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Phytochemical components of methanolic fruit extract of *Dennettia tripetala*

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Abstract

Therapeutic plants are rich source of bioactive compounds which are greatly useful in combating different human diseases. This present study examines the phytochemical components of the methanolic extract of *Dennettia tripetala* (D. tripetala) (pepper fruit) using standard methods and by means of Gas chromatography-Mass spectrometry (GC-MS) analysis. Important secondary metabolites like terpenoids, tannins, alkaloids, steroids, cyanogenic glycosides, flavonoids and saponins linked with several medicinal attributes and serving as a pointer to the folkloric usage of this fruits plant were identified.

The GC-MS analysis of the fruit oil of *Dennettia tripetala* shows that it is rich in constituents like oleic acid (29.30%), palmitic acid (23.4%), stearic acid (8.02%), estragole (7.42%) and phytol (6.14%).

Keywords: Phytochemicals, GC-MS, *Dennettia tripetala*, Oleic acid and Palmitic acid.

1 Introduction

Dennettia tripetala (pepper fruit) usually found in southern, eastern and western areas of Nigeria belong to the family: Annonaceae, kingdom: plantae, genus: denettia; species: *Dennettia tripetala* and class: magnolidae (1, 2). This plant is not only predominant in Nigeria but also found in western Cameroun and Ivory Coast. It is a native medium-sized evergreen fruit tree with a height of about 12-15 m; it is characterize with a spicy aroma and have several therapeutic benefits.

The fruit of this plant is elliptic in shape composed of seeds and a fragment of hard spicy flesh, they are green in the unripe stage; appear red when ripe and black when dried, the major part of the fruit fit for human consumption is found in the matured fruits. The sharp and pepperish taste of the leaves, root, barks and fruits of this plant is majorly due to a class of compound known as esters made up of complex mixtures found in the components of this plant parts.

The matured fruit of *D. tripetala* are chewed when green, ripened red or black dried to serve as a mild stimulant to the consumer (3). The different parts of this plant are used as seasoning and condiments in Nigeria to prepare food such as soup sausage, pepper soup, local vegetable dishes and used to preserve cooked meat in rural areas where there is no steady electricity to prevent it from decomposing (4).

Traditional healers in most rural parts of Nigeria give their client the concoction made from this plant part blended with other therapeutic plants to treat ailment like convulsion, typhoid, vomiting, stomach upset, cough, fever, infantile, swelling, oedema, diabetes, ulcer and use it as diuretic in the treatment of high blood pressure, this set of people prefer to solve their health problem this way as a result of their poor economic condition in preference to modern synthetic drugs that are a bit expensive to them (3, 5-6).

Pregnant and post natal women from rural settings of Nigeria believes that the leaves when used to prepare pepper soup delicacies and as a condiment in their local dishes or oral ingestion of its decoction induce strong uterine contraction while records have it that *D. tripetala* plant drastically reduce intra ocular pressure in persons with glaucoma (7-9).

Some components like uvariopsine, argentinine, dennettine, a new 2, 6-dimethoxychromene have been isolated and characterizes from the root of this plant (10). 1-nitro-2-phenylethane and linalool have been found to be part of the phytochemical components of this plant (11). Phytochemical components from the ethanolic extract of the fruit of this plant has been traditionally used in Nigeria to combat *Ostrinia nubilalis* growth that drastically have an effect on corn, cottons and some vegetable crops (12).

Oyemitan *et al*. (13) document the antinociceptive and anti-inflammatory potency of the volatile oils of the fruit in mice which tend to justify the ethnomedicinal importance of *D. tripetala* in the treatment of cough, fever and vomiting (3). In addition, the volatile oils derived from the stem bark and leaves have been found to have antimicrobial action against *Staphylococcus aureus* (14).

Herbal medicines obtained from plant extracts serves as an alternative health treatment for several people particularly in developing countries of the world.

Plants are complicated chemical storehouses of concealed biodynamic compounds with untapped potential for use in modern medicine (15). The products obtained from plants have been used as drugs to cure a variety of ailment since

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the dawn of civilization because plant serve as a reservoir of valuable chemical components referred to as phytochemicals.

