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Phytochemical profiling and antioxidant activities of *Monodora myristica* and *Dennettia tripetala* against lipid peroxidation in rat heart

Kayode Olayele Karigidi[1], Emmanuel Sina Akintimehin[1], Damilola Alex Omoboyowa[2] and Foluso Olutope Adetuyi[1]

1 Department of Chemical Sciences, Olusegun Agagu University of Science and Technology, Okitipupa, Ondo state
2 Department of Biochemistry, Adekunle Ajasin University, Akungba Akoko, Ondo state

ABSTRACT

This study was carried out to investigate the phytochemical profiling and *in vitro* antioxidant activities of methanol extract of *Monodora myristica* (MM) and *Dennettia tripetala* (DT) against lipid peroxidation in rat's heart. The antioxidant activities and lipid peroxidation inhibition were evaluated with spectrophotometric methods. Thereafter, the extracts were profiled using high performance liquid chromatography (HPLC) method. The results showed that MM and DT possessed polyphenol content which culminated in antioxidant activities. However, MM exhibited significantly higher ($p < 0.05$) antioxidant activities than DT. Also both extracts were able to inhibit lipid peroxidation in rat's heart *in vitro*. The extracts profiling showed the abundance presence of myristyl chloride, linalool and nerolidal in MM extract and elemicin, myristicin, eugenol and pinene in DT extract. Therefore, it can be concluded that MM and DT may be used against oxidant related diseases of the heart. Also, there might be need to isolate these compounds and evaluate them for probable drug lead

Key words: Antioxidant, *Dennettia tripetala,* lipid peroxidation, *Monodora myristica*, HPLC

Corresponding author:
Department of Chemical Sciences,
Olusegun Agagu University of Science and Technology, Okitipupa, Ondo state.
Email: ko.karigidi@osustech.edu.ng
ORCIDs:
Kayode Karigidi: 0000-0002-5394-430X
Emmanuel Akintimehin: 0000-0003-0332-1208
Damilola Omoboyowa: 0000-0002-1740-9764
Foluso Adetuyi: https: 0000-0001-9162-6188
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INTRODUCTION

The contribution of free radicals in the aetiology of cardiovascular diseases such as hypertension and myocardial infarction has been well established [1,2]. These diseases are mediated via reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase–driven production of reactive oxygen species (ROS) [2]. Atherosclerosis, the hardening of arteries under oxidative stress is a chronic process initiated from the deposition of oxidized low density lipoprotein (LDL) underneath the artery wall. The polyunsaturated fatty acids in LDL are the primary target of this free radical-induced lipid peroxidation in the artery [3,4]. Recent studies have highlighted lipid peroxidation products (oxidized phospholipids) as biomarker and therapeutic target in cardiovascular diseases[5]. The elevation in free radicals generation which cause an increase in the lipid peroxidation can be averted or minimized by antioxidants which protect the body against free radicals induced damage [6, 7]. However, plants have been shown as natural reservoir of many phytochemicals with vast antioxidant properties [8, 9].

Monodora myristica Dunal (MM) is a member of Annonaceae, popularly known as African nutmeg. It is one of the most important trees in the evergreen forest of southern Nigeria as almost every part has economic importance but the seed is the most economically important part because of its spicy aroma [10]. Traditionally, the seed is usually used as condiment to prepare pepper soup, to treat constipation and to control intra-uterine bleeding in women immediately after child birth [11]. Apart from this, it is use in the treatment of hypertension and diabetes mellitus [12, 13]. Previously, different extracts of MM have been shown to possess numerous phytochemicals and antioxidant potentials [14, 15].

