Available online on www.ijppr.com
International Journal of Pharmacognosy and Phytochemical Research 2015; 7(6); 1262-1266

ISSN: 0975-4873

Research Article

Major Compounds from the Essential Oil of the Fruit and Comparative Phytochemical Studies of the Fruits and Leaves of *Dennettia tripetala* Barker F. Found in North Central Nigeria

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Available Online:30th November, 2015

ABSTRACT
Dennettia tripetala is a spicy medicinal plant commonly consumed in Nigeria for its spicy fruit and leaf. In most communities practitioners of herbal medicine prescribe both fruit and leaf of this plant alike. In this study, the fruit essential oil, fruit and leaf of *D. tripetala* found in North central Nigeria were analysed to identify major chemical components of the oil and the secondary metabolites of the fruit and the leaf using standard procedures. GC-MS analysis of the fruit essential oil revealed three major compounds constituting about 95% of the total eighteen (18) constituents of the oil. The compounds were 2-phenyl nitroethane (72.41%), linalool (18.01%) and (6E)-nerolidol (4.51%). Both fruit and leaf tested positive for carbohydrate, tannin, alkaloids, terpenes, flavanoids, and phenol, while sterol and balsams tested positive in only the fruit. Saponin and resin were not detected in the leaf and fruit. The proximate analysis of the leaf showed a moisture content of 12.37%, water extractive value of 16.16% and an alcoholic extractive value of 16.67%. The 2-phenyl nitroethane which constituted the major component for most reported *D. tripetala* fruit oil in literature was also found in the same quantum in this study. Hence it is a as chemical marker for the fruit essential oil. Thus both parts of the plant hold similar and high medicinal or pharmacological potentials.

Keywords: *Dennettia tripetala*, 2-phenyl nitroethane, Linalool, Nerolidol, Chemical marker, Phytochemicals, Fruits.

INTRODUCTION

Pepper fruit botanically known as *Dennettia tripetala* Baker F. and locally called *ako* in Edo, *opipi* in Idoma, *nkarika* in Ibibio, *mmimi* in Igbo and *ata igbere* in Yoruba language, is a well-known medicinal and aromatic tropical African tree common in the southern parts of Nigeria and mostly found in the mangrove forest. The unripe fruit is green, then, turns red or pink on ripening around April and May. The fruits, leaves and roots of the plant posses' strong pepperish and pungent taste. The fruit are mainly chewed raw in different forms (fresh green, fresh ripened, black dry fruit and dry seeds.) because of its pepperish spicy taste which serves as stimulants for the consumers[1,2]. *Dennettia tripetala* tree is medium in size and belongs to the annonaceae family[3]. The leaf is alternate, oblong cociaceous, apex acuminate with a base cumate margin. It exhibits a symposia growth with a chasmogamous flower. The sapels are green, very broad pubescent and are three in numbers. The petals are fleshy light yellow apocarpous fruit[4,5]. The leaves and roots are commonly used by the local herbalist in folk medicine in combination with other medicinal plants to treat various ailment including fever, infantile convulsion, typhoid, worm infestation, vomiting and stomach upset[6]. The leaves are also used in pepper

Figure 1: Fruits of *D. tripetala*

soup delicacies and as condiment in some local dishes for pregnant and postnatal women during which time, the spices and herb aid in uterine contraction[7,8]. *Dennettia tripetala* had been scientifically proven to significantly lower intra ocular pressure in individuals with glaucoma by up to 25% [9]. Elemental analysis, have showed that the fruit contains mainly calcium and iron, and other elements such as magnesium, zinc, manganese and copper. The fruit had been reported to contain minerals, vitamins, oil, flavor, crude, protein fiber, ash, carbohydrate and sulphur[10,11]. Dennettine, a new 2,6-dimethoxychrome, uvariopsine, stephenanthrine, argentine, phenolics and vanillin had been isolated and characterized from the root of *Dennettia*

