric acid
(c) Dragendorrf's reagent (Bismuth potassium iodide)

3.1.2. TEST FOR GLYCODSIDES (HYDROLYSIS TEST)

0.1g of 2ml of aqueous solution methanolic extract of the powder was boiled with 3ml of dilute sulphuric acid for 15 minutes. The filtrate was neutralized with 20% a sodium hydroxide and about 5ml of equal volumes of fehling's solution A and B mixture was added to the mixture and boiled. This was observed when cold for some brick red precipitate.

3.1.3. TEST FOR TANNINS

2ml methanol extract was diluted with 2ml of distilled water. Few drops of ferric chloride solution were then added and observed for a blue-black precipitate.

3.1.4. TEST FOR FLAVONIODS

0.1g of the extract was extracted with 5ml ethylacetate and filtered.

(i)To 2ml of the filtrate, 1ml of dilute ammonia solution was added, shaken, allowed to separate and the colour of the ammonia layer observed for yellow colouration.

(ii) 2ml of the extract was shaken with 1ml of 1% aluminum chloride solution and the colour in aluminum chloride was observed on standing for a yellow colouration.

3.1.5. TEST FOR SAPONINS (FROTHING TEST)

5ml aqueous solution of the extract was heated on a water bath. 1ml of this was diluted with 4ml of distilled water, shaken vigorously and observed on standing for persistent profuse frothing.

3.1.6. TEST FOR STEROLS AND TRITERPENOIDS

0.1g of the extract was dissolved in 5ml chloroform.

(i)2ml chloroform solution was evaporated to dryness, and re-dissolved with a 5 parts conc H_2SO_4 – 1 part water solution and then observed for a dark green colour. (molechott test).

(ii)2ml chloroform solution was concentrated and about 1ml of conc H_2SO_4 was added and the mixture observed for a brown colour at the interface. (Salkowski's test).

3.1.7. TEST FOR ALDEHYDES (TOLLEN'S REAGENT TEST)

To 2ml methanol extract was added about 1ml of tollen's reagent (prepared by precipitating the Ag from $AgNo_3$ with NaoH and re-dissolution with ammonium solution) and heated for about 20 minutes. This was then observed for the precipitation of silver ions (sliver mirror).

3.1.8. TEST FOR STEROID AGLYCONES (BUTCHARD LIEBERMAN'S TEST)

To 0.5ml of an acetic acid extract of the sample was added 1ml of the chilled colour reagent and observed over a period of 35 minutes for any colour change. The colour reagent was prepared by chilling out 19 parts by volume of

acetic anhydride and mixing with 1 part by volume of conc sulphuric acid.

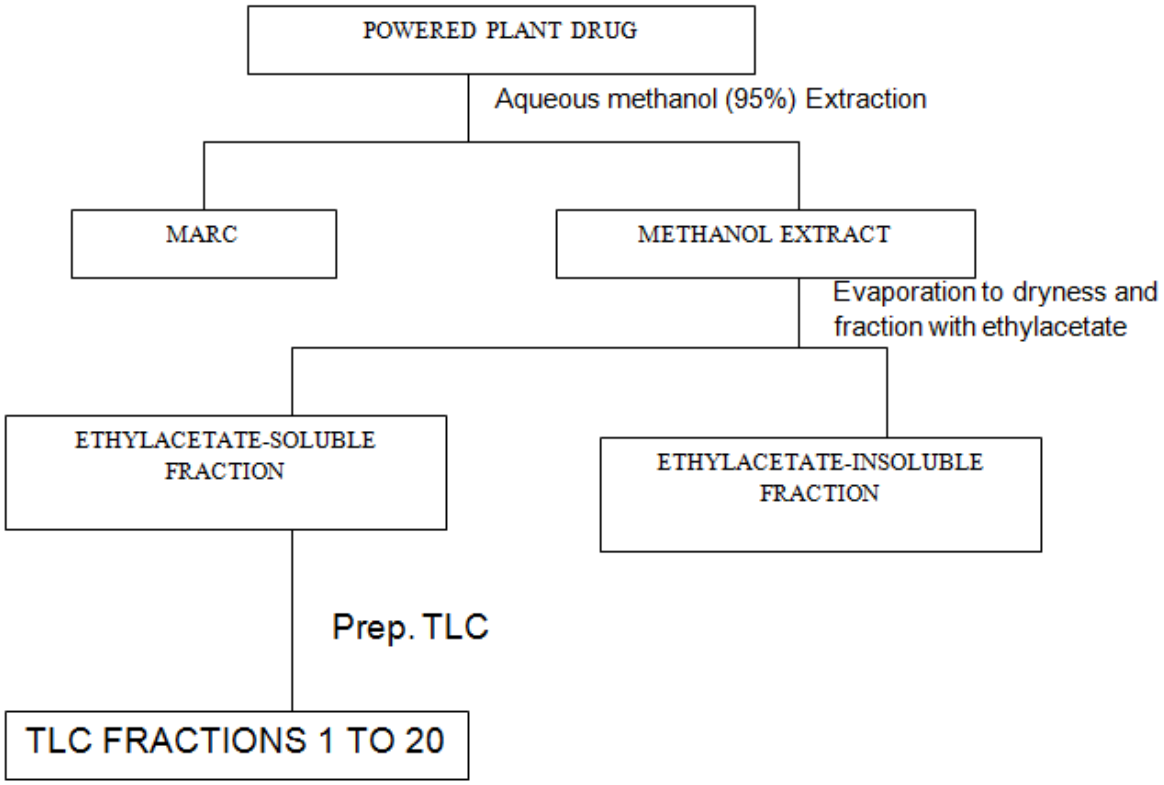
3.1.9. TEST FOR CARBONYL COMPOUNDS (2, 4-DINITROPHENYLHYDRAZINE)