Dennettia tripetala G. Baker (DT), also a member of the family Annonaceae is popularly called Pepperfruit because of its spicy taste. It is well eaten among the people of southern Nigeria not only for its spicy taste but it is believed to increase alertness in the community and also medicinal [16,17]. Folklorically, it is used in the prevention and management of sore throat, cough, nausea, hypertension, diabetes and as purgative [16, 17]. Like MM seeds, the seeds of DT are important in the diet of postpartum women because it is believed to aid contraction [18]. Previously, Omage et al (2018) have reported the presence of phytochemicals like phenols, flavonoids, saponins, tannins, alkaloids in extracts of DT fruits [16].

Despite the wide traditional uses of these plant products for treatment and management of diseases especially hypertension, there is paucity of information on their mechanisms of action in heart-related diseases. Therefore, this study is designed to investigate the *in vitro* antioxidant activity of *Monodora myristica* and *Dennettia tripetala* against lipid peroxidation in rat's heart.

METHODOLOGY

Plant materials

Seeds of *Monodora myristica* (African nutmeg) and *Dennettia tripetala* (Pepper fruit) used in the study were purchased from the Okitipupa local market, Ondo State, Nigeria. Identification and authentication were carried out at the Herbarium of the Department of Biological Sciences, Olusegun Agagu University of Science and Technology, Okitipupa, Ondo State, Nigeria. The herbarium numbers (OSUSTECH/568 and OSUSTECH/560) respectively were deposited in the herbarium. Seeds of African *Monodora myristica* and *Dennettia tripetala* were dehulled, shade dried at ambient temperature and pulverized into powdery form using a laboratory blender.

Preparation of *Monodora myristica* and *Dennettia tripetala* extracts

One hundred gram of powdered *Monodora myristica* and *Dennettia tripetala* seeds were soaked separately in 2 L of methanol for 72 hours. Thereafter the extracts were filtered using clean cheese cloth and concentrated with rotary evaporator. The percentage yield of MM and DT concentrated extracts were 15.66 % and 13.40 % respectively and stored in the freezer until use[19, 20].

Polyphenols content

The total phenolic content (TP) of the extracts was evaluated by the Folin-Ciocalteu phenol reagent method of Kim et al (2003). The TP was calculated from gallic acid calibration curve and expressed as mg per 100g gallic acid equivalent (mg GAE/100g) [21].

The Total flavonoid content (TF) of the extracts was evaluated using the method of Park et al (2008). The flavonoid content was calculated from quercetin standard curve and expressed as mg per 100g quercetin equivalent (mg QUE/100g) [22].

In vitro antioxidant assays

The total antioxidant capacity (TAC) of the extracts was determined using the phosphomolybdate method of Prieto et al (1999). The total antioxidant capacity was calculated from ascorbic acid standard curve and expressed as mg per 100g ascorbic acid equivalent (mg AAE/100g) [23].

The 2,2-Diphenyl-1-picrylhydrazyl scavenging activity (DPPH) activity of extracts was determined by the method of Gyamfi et al (1999) and expressed as percentage inhibition[24].

The reducing power (RP) of extracts was evaluated using the method of Oyaizu (1986). The reducing power was calculated from ascorbic acid standard curve and expressed as mg per 100g ascorbic acid equivalent (mg AAE/100g) [25].

The 2, 2-azobis-3-ethylbenzothiazoline-6-sulfonate radical scavenging ability (ABTS) of the extracts was evaluated using the method of Re et al (1999) [26]. Trolox was used as standard and trolox equivalent was subsequently calculated as mg TEAC/100g

The Ferric reducing antioxidant power (FRAP) of the extracts was evaluated using the method of Benzie and Strain (1996). The FRAP was evaluated from ferrous sulfate calibration curve and expressed as mg Fe^{2+}/100g [27].

Nitric oxide scavenging ability of the extracts was determined by the method of Modal et al (2006) and expressed as percentage inhibition [28].

Experimental animals

Six healthy male Wistar rats (120-140) g were obtained from the Department of Physiology animal holding facility, University of Ibadan. The animals were acclimatized for 2 weeks before used for the experiment. They were given standard pellet diet and water *ad libitum*.