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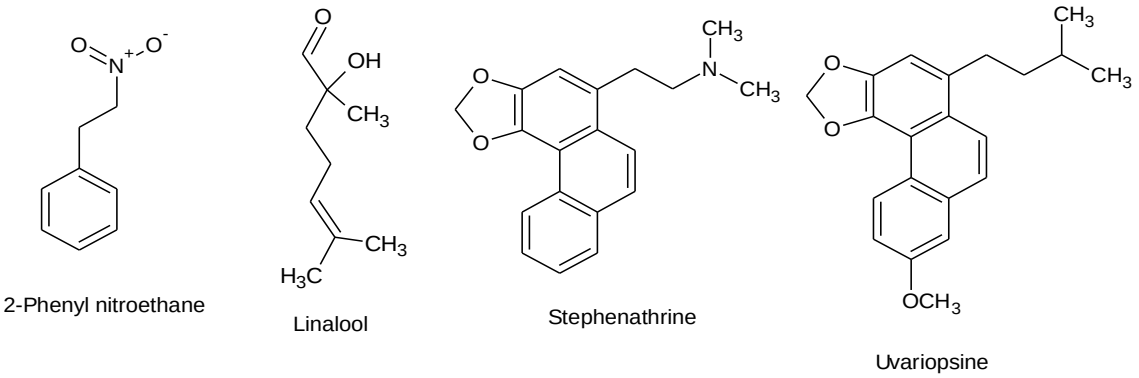
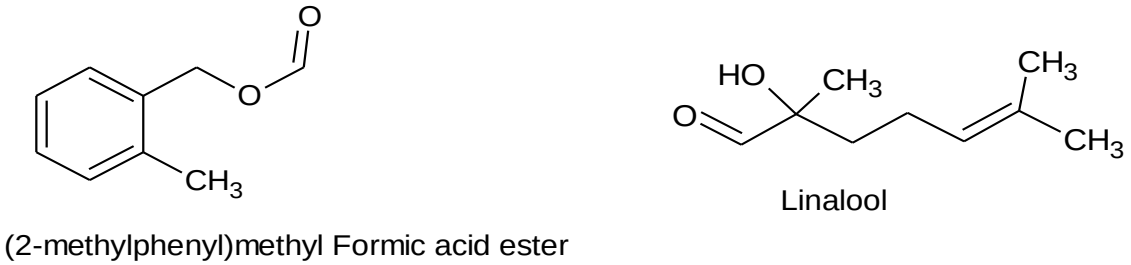


