INHIBITORY EFFECTS OF UNRIPE, RIPE PEELS AND SEED VOLATILE OILS OF DENNETTIA TRIPETALA ON SOME PATHOGENS AND FREE RADICALS

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ABSTRACT

Dennettia tripetala is used traditionally as a spice, wound healing, and as an antidepressant agent in Nigeria. We studied *D. tripetala* unripe, ripe fruit peels and seeds volatile oils (VOs) inhibition effects on DPPH, different oxidative radicals (DORs) and pathogens. The VO potency to inhibit DORs and bacterial growth was investigated by spectrophotometric and micro-dilution methods respectively. The IC_{50} value of the ripe seed and unripe peel was 1.03 and 2.24mg/mL respectively in scavenging DPPH radical.

The MIC against *Listeria ivanovii*, *Mycobacterium smegmatis*, *Staphylococcus aureus*, *Enterobacter cloacae*, and *Escherichia coli* ranged from3.25 - 0.10 mg/mL. The unripe seed was bactericidal against *L. ivanovii* at 0.20mg/mL. GC/MS revealed methyl phenyl acetate (MPA) and linoleic acid (LA) as the valuable compounds in the seeds lacking in the peels. This study indicates that besides the local uses of the plant, the seed VO has noteworthy bioactive compounds e.g. MPA, LA and may be good candidates in the hunt for lead phytochemicals for the synthesis of potent antibiotics in this era of emerging multi-drug resistant pathogens.

KEYWORDS: D*ennettia tripetala,* volatile oil, radical inhibitor, antibacterial

INTRODUCTION

Antibacterial resistance has been listed as one of the top three threats to global public health[1]. Bacterial strains including *Escherichia coli*, vancomycin-resistant *Enterococcus faecalis,Vibrio* species andmethicillin-resistant *Staphylococcus aureus* (MRSA) are known public threats in the hospitals[2].They have become more resistant because of natural selection processes, and over-prescription and misuse of antibiotics[3]. Therefore,the rise of multi-drugresistant microorganism strains has ensued,making treatment of pathogens that were previously susceptible to these antibiotics increasingly difficult to treat.A new potential therapeutic agent is needed, which will be unrelated to existing classes of antibiotics, to alleviate problems with cross-resistance and co-resistance of microbes [4].

In a biochemical process, endogenous antioxidants, comprising glutathione peroxidase, superoxide dismutase as well as catalase, inhibits radicals. However, most often, radicals such as hydroxyl (HO), lipid peroxyl (LP), nitric oxide (NO) and superoxide (O_2) produced from metabolism and ecological activities overpowers these naturally-formed antioxidants. Consequently, oxidative stress-related diseases (OSD), such as cancers, arthritis, and cardiovascular, several more diseases have been on the increase globally[5]. In the last three decades, the use of radical scavenger (antioxidant), as a food and cosmetics flavour as well as a preservative has considerably reduced the occurrence of OSD[6]. However, studies have shown side effects in some of the over the counter (OTC) antioxidants such as butylated hydroxyl toluene (BHT), β-carotene, among others [7,8]. Hence,

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there is an urgent need to find new potent radicals scavenger from the plant source, especially from herbs, and spices. Plant's volatile oil (VO) constituent could function as a credible option to man-made antibiotics because they are biocompatible, biodegradable and can effectively scavenge radicals as well as inhibit bacterial growth[9-11]

Dennettia tripetala G. Baker (pepper fruit tree) is an admired tasty food spice in West Africa, endemic in the southeast and southwest Nigeria[12]. The nutritive and pharmacological properties of the solvent leaf, root and fruit and extracts reports of *D. tripetala* (DT) are well documented[13-16].The uses and medicinal activities of DT seed and fruit have been widely reported. Our earlier studies revealed that the DT ripe fruit essential oil (EO) had stronger antioxidant capacity compared to the unripe fruit oil[16]. However, the vivid difference of the phytochemicals in the ripe and unripe fruit peels, as well as the seeds volatile oil (VO) is lacking. Neither were the comparative effects of these four VOs on known free radicals and pathogens associated with non-communicable and communicable diseases examined. This data is fundamental for the biomedical and economic worth of different parts of DT. We aimed to assess the inhibitory effects of the ripe and unripe fruit peels and seeds VOs of DT and compare eachVO efficiency with some commercial antibiotics against selected infectious reference bacterial strains and oxidative stress radicals.