Statement of ethics

The experimental protocols were conducted according to the guidelines of National Institute of Health on the handling and use of laboratory animals (NIH Publication No. 80-23) revised in 2011 [29]. This protocol was approved by the Research Ethics Committee of Olusegun Agagu University of Science and Technology (OAUSTECH/ETHC-BCH/2020/01)

Lipid peroxidation (LPO)

The heart homogenate was prepared according to the method described by Akinyemi et al (2013). The experimental rats were anesthetized and decapitated using sodium pentobarbitone. The heart tissue was removed and weighed on ice. The tissue was homogenized with cold normal saline (1:4 w/v) on ice. The homogenate was centrifuged at 3,000 rpm for 10 min and the supernatant was used for determination of lipid peroxidation[30].

The Lipid peroxidation inhibition capacity of the extracts was determined using the method of Ohkawa et al (1973) and expressed as percentage inhibition [31].

Phytochemical profiling by HPLC-DAD

The phytochemical profiling was done using the method [32]. Twenty grammes of

samples were extracted with 15 ml acetonitrile and stabilized with ethyl acetate and made up to 25ml; filtered through 0.45 mm membrane filter and then degassed by ultrasonic bath prior to use. Stock solutions of standards were prepared in the HPLC mobile phase in the concentration range of 0.030–0.500 mg/ml. The flow rate was maintained at 500 µl/min. Before injecting the sample, standard (cayophyllene, phytol, myristyl chloride, linalool, nerodial, copaene, squalene, estragole, camphene, elemicin, cineol, sabinene, myristicin, eugenol, pinene, methyleugenol, limonene and terpene) form of analytes were first injected to generated a chromatogram of given peak area and peak profile that was used to create a window in the HPLC for the test sample analysis. Briefly, an aliquot (5 µl) of the extracted sample (0.8mg/ml) was injected into the HPLC to obtain a corresponding peak area and peak profile. Identification of the compound was achieved by comparing the peak area of the sample to the registered standard spectra. The concentration of the sample was calculated using the formula:

Concentration= {Peak area of the analyte in sample × Analyte (standard)}/ Peak area of Standard

Statistical analysis

Results are presented as the mean ± SD of three measurements. The results were analysed using analysis of variance (ANOVA) and significance was established with least significant difference (LSD) post hoc treatment at $p < 0.05$. Correlation was calculated using Pearson correlation test and significance was determined at $p < 0.05$

RESULTS AND DISCUSSION

The results of total phenolic (TP), total flavonoid (TF), total antioxidant capacity (TAC), ferric reducing antioxidant power (FRAP), 2, 2-azobis-3-ethylbenzothiazoline-6-sulfonate (ABTS) and reducing power (RP) are presented in Table 1. Total phenolic is one of the secondary metabolites vastly found in plants. They are synthesized from tyrosine and phenylalanine and they exhibit different biological activities such as antioxidant, anti-inflammation and antimicrobial [33]. In this study, the TP of MM (25.97 mg GAE/100g) was significantly higher ($p < 0.05$) than that of DT 14.23 mg (GAE/100g). Some studies have reported a strong positive correlation between total phenolics and scavenging capacities [34, 35]. The TP of our extracts was higher when compared with the result reported for different varieties of spice (*Curcuma longa*), which ranged from 4.52-16.07 mg GAE/100g [36]. Flavonoids are one of the polyphenols widely found in human diets with many antioxidants and health benefits

[37]. Also like the total phenolics, MM (14.41 mg QUE/100g) possessed significantly higher ($p < 0.05$) flavonoid content than DT (10.73 mg QUE/100g). The flavonoid content of both samples ranked well when compared with flavonoid content of other spicy plants reported by Do et al., (2014) [38]. The contents of TP and TF in this study were higher than the ones reported for the leaves and bark of MM by Moukette et al (2015) [39]