Figure 2: Some reported compounds from *D. tripetala*



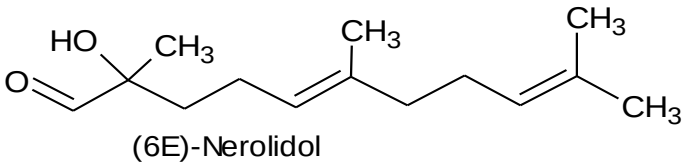


Figure 3: Major compounds of the essential oil of *D. tripetala* fruit

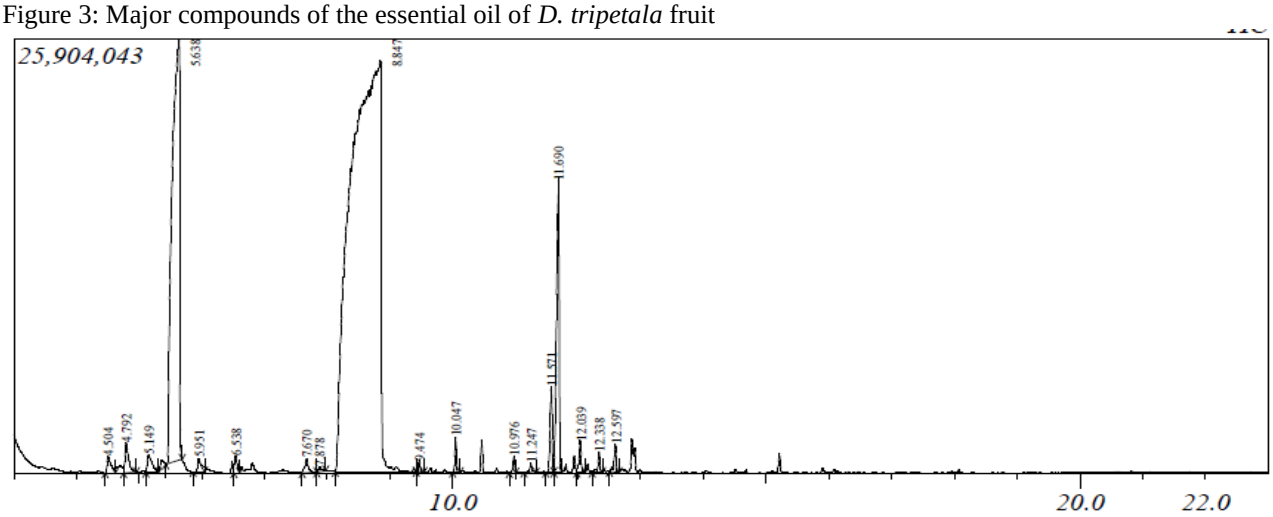


Figure 4: GCMS chromatogram of the essential oil of *D. tripetala* fruit

tripetala[16]. The essential oil of the leaf had been reported to contain 2-phenyl nitroethane as its preponderant component (over 70%), in addition to linalool (17.8%)[12]. The leaf and stem bark oils had been demonstrated to possess antimicrobial activity against staphylococcus aureus[13]. Oyemitan *et al.* reported the antinociceptive and anti-inflammatory activities of the essential oil of the fruit in mice. The results further justified the use of the plant in ethnomedicine for treating fever, cough and vomiting[6]. The ethanolic extract has been used traditionally in Nigeria to combat the growth of *Ostrinia nubiliries* that affects significantly corn, cotton as well as other vegetable crop[14]. Adedayo *et al.* however, reported that the unripe fruit contained significantly more phenol than the ripe fruit and also exhibited better antioxidant activity[15].

The fruit extract had been shown to possess strong antifungal activities against *Saccharomyces cerevisiae, Candida tropicalis, Candida sp, Cryptococcus sp, Geotrichum sp, Rhizopus stolonifer*, *Aspergillus* and *Fusarium sp.*[17]. Elemental analysis showed that *Dennettia tripetala* fruit contains mainly calcium and iron. Others are Magnesium, zinc, manganese and copper. Generally, the

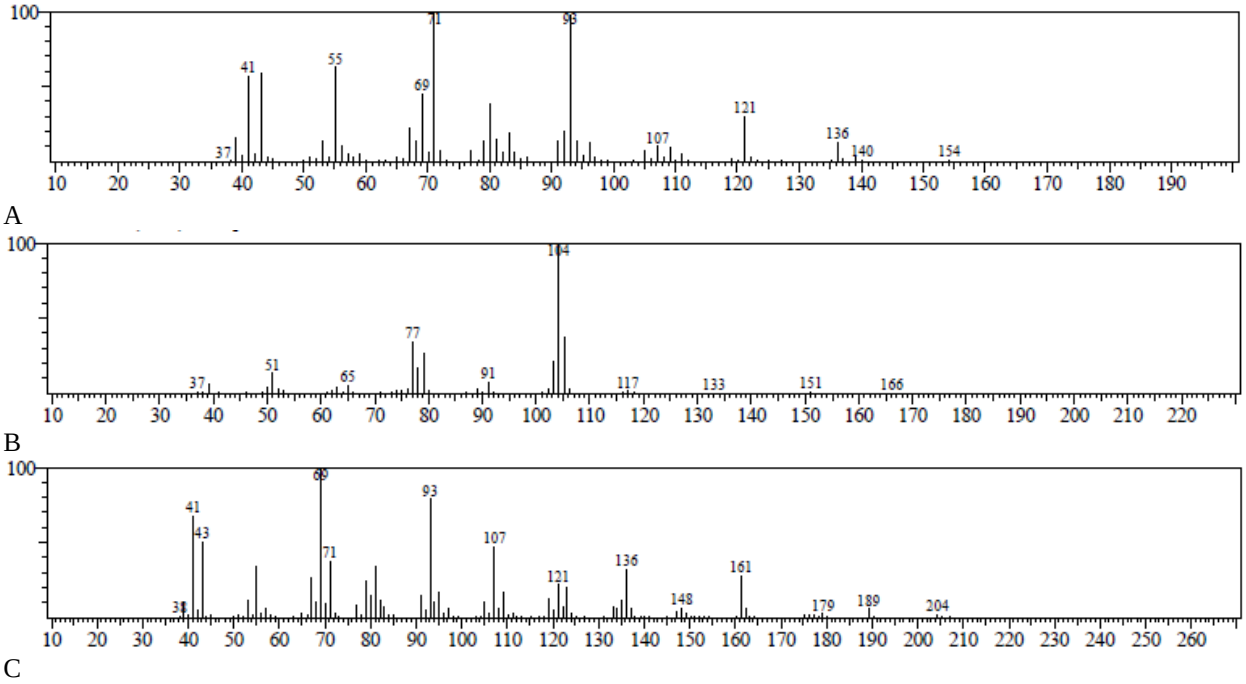


Figure 5: MS profile of linalool (a), 2-phenyl nitroethane (b) and (6E)-nerolidol (c)