A saturated solution of the reagent was made in 2N Hcl in a test tube. To about 2mls of the aqueous extract of the sample, 1ml of the reagent was added and shaken vigorously. The mixture was then heated to boil. Yellow insoluble adducts indicate the presence of carbonyl compounds.

3.1.10. TEST FOR UNSATURATION (BROMINE WATER TEST)

To about 2ml. aqueous ethanol solution of the sample was added about 2ml of bromine water. This was then observed for bromine water decolourization.

Figure 1
FLOW CHART FOR ISOLATION



4. RESULTS

Table 1
Results of the Phytochemical tests on the crude Monodora myristica extracts.

S/N	PHYTOCHEMICAL CLASS	TEST RESULT
1	ALKALOIDS	Negative
2	**GLYCOSIDES**	**Positive**
3	TANNINS	Negative
4	SAPONINS	Negative
5	FLAVONOIDS	Negative
6	**STEROLS AND TRITERPENOIDS**	**Positive**
7	**ALDEHYDES**	**Positive**
8	STEROIDAL AGLYCONES	Negative
9	CARBONYL COMPOUNDS	Negative
10	**UNSATURATED COMPOUNDS**	**Positive**

5. DISCUSSION

The phytochemistry of the crude extracts showed the presence of glycosides, sterols, triterpenoids, aldehydes and unsaturated compounds. However the test for alkaloids, tannins, saponins, flavonoids, steroidal aglycones and carbonyl compounds gave negative results. Glycosides are a large and varied group of polar compounds occurring mainly in higher plants and also to a small extent in lower forms of plant life. They are characterized by the possession of a glycosidic linkage between one or more sugar moieties and a non-sugar moiety (the aglycone or genin) in the molecule. Though the possession of a sugar unit gives them a natural group identity, they however vary much in their physical, chemical and pharmacological properties due to the varied nature and complexity of their aglycones. Their pharmacological actions include purgation, cardioactivity and antimicrobial activities. Sterols are modified steroids in which the side chain is an aliphatic one containing one or more hydroxyl groups attached in alycyclic linkage. Steroids form a group of structural compounds widely distributed in animals and plants. They include sterols, vitamin D, the bile acids, a number of sex hormones, the adrenal cortex hormones, some carcinogenic hydrocarbons, certain sapogenins etc. Triterpenoids are terpenoids whose carbon skeleton are based on six isoprene units derived from the acyclic C_{30} hydrocarbon, squalene. They are colorless, crystalline, optically active often with high melting point and generally difficult to characterize due to their chemical unreactivity. They occur in waxy coatings of leaves and on fruits such as apple and pear. They serve a protective function in repelling insects and microbes. Large quantities of triterpenes are found in the latex and resins of many plants and they serve as a chemical defense against pathogens and herbivores. They are expected to act against certain human and animal pathogens but for their their hydrophobic nature which limits their therapeutic application. The recent advances in drug solubilization techniques is expected to overcome this problem. The biological activities of triterpenes include anti-tumour anti-inflammatory, antiviral and antibacterial activity. Aldehydes are simple organic compounds which contain a carbonyl group – carbon-oxygen double bond. They are simple in the sense that they don't have other reactive groups like –OH or –Cl directly attached to the carbon atom in the carbonyl group. Eg methanal, ethanol, propanal, 2-methylbutanal.[4] They are formed by partial oxidation of primary alcohols and form carboxylic acids when further oxidized. They are used for the manufacture of synthetic resins like Bakelite, and for making dyestuffs, flavourings, perfumes and other chemicals. Some are used as preservatives and disinfectants.[5] Unsaturated compounds are chemical compounds that contain carbon-carbon double bonds or triple bonds.[6,7] They can also have functional groups. Thus the constituents of M. myristica seed may be triterpenoids existing as steroidal aglycones or glycosides. The authors however are conducting further studies on this plant by separating its constituents and analyzing the antimicrobial and phytochemical characteristics of the isolates.

6. CONCLUSION

The study concluded that the constituents of Monodora myristica may be triterpenoids existing as steroidal aglycones or glycosides.

7. ACKNOWLEDGEMENT

Mr Paulinus Ugwu and Mr J.E Ekekwe of Botany department, University of Nigeria, Nsukka for assisting in the identification and collection of the plant materials.

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