Table 1. Polyphenols and antioxidant capacity of methanol extracts of MM and DT

	MM	DT
TP (mg GAE/100g)	25.97± 1.13[a]	14.23± 0.91[b]
TF (mg QUE/100g)	14.41 ± 0.32[a]	10.73± 0.47[b]
TAC (mg AAE/100g)	90.67 ± 6.79[a]	75.60 ± 3.92[b]
FRAP (mg Fe^{2+}E/100g)	18.03 ± 1.04[a]	16.59 ± 1.52[a]
ABTS (mg TEAC/100g)	1.09 ± 0.02[a]	1.01 ± 0.03[a]
RP (mg AAE/100g)	14.80± 1.71[a]	13.18 ± 1.22[a]

Data were presented as Mean ± SD. Values with same alphabet across the row are not significantly different ($p < 0.05$). TP: Total phenolics, TF: Total flavonoid, TAC: Total antioxidant capacity, FRAP: Ferric reducing antioxidant potential, ABTS: 2, 2-azobis-3-ethylbenzothiazoline-6-sulfonate, RP: Reducing power

Antioxidants are substances that scavenge or slow down the activities of free radicals thereby inhibiting oxidative mechanisms that lead to chronic and degenerative diseases in the body [40]. Antioxidant ability assays can be divided into two; hydrogen atom transfer (HAT) and single electron transfer (SET) assays. Most of the HAT assays are kinetics based and involve a competitive reaction scheme while SET assays measure the ability of an antioxidant to reduce an oxidant with corresponding colour change when reduced [40,7]. Examples of SET assay include DPPH, FRAP, TAC, RP ABTS, NO and LPO. Many SET assays were employed in this study because of their wide uses, accuracy and precision in *invitro* studies. In the present study, MM exhibited higher antioxidant activities in all antioxidant models than DT, though, the differences were not significant ($p < 0.05$) in some models (FRAP, RP and ABTS).

The result for DPPH assay is presented in Figure 1, a concentration-dependent relationship was found in the DPPH scavenging ability of MM and DT. Their IC_{50} (Table 2) showed that MM (0.34 mg/ml) had a significantly ($p < 0.05$) lowered IC_{50} than DT (0.55 mg/ml), which is an indication of higher DPPH scavenging ability of MM. The higher ability of MM corroborated with the results of total phenolic and total flavonoid as many studies have reported strong posi-

tive correlation between these polyphenolic compounds and DPPH scavenging effect [42, 43, 44, 45, 46]. The IC_{50} reported for MM and DT in this is lower than the one reported for aqueous and ethanol extracts of DT by Josiah et al., (2016) [47].

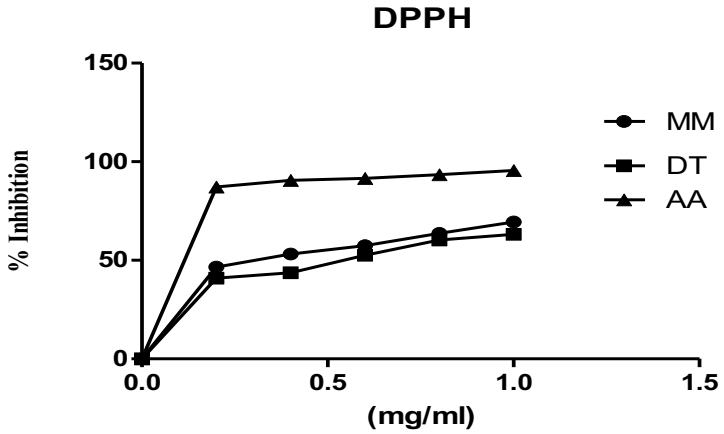

Figure 1. DPPH scavenging activity of methanol extracts of MM and DT

Table 2. IC_{50} (mg/ml) of DPPH, NO and LPO

	MM	DT	AA
DPPH	0.34 ± 0.02[a]	0.55 ± 0.03[b]	0.13 ± 0.01[c]
NO	0.55 ± 0.04[a]	1.01 ± 0.08[b]	0.18 ± 0.01[c]
LPO	0.78 ± 0.02[a]	1.69 ± 0.14[b]	0.11 ± 0.01[c]

Data were presented as Mean ± SD. Values with same alphabet across the row are not significantly different ($p < 0.05$). DPPH: 2,2-diphenyl-1-picrylhydrazyl scavenging activity, NO: Nitric oxide scavenging ability, LPO: Lipid peroxidation inhibition. AA= Ascorbic acid

Nitric oxide radical and lipid peroxidation have been two of the prominent sources of free radicals in cardiovascular diseases causing atherosclerosis [48]. Nitric oxide (NO•) is a reactive radical that has been implicated in many physiological processes which include neurotransmission, blood pressure regulation, immune regulation, defence mechanisms, and smooth muscle relaxation [48]. However, overproduction of this reactive species leads to a condition known as nitrosative stress (RNS) in the body [49]. The result of NO-radical scavenging ability is presented in Figure 2. The extracts of DT and MM were able to inhibit (dose dependent) the generation of nitric oxide at physiological pH from sodium nitroprusside (SNP). The IC_{50} of MM (0.55 mg/ml) was significantly ($P < 0.05$) lower than that of DT (1.01 mg/ml). The IC_{50} of MM was lower while DT was higher than the one reported for aqueous extract of DT[46]. The capacity

of these extracts to inhibit the generation of this radical might be due to their antioxidant activities. This study is in agreement with the studies of some researchers that reported extract(s) rich in phenolics and flavonoid to possess NO scavenging ability [50, 51]. Also, correlation analysis (Table 5) showed a positive relationship between polyphenol and NO scavenging ability.

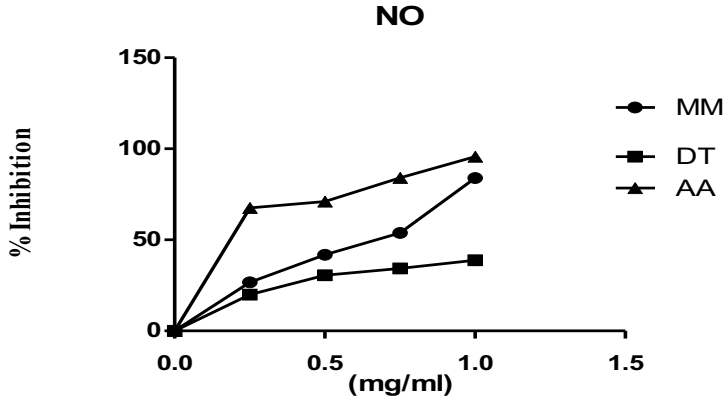

Figure 2. NO scavenging ability of methanol extracts of MM and DT

In this study, the lipid peroxidation on rat's heart homogenate was initiated by the pro-oxidant effect of transition metal, which produced lipid peroxides by stimulation of the oxidative machinery (OH.) through haber-weiss reaction [52]. The extent of lipid peroxidation is determined by the thiobarbituric acid reactive species (TBARS) formed. In this study, incubation of rat's heart homogenate with ferrous sulphate led to increased production of TBARS in heart homogenate. However, MM and DT were able to significantly ($p < 0.05$) inhibit the production of TBARS in a dose dependent manner but inhibition was significantly higher in MM than DT (Figure 3). The IC_{50} of MM (0.78 mg/ml) is significantly ($p < 0.05$) lowered than that of DT (1.69 mg/ml). The capacity of the extracts to inhibit the generation of lipid peroxides could be due to their phenolic contents as correlation analysis (Table 5) showed a significant ($p < 0.05$) positive relationship ($r=0.95, 0.94$) between them. The IC_{50} obtained in this study is higher when compare with the one reported by Assadpour et al., (2016) for *Allium rotundum L* [53]. This study corroborated the previous study of Oyetayo and Ojo (2017), where DT seeds inhibited lipid peroxidation in liver and brain homogenates [54].

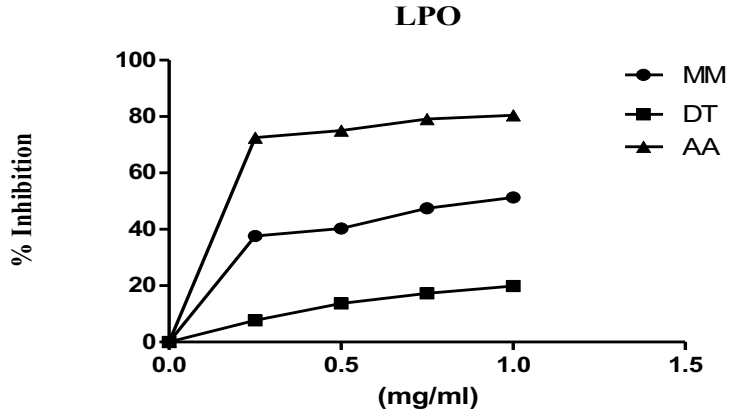

Figure 3. Lipid peroxidation inhibitory activity of methanol extracts of MM and DT

Table 5: Correlation among the polyphenols, antioxidant abilities and lipid peroxidation in rat's heart

	TP	TF	TAC	DPPH	RP	ABTS	FRAP	NO	LPO
TP	1								
TF	0.99*	1							
TAC	0.92*	0.91*	1						
DPPH	0.66	0.62	0.48	1					
RP	0.51	0.56	0.26	0.06	1				
ABTS	0.82*	0.84*	0.56	0.36	0.73	1			
FRAP	0.66	0.69	0.78	0.07	0.24	0.49	1		
NO	0.78	0.78	0.77	0.86*	0.09	0.56	0.42	1	
LPO	0.95*	0.94*	0.84*	0.76	0.37	0.80	0.50	0.90*	1

Significant is established at ($p < 0.05$). TP: Total phenolics, TF: Total flavonoid, TAC: Total antioxidant capacity, DPPH: 2,2 diphenyl-1-picrylhydrazyl scavenging activity, RP: Reducing power, ABTS: 2, 2-azobis-3-ethylbenzothiazoline-6-sulfonate scavenging activity, FRAP: Ferric reducing antioxidant potential, NO= Nitric oxide scavenging ability, LPO= Lipid peroxidation inhibition

The extracts profiling by HPLC (Figures 4 and 5) showed ample presence of myristyl chloride, linalool and nerolidal in MM (Table 3) and elemicin, myristicin, eugenol and pinene in DT (Table 4). The ability to inhibit lipid peroxidation by these extracts might not be unconnected to the presence of these compounds; linalool has be shown to reduce lipid peroxidation in uremia induced vascular calcification and acrylamide-induced neurotoxicity in rats [55,56], Pinene has also reduced lipid peroxidation in the brain of rats following induced focal ischaemic stroke in rat[57]. Also, myristicin has been proven to protect against ulcerative colitis induced by acetic acid in mice by mitigating lipid peroxidation[58]. Also, there might need in future to isolate these compounds from the extracts (MM and DT) and evaluate them for probable drug lead. Conclusively,

the present study has shown that MM and DT has considerable polyphenolic content which culminated into antioxidant activity and ability to inhibit nitric oxide radical and lipid peroxidation in rat's heart homogenate *invitro*. Therefore, the extracts can serve as natural antioxidants against free radical-induced cardiovascular injury.

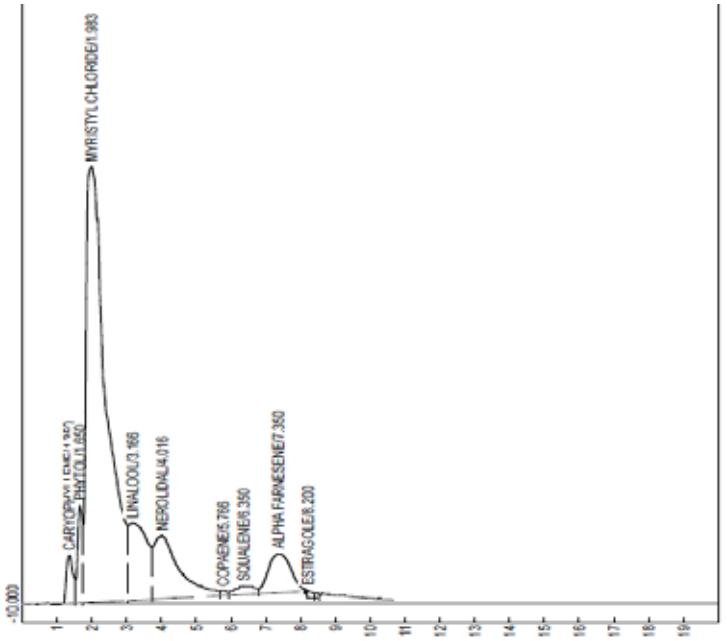

Figure 4. Chromatogram of MM using HPLC

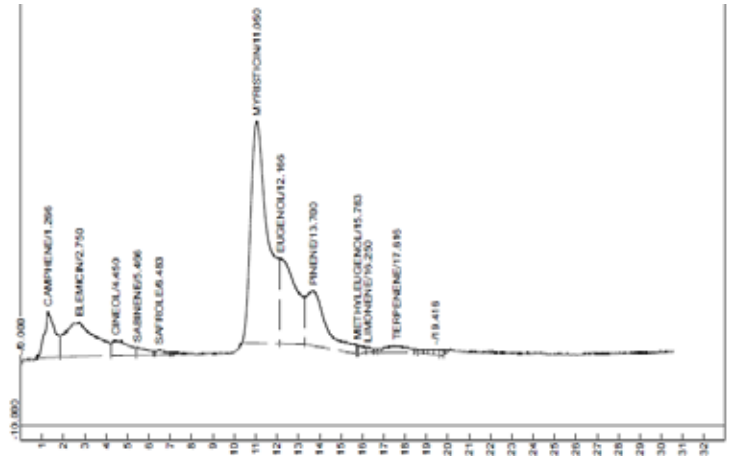

Figure 5. Chromatogram of DT using HPLC

Table 3. Phytochemicals found in MM using HPLC

	Compound	mg/g
1	Caryophyllene	0.04 ±0.00
2	Phytol	0.06 ±0.01
3	Myristyl chloride	1.12 ±0.05
4	Linalool	0.18 ±0.01
5	Nerolidal	0.21 ±0.02
6	Copaene	0.01 ±0.00
7	Squalene	0.02 ±0.00
8	Alpha farnesene	0.11 ±0.01
9	Estragole	0.01 ±0.00

Values were presented as Mean ± SD.

Table 4. Phytochemicals found in DT using HPLC

	Compound	mg/g
1	Camphene	0.10 ±0.01
2	Elemicin	0.22 ±0.04
3	Cineol	0.05 ±0.00
4	Sabinene	0.02 ±0.00
5	Safrole	0.01 ±0.00
6	Myristicin	0.78 ±0.05
7	Eugenol	0.30 ±0.03
8	Pinene	0.23 ±0.03
9	Methyl Eugenol	0.01 ±0.00
10	Limonene	0.01 ±0.00
11	Terpenene	0.03 ±0.00

Values were presented as Mean ± SD.

CONFLICT OF INTEREST

The authors declare there is no conflict of interest

AUTHOR CONTRIBUTIONS

KOK and FOA designed the study; KOK, ESA and DAO accumulated the data; KOK, ESA, DAO and FOA drafted the manuscript.

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