fruit contains minerals, vitamins, oils, iron flavours, crude proteins, fibre, ash, carbohydrate, sulphur. Some of the fruit extracts have been shown to be active as antifungal agents against *Saccharomyces cerevisiae Candida tropicalis, Candida Sp., Cryptococcus Sp., Geotrichum Sp., Rhizopus stolonifer, Aspergillus and Fusarium Sp*. It can also serve as insecticide against 3rd instars larvae of *Aedes aegypti* mosquito, bio insecticides for the control of rice weevil *Sitophilus zeamais.* Medicinally, the leaves and fruits of *D. tripetala* are used for cough treatment and enhancing appetite[18]. This study sort to identify the major chemical constituents of the essential oil of the fruit of *D. tripetala* found in North central Nigeria, and also to undertake a comparative study of the secondary metabolites of the leaf and fruit with a view towards establishing their similarities and differences, which may be beneficial in their medicinal or/and industrial applications.

MATERIALS AND METHODS

Plant collection

Fresh leaves and fruits of *dennettia tripetala* were collected on the 23rd of May 2013 from Effekwo village in Otukpa at Ogbadibo local government area of Benue state. They were identified at the Herbarium of NIPRD and allowed to air-dry for ten days after being chopped into smaller piece. The dried material was pulverized with mortar and pestle and kept in clean airtight cellophane until required.

Extraction and GC-MS analysis of the Fruit's Essential oil

The fresh fruit (200g) was chopped into smaller pieces with a sharp knife and hydrodistilled using a Clevenger-type apparatus. The volatile oil obtained was dried with the addition of some grams of sodium sulphate and filtering. The filtrate was subjected to GC-MS analysis on a Shimadzu GC-MS model QP2010 SE (Japan) at the Shimadzu Training Center (STC) for Analytical

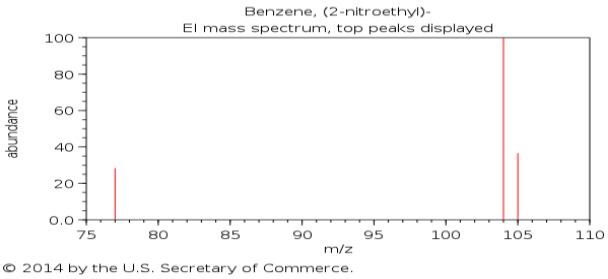


Figure 6: MS spectrum of 2-phenyl nitroethane (*source NCBI*)

Instruments, Lagos, Nigeria. Gas chromatography/mass spectrometry (GC-MS) was equipped with Optima 5MS capillary column of length 30 m, internal diameter 0.25 mm and a film thickness of 0.25µm. The carrier gas was Helium with a flow rate of 6.2 mL/min. The injector mode was split (1.0). The injector temperature was 250 °C and the detector (ion source) temperature was 200 °C. The conditions for analysis were set as follows; column oven temperature was programmed from 60-280°C (temperature at 60°C was raised to 180°C at 10°C/min and held for 2 min, and then finally to 280°C at 15°C/min and held for 4 min). The M/Z was set at 40-600. The constituents of the essential oil were identified by matching their mass spectra with NIST 11 mass spectral library collection.

Phytochemical Screening

Phytochemical analysis to determine the presence of secondary metabolites which include saponins, tannins, resins, alkaloid, glycosides, terpenes, flavonoids and sterols on the crushed dried samples of both leaves and fruits of *Dennettia tripetala* was undertaken using standard methods[19].