Plant chemicals known as phytochemicals possess bioactive compounds which are useful in combating various ailments in human. They are classified into primary and secondary metabolites. The most important of these bioactive constituents are alkaloids, tannins, flavonoids, terpenoids and phenolic compounds (16). Alkaloids are good anesthetic, analgesic and antispasmodic agent; they also show some metabolic role and regulate the development in living system (17-19). Flavonoids act as a good antioxidant and help to stall the initiation, promotion and progression of tumors (20-21). Tannins have a broad spectrum of action against pathogenic fungi, viruses and bacteria; they also assist in the healing of wounds and inflamed mucous membrane (22). Steroids helps to reduce the risk of coronary heart and neuro-degenerative diseases in hale and youthful post-menopausal women (23) while terpenoids exhibit significant therapeutic action like anti-inflammatory, anti-cancer, anti-viral, anti-bacteria and anti-malaria potencies (24)

Owing to the significance function of these secondary metabolites mentioned above, preliminary phytochemical screening of plants is the need of the hour in order to discover and develop novel therapeutic agents with improved efficacy.

2 Experimental

The fully matured fruit extract of *D. tripetala* were collected on the 30th of May, 2017 from a farm in Ogugu, Olamaboro L.G.A of Kogi State Nigeria, they were authenticated at the Herbarium of Biological Sciences Department, Kogi State University, Anyigba, Nigeria.

2.1 Extraction Procedure

Thirty grams of the matured fruit was grounded using a mechanical grinder and this was exhaustively extracted in 800 mL of methanol with soxhlet extractor, it was further concentrated using rotary evaporator about 10 mL of the extract was used for the phytochemical screening while the remaining was used for GC-MS determination of the phytochemical constituents.

2.2 Qualitative phytochemical screening of the methanolic extract of *D. tripetala*

Chemical tests for the screening and detection of bioactive chemical constituents in the therapeutic plant of *D. tripetala* were carried out on the methanolic extracts. The technique of Harborne (1998) (25) and Sofowora (1993) (24) were used in the qualitative screening of saponins, alkaloids, tannins, flavonoids, cyanogenic glycosides, steroids and terpenoids. Meyer's tests was used to screen for alkaloids, flavonoid and tannins by ferric chloride test, terpenoid and steroids by salkowski's test, saponin by frothing test and cyanogenic glycosides by Keller killiani's test.

2.3 GC and GC-MS Analysis

The GC and GC-MS analysis of the fruit oil of *D. tripetala* (pepper fruit) was carry out by means of a multi dimensional gas chromatography attached with gas chromatography-mass spectrophotometer, having a non-polar and polar double capillary columns (25.0 m× 0.25 µm i.d., 0.25 µm df). The carrier gas used for the analysis was high purity helium at a constant flow rate of 0.99ml/min. Injection of 1 µL of the methanolic extract was injected (split ratio 100:1) into GC and GCMS using AOC-20i; auto injector for analysis. The original temperature was set at 60 °C, heated at a rate of 3 °C/min to 280 °C and held isothermally for 6minutes. Ion source temperature was regulated to 200 °C while the interface was set at 250 °C; solvent cut time was 3 minutes. Electron impact (EI) ionization mode was 70ev and the column linear speed was set at 36.8 cm/sec (26)

The detection of the different components were achieved based on similarity of their mass spectra with those of Nist Library mass Spectra data base and mass spectra from Literature.

3 Results and Discussion

Table 1: Preliminary qualitative phytochemical analysis of methanolic extract of *D. tripetala*

Name of Phytochemicals	Test
Tannin	+
Cyanogenic glycosides	+
Saponins	+
Alkaloid	+
Flavonoids	+
Terpenoids	+
Steroids	+

Key: (+) = Present

3.1 Phytochemical components of the methanolic extract of *D. tripetala*

From table 1, the phytochemical screening of the methanolic extract of the fruit of *D. tripetala* was carried out in order to analyses the presence of the secondary metabolites such as flavonoids, alkaloids, tannins, steroids terpenoids, cyanogenic glycosides and saponins by utilizing the standard methods. Phytochemicals like Tannin, cyanogenic glycosides, saponins, alkaloid, flavonoids, terpenoids and steroids were present in the methanol extract of this plant. A number of of these classes of phytochemicals had earlier been reported in some of our previous work on *M. aboreus* and *C. sativa* [27, 28]. Among these phytochemical compounds, tannin, saponins, alkaloid, flavonoids, steroids and terpenoids etc are thought to be accountable for the acclaimed exceptional properties exhibited by this pepper fruit plant. Phytochemicals have been formerly documented to

contain some biological and medicinal properties which support the usage of this fruit plant (27-30). Plants rich in terpenoids have been documented to possess anti inflammatory, antimalaria, antiviral, inhibition of cholesterol synthesis and antibacterial activities (Sofowora, 1993), while those rich in alkaloid are used in medicine to reduce headache and fever in addition to their antibacterial and analgesic properties (31).