MATERIALS AND METHODS:

Analytical Chemicals/ Reagents:

We used 2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), DPPH, thiobarbituric acid, sodium dodecyl sulfate, trichloroacetic, sodium nitroprusside and Griess reagent bought from Sigma - Aldrich St Louis, USA);Methanol and DMSO (Dimethyl sulfur oxide) bought from Fluka Chemicals (Buchs, Switzerland); Mueller Hinton agar from Oxford Ltd (Hampshire, England) for the experiments and they were all analytical grade. The bioassays were carried out in the AEMREG laboratory, Department of Biochemistry and Microbiology, University of Fort Hare, Alice, Campus, South Africa.

Collection and processing of plant material:

The green (fresh unripe fruit) and yellow (fresh ripe fruit) of DT were purchased in Mushin spice, fruit/vegetable local market, Lagos, Nigeria. The two samples were authenticated by a plant taxonomist in the Department of Botany, University of Lagos, while LUH17SO1 and LUH17SO2 were marked on the ripe and unripe sample respectively and preserved in the Lagos University Herbarium (LUH). Thereafter, the peel from each sample was carefully removed from the fruit by hand with a sterilized knife and separated from the respective seed. Then, the unripe and ripe peels, as well as their seeds were separately washed with deionized water. To obtain moisture -free samples, the peels and seeds were air-dried for seven and four days respectively at ambient temperature. We then subjected each powder sample (250g each) in a Clevenger-type apparatus to hydro-distillation for 3 h and VO was obtained. Distillation from each fresh powdered sample was performed thrice to produce sufficient VO for use in different bioactive tests. Anhydrous sodium sulfate was used to dry the extracted VO and dispensed into sample tinted vials and kept at four degrees centigrade (4°C). Each VO yield was estimated in w/w% of the powdered sample.

Analysis of the volatile oils extracted:

The four volatile oils (USVO, RSVO, UPVO and RPVO) were analysed using Agilent gas chromatography-mass spectrometry (GC/MS) as described in our previous report[17]. The GC-MS conditions were programmed in a Hewlett-Packed HP 5973 mass spectrometer interfaced with an HP 6890 gas chromatograph. The conditions of temperature and column of the GC/MS used were set as equilibration time 3.00 min, ramp 4 °C/min, initial temperature 70°C, final temperature 240°C; inlet: splitless, initial temperature 220°C, pressure 8.27 psi, purge flow 30 mL/min, purge time 0.20 min, helium gas; column: capillary, 30 m X 0.25 mm,

internal diameter 0.25 μm, film thickness 0.7 mL/min, average velocity 32 cm/sec; MS: EI method at 70 eV. Thereafter, the identity of each constituent was carried out by the conformity of their mass spectra data (MSD) with the reference held in the computer library (Wiley 275, New York). Furthermore, the retention index (RI) of each constituent was literature matched to confirm each constituent identity, while the total percentage composition of each VO was its peak area[16].

Antibacterial assay:

Six reference bacteria strains (RBS) and one bacterial isolate from our laboratory stock culture, confirmed as a multi-drug resistant (MDR)[17,18], were used for the antibacterial assay.*Staphylococcus aureus* (NCIB 50080),*Mycobacterium smegmatis (*ATCC 700084), *Listeria ivanovii, (*ATCC19119), *Enterococcus. faecium*(ATCC19434)and *Escherichia coli* O157 (ATCC 700728), *Enterobacter cloacae* (ATCC 13047) were the four (4) Gram- positive and two Gram-negative RBS, as well as *Vibro paraheamolyticues*shown resistant to q cefuroxime, cephalexin, nalidixic,sulphamethoxazole, streptomycin, tetracycline and streptomycin[17],were examined on the four VOs and ciprofloxacin (CF), using standard procedures[19].

Evaluation of minimum inhibitory concentration (MIC) of extracted oils:

The MIC of the four VOs was performed following CLSI 2014 guidelines as described by Omoruyi et al.[20] with few modification (DMSO as diluent). The bacterial suspensions were prepared by inoculating a fresh stock culture of the seven test bacteria strains into tubes containing 5 mL of sterile Luria Bertani broth (LBB) and incubated at 37 °C for 24 h. Thereafter, cultures grown in sterile LBB were inoculated into Mueller-Hinton Agar (MHA) incubated for 24 h at 37 °C. Then, single colonies were transferred from MHA plates into 4 mL of normal saline solution determined at 580 nm as previously reported by Igwaran et al.[21] and the dilutions matching with 0.5 Mc-Farland standard were used for the test. Firstly, the n-hexane content in USVO, RSVO, UFPVO and RFPVO was evaporated by shaking the vial in warm water, thereafter each stock VO was separately dissolved in DMSO (500 μL) and the solution then properly vortexed. Then,two-fold serial dilutions were performed aseptically in sterile micro centrifuge tubes in a total volume of 100 μL of Muller Hinton (MH) broth mixed with the VO of various concentrations ranging from 0.10 - 3.25 mg/mL. Thereafter, 20 μL of each of the inoculums dilution matching 0.5 Mc-Farland standard was added into the tubes of various concentrations and vortexed. DMSO and ciprofloxacin were used as negative and positive controls respectively, the samples were then incubated for 24 h at 37°C. Thereafter, the assay was carried out in duplicate and the tube with the smallest concentration (SC) without visible growth (WVG) was recorded as the MIC.

Evaluation of minimum bactericidal concentration (MBC) extracted oils:

The minimum bactericidal concentration was determined by poured plate method[17]. Briefly by streaking out the tubes WVG in the MIC procedure (described above) into new nutrient agar plates. Thereafter, at 37°C the culture was incubated for 24 h. The concentration of USVO, RSVO, UPVO and RPVO and ciprofloxacin that did not on the solid medium yield any growth after 24 h was recorded as the MBC[18] of each sample.

Antioxidant Property:

Applying a multiple-assay approach, the radical inhibitory efficacy of USVO, RSVO, UPVO and RPVO was each tested on DPPH, ABTS, nitric oxide (NO) and lipid peroxyl (LP) radicals. The four VOs effects on the four different radicals were investigated at increasing concentrations (0.02 - 0.40mg/mL). Thereafter, the IC_{50} value (antioxidant capacity) of each VO was determined by regression equations and the result was compared with two known commercial antioxidants (β-carotene and Vitamin C) as positive control/reference compounds (RCs).

DPPH radical assay:

The DPPH procedure of Liyana-Pathirana and Shahidi[22] as described in our previous report[17]was used to evaluate each VO

inhibitory potential against DPPH radical with few modifications (DMSO as the diluent for VO). The test was performed in triplicate and each oil potency to inhibit DPPH was determined as % radical inhibitory effects with the equation (1) below.

$$\% \text{ Radical inhibitory effects on DPPH}^{\bullet} = \frac{(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}})}{(\text{Abs control})} \times 100 \text{ --------- (1)}$$

Where $\text{Abs}_{\text{sample}}$ is the DPPH $^{\bullet}$ + VOs or RCs (reference compounds) absorbance; $\text{Abs}_{\text{control}}$ DPPH radical + DMSO absorbance.

ABTS radical assay:

The modified mono cation ABTS radical protocol by Re [23] was applied at increasing concentrations (0.02 -0.40 mg/mL) to evaluate radical inhibitory effects of the four hydro-distilled VOs as previously described[16]. The test was performed in parallel triplicate for the four VOs as well as for the RCs. The effectiveness for each VO to inhibit $ABTS^{+}$ was expressed as % inhibitory effect on ABTS radical with the equation (1) described above.

Lipid peroxyl radical assay:

The effectiveness of the four VOs to inhibit lipid peroxidation radical (LP) was determined applying the thiobarbituric acid (TBA) technique[24] with few modifications, using egg yolk as a known lipid-rich source. Concisely, five hundred microliters (µL) of egg yolk homogenate (10%) was poured into 100 µL of the VO in DMSO at increasing dose (0.02 – 0.40mg/mL). DMSO was then added to bring the volume of each reaction solution to 1000 µL. Thereafter, 50 µL of $FeSO_4$ (0.07 M) was added to induce the lipid peroxidation reaction and the resultant solution was incubated for 30 min at ambient temperature. Subsequently at a pH 3.50, 1.5 mL acetic acid (10%) was added, followed by addition 0.08 %of TBA (1.5 mL)in sodium dodecyl sulfate (1.1 %) and trichloroacetic acid (20%).The solution was properly vortexed and warmed to 65°C for 1h and left at ambient temperature for cooling, then n-butanol (500 µL) was added and centrifuged for 10 min at 3000 rpm. This was followed by carefully removing the upper organic layer and at 532 nm the solution absorbance was read. The radical inhibitory effect of each VO on LP at increasing concentrations was then assessed with the equation (1) above. The test was carried out in triplicate for the VOs, as well as for the controls, and then the average % value was recorded.

Nitric oxide radical assay:

The sodium nitroprusside (SNP) nitric oxide radical (NO) induced protocol, as described by Makhija et al.[25] was used to evaluate the radical inhibitory potency of the VOs with few modifications (DMSO used as a diluent for each VO). Briefly, at pH 7.2 the solution 1000 µL of SNP (10 mM) was added to the VO (1 mL) prepared in DMSO at increasing dose (0.02 – 0.40mg/mL) and the mixture was then incubated at the ambient temperature for 2 h. Subsequently,1 mL of the brownish stable nitrate and nitrite mixture formed was then measured by adding Griess reagent[26], consisting of 1% sulphanilamide, and 1% N-naphthyl-ethylenediamine hydrochloride in 2% ortho-phosphoric acid. At 546 nm, the absorbance of the resultant colour was read at against the reagent blank. The experiment was performed in parallel triplicate, while the average % absorbance obtained at increasing concentrations was used as each VO inhibitory effects on NO, utilizing the equation (1) above. The IC_{50} (mg/mL) value for each VOs and controls in the four antioxidant assays were calculated from regression equation generated in the individual standard curve as described in our previous study [16].

Data analysis and calculations

The statistical analysis was carried out with SPSS24.0 for Windows (with the University of Fort Hare, Reg. number 2012/1786646/07, IBM SPSS Inc.). The antibacterial and antioxidant assays were carried in parallel duplicate and triplicate respectively. The results were expressed as means ± S.D. The standard curve obtained for each VO was used to produce a regression equation and calculate the IC_{50} value each volatile oil, whereas the

correlation between the concentration and % inhibitory activity was performed by t-test correlation analysis. A confidence level of $P < 0.05$ was considered as significant.

RESULTS:

Inhibitory effects of the volatile oils on bacterial strains:

The seven test bacterial strains (BS) were susceptible to the unripe fruit peel (UPVO), ripe peel (RPVO), seed VOs from the unripe and ripe fruits (USVO and RSVO). The USVO displayed stronger inhibitory effect compared to RSVO, as well as the peels VOs, with MIC ranging between 0.10 - 0.40 ± 0.04 mg/mL for USVO, 0.20 - 0.81 ± 0.02 mg/mL for RSVO, 0.40-3.25 ±0.02 mg/mL for UPVO and 0.81 – 3.25 mg/mL ± 0.03 for RPVO. The inhibitory effects of the UPVO and RPVO on the test BS were significantly different ($P<$ 0.05), whereas for USVO and RSVO was similar, except against*E.coli*057 and *V. Paraheamolyticues* (Table1). Interestingly, the USVO exhibited bactericidal effects against all test bacteria at 0.20 mg/mL after 24 hr, except against *E. coli* and *S. aureus.* Conversely, the RSVO displayed bacteriostatic effect except against *M. smegmatis* (Table 2). It is worthy of note that antibacterial effects of the two seed oils (USVO and RSVO) was significantly different (P < 0.05) from the fruit peels (UPVO and RPVO) against test bacterial strains, with the seeds oils exhibiting superior inhibitory capacity (Table 1 and 2). Even at much higher concentrations (>0.80 mg/mL) the peel oils (UPVO and RPVO) exhibited bacteriostatic effects on the 7 test BS at (Table 2). In addition, the seed oils exhibited higher inhibitory effects against the test Gram - positive BS than the Gram-negative bacteria strains.The greater antibacterial activity of the USVO corroborates our previous report on the bactericidal activity of DT unripe fruit [16], which may be attributed to the varied chemical profiles of the four VOs in this present study. Notably, there is higher quantity of methyl phenylacetate, longifolene, phenylethyl alcohol, among others in the seeds (USVO and RSVO), whereas the content of these compounds was significantly lower ($P<$ 0.05) in the peels VOs. Furthermore, known bioactive compounds we found in the seed oils including α-terpinene[27,28], linoleic acid[29], squalene[30], among others were not detected in the peel oils (Table 4).

Inhibitory effects of the volatile oils on radicals:

The radical inhibitory effects of the four volatile oils of DT and RCs were assayed on four different radical's paradigms. The inhibitory activities of the VOs and RCs were as presented in Figure 1 to 4.In the DPPH assay (Figure 1), the inhibitory effects of the unripe and ripe seeds oils (USVO and RSVO) were stronger than the unripe and ripe peels (UPVO and RPVO) as well as vitamin C at all concentrations (0.02, 0.05, 0.10, 0.20 and 0.40 mg/mL) but significant different ($p < 0.05$ from β-carotene at 0.02-0.10 mg/mL. The higher inhibitory efficacy of the seeds oils against DPPH radical, validates their greater antibacterial strength than the peels oils.

To determine each oil specific radical inhibitory effectiveness, LP, NO and a monocation radical ($ABTS^{+}$) were quantitatively determined at increasing concentrations (0.02-0.40 mg/mL). In the 4 assays, the four VOs showed strong inhibitory effects against the different radical species, signifying that the seed and peel VOs of DT are good inhibitors of ABTS, NO radicals (Figure 2 and 4) and displayed noteworthy inhibitory property against DPPH and LP radicals (Figures 1 and 3). The concentration required to inhibit 50 % of each radical (IC_{50}value) for individual VO was calculated from the regression equation generated for each oil produced from the standard curve (Figure 1.1 and 3.1 SM), and t-test analysis was used to assess the correlation between% radical inhibition and concentration. The four VOs achieved 50 % reduction of DPPHto DPPH-H (none radical molecule) with an IC_{50} value of 1.03 ± 0.03 mg/mL for RSVO, and 1.48 ± 0.04 mg/mL for the USVO. The inhibitory capacities (IC_{50}) for the ripe and unripe seeds VOs were significantly ($P<0.05$) superior to the ripe and unripe peels oils, while vitamin C had the least inhibitory capacity (3.03 mg/mL) to

reduce DPPH into a none radical molecule. The VOs inhibitory effects (Figure 2) against the monocation radical ($ABTS^{+}$) indicated a weaker property when compared to DPPH radical. Again, the antioxidant strength (IC_{50}) of seeds VOs (≤ 2.53 mg/mL) were better than the peels VOs (≥2.73 mg/mL). This confirmed greater antioxidant effects of the seeds over the peels VOs as well as the vitamin C (2.65 mg/mL) as revealed in DPPH assay. The RSVO demonstrated remarkable superior antioxidant strength against LP and NO radicals (1.40 and 1.70mg/mL),when compared to unripe VOs, vitamin C as well as β-carotene in lipid peroxidation test (Table 3).The *D. tripetala* VOs inhibitory effects on NO were similar to LP test, except that at higher concentrations (0.2 and 0.40 mg/mL), vitamin