Table 1: Results of GCMS analysis of the essential oil of *D. tripetala* fruit

S/N	Retention Time (min)	% Composition (Peak Area)	MW (Da)	Names of identified compounds
1	4.504	0.39	134	o-cymene
2	4.792	0.63	136	cis-β-Ocimene
3	5.149	0.51	170	α-methyl-α-[4-methyl-3-pentenyl]oxiranemethanol
4	5.638	18.01	154	Linalool
5	5.951	0.30	117	α-Cyanotoluene
6	6.538	0.26	170	Epoxylinalol
7	7.670	0.29	156	(E)-5-Decen-1-ol,
8	7.878	0.09	158	1-Decanol
9	8.847	72.41	226	2-phenyl nitroethane
10	9.474	0.09	204	Copaene
11	10.047	0.31	204	Caryophyllene
12	10.976	0.15	204	α-Farnesene
13	11.247	0.12	204	Cadina-1(10),4-diene
14	11.571	0.97	222	[1R-(1α,3α,4β)]-4-trimethyl-3-(1-methylethenyl)-α,α-4-ethenyl-cyclohexanemethanol
15	11.690	4.51	222	(6E)-Nerolidol
16	12.039	0.38	220	Caryophyllene oxide (isomer)
17	12.338	0.24	220	Caryophyllene oxide (isomer)
18	12.597	0.34	222	⅟- Eudesmol

Table 2: Results of phytochemical screening of the Fruits and Leaves of *D. tripetala*

Metabolites	Fruit	Leaf
Carbohydrate	+	+
Tannins	+	+
Saponins	_	_
Alkaloids	+	+
Sterols	+	_
Terpenes	+	+
Flavonoids	+	+
Balsams	+	_
Resin	_	_
Phenol	+	+

Key: + = detected; - = not detected

Pharmacognostic analysis

The moisture content, water extractive value and alcohol extractive value of the dried leaf material of *Dennettia tripetala* were investigated using standard procedure[19].

RESULTS AND DISCUSSION

The yield of essential oil of the fruit of *D. tripetala* was 0.002% v/w. The results of GC-MS analysis (Table 1) revealed three major compounds constituting about 95% of the total eighteen (18) constituents of the essential oil. The compounds were 2-phenyl nitroethane (72.41%), linalool (18.01%) and (6E)-nerolidol (4.51%) (Figure 3). The GC-MS chromatogram and the MS of the three major compounds are shown in Figure 4 and 5, respectively. The 2-phenyl nitroethane was identified after thorough comparison with the NIST enrty number 413703 (Figure 6) and not (2-methylphenyl)methyl formic acid ester as predicted by the GC-MS NIST 11 library[20] The profile of identified major compounds is comparable to that obtained by Adeoti *et al.*, which reported 70% 2-phenyl nitroethane[12]. (6E)-nerolidol found to be 4.51% in this study is a structural analogue of linalool which was 18.01% and corresponded with 17.8% reported by Adeoti *et al.*[12]. Adjalian *et al.* (2014) reported 52.6% and 26.85 for 2-phenyl nitroethane and linalool respectively[21]. The results obtained from the phytochemical screening of both fruit and leaves of *Dennettia tripetala* is as showed in Table 2. The fruit tested positive to carbohydrate, tannins, alkaloids, sterols, terpenes, flavonoids, balsams and phenols while saponins and resin were not detected. The leaf showed similar profile to the fruit except that sterols and balsams were also not detected. The difference in the phytochemical profile may suggest differences in biological activity or at least in the potency of activity. However, the presence of tannins, alkaloids, flavonoids and terpenes suggest that the fruits and leave could possess beneficial pharmacological activity such as antioxidant, anti-allergic, anti-inflammatory and antimicrobial activity for which most compounds belonging to these classes of secondary metabolites had been known to exhibit good pharmacological activities[22]. The pharmacognostic analysis of the leaf gave a moisture content value of 12.37 % which was within the recommended range of 8-14% for vegetable drug. The water and alcohol extractive value were 16.16 % and 16.67 %, respectively, suggesting that both solvents would be good extraction solvent for the leaf material.

CONCLUSION

The fruit oil of *Dennettia tripetala* from in North central was found to be rich in 2-phenyl nitroethane, linalool and (6E)-nerolidol. The chemical profile of the essential oil of the fruit suggests that 2-nitroethyl benzene remains the major constituent of *D. tripetala* grown in most regions of the world. The compound may be responsible for most of the biological actions of the essential oil, and could be used

as a chemical marker for identification and authentication of the fruit sample. The leaf and fruit were also found to possess secondary metabolites like carbohydrate, tannin, alkaloids, terpenes, flavanoids, and phenol. The similar profile of the metabolites suggested that both part could possibly be used for the same ethnomedical purpose. It is no wonder that animals are known to eat both fruits and leaves alike. The moisture content of the leaf suggested that it could be stores dried for a reasonable period without microbial attack. It is recommended that further work be done to determine and quantify the major chemical compound in the plant, which may serve as alternative natural source for such biomolecules.

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