Table 2: GC-MS Components of the methanolic extract of *D. tripetala*

Name of compounds	Retention Index	Peak area	MF	MW	SI % to TC
1-Decene	1005	0.72	$C_{10}H_{20}$	140	88
Glycerine monoacetate	1091	3.02	$C_5H_{10}O_4$	138	90
Estragole	1172	7.42	$C_{10}H_{12}O$	148	97
Dodecane	1214	0.33	$C_{12}H_{26}$	170	89
Tetrahydrogeranyl acetate	1321	0.98	$C_{13}H_{26}O$	198	90
β-farnesene	1440	1.58	$C_{15}H_{24}$	204	98
(Z,E)-α-farnesene	1458	2.61	$C_{15}H_{24}$	204	99
Cyclohexyl carbinol	1522	0.76	$C_{15}H_{26}O$	222	90
Z- α-Bisabolene epoxide	1531	3.25	$C_{15}H_{24}O$	220	98
Myristyl chloride	1638	1.02	$C_{14}H_{29}Cl$	232	89
Pentadecanoic acid, 14-methyl, methyl ester	1814	0.57	$C_{17}H_{34}O_2$	270	88
Palmitic acid	1908	23.4	$C_{16}H_{32}O_2$	256	99
Phytol	2045	6.14	$C_{20}H_{40}O$	296	97
Methyl petroselinate	2085	0.82	$C_{19}H_{36}O_2$	296	95
Stearic acid	2167	8.02	$C_{18}H_{36}O_2$	284	98
Oleic acid	2175	29.30	$C_{18}H_{34}O_2$	282	99
Cholest-5-ene, 3- methoxy-3β	2545	4.37	$C_{28}H_{48}O$	400	97
Squalene	2914	5.45	$C_{30}H_{50}$	410	98

RI = refraction Index MF = Molecular Formula, MW = Molecular Weight, SI% = Similarity Index, TC = Target Compound.

The results obtained by GC–MS analysis of the methanolic extract of *D. tripetala* are presented in Table 2 above. Eighteen (18) components were identified in the methanolic extract of this fruit plant. The major constituents are oleic acid (29.30 %), palmitic acid (23.4 %), stearic acid (8.02 %), estragole (7.42 %) and phytol (6.14 %).

The key component recorded in the GC-MS analysis of the methanolic extract of the fruit of *D. tripetala* is Oleic acid (29.30 %), it is a widespread monounsaturated fat found in human diet. The oral intake of monounsaturated fat is linked with decreased low-density lipoprotein (LDL) cholesterol, and perhaps increased high-density lipoprotein (HDL) cholesterol (32). Oleic acid a fundamental omega-9 monounsaturated fatty acid is employed as an emulsifying agent, it has also been described as being hypotensive (33) and known to inhibit the progression of adrenoleukodystrophy, a serious disease that affect the brain and adrenal glands (34)

Palmitic acid (23.4 %) which is the second main component of the fruit extract has been shown to demonstrate antioxidant properties and this has help to prevent atherosclerosis in rats (35).This may possibly be accountable for the anti-atherosclerotic properties of the *D. tripetala* fruit pepper.

Stearic acid (8.02 %) being one of the chief constituent of the fruit extract of this plant has been documented in epidemiologic and clinical studies to be linked with lowered LDL cholesterol in contrast with other saturated fatty acids (36). Stearic and Palmitic acid had been shown to exert a neutral or hypocholesterolemic result on blood cholesterol levels in experimental animals (37) and this might be linked to the hypocholesterolemic effect of the fruit of *D. tripetala* on blood cholesterol of human being.

Estragole detected as one of the major constituent of this pepper fruit is a natural organic compound having a methoxy and a propenyl groups attached to its benzene ring, it is a colourless liquid found as a component of

different trees and plants. It is a major constituent of tarragon volatile oil and used in the production of perfumes and as food additives for flavor (38).

The antinociceptive action of phytol in mice which constitute one of the major constituent of *D. tripetala* has been documented and this has been linked to the antioxidant activity of phytol. Phytol a diterpene alcohol is a product of chlorophyll metabolism and they are generally abundant in nature (39). It has been found to inhibit the growth of *Staphylococcus aureus* and block the teratogenic effects of retinol (40-41).

4 Conclusions

Phytochemical screening of *D. tripetala* fruit revealed the presence of valuable biological components like flavonoids, alkaloids, tannins, steroids terpenoids, cyanogenic glycosides and saponins thereby providing knowledge of the rich phytochemical metabolite in this plant. The various identified phytochemicals in this plant of study may be responsible for the pharmacological activities in the fruit of *D. tripetala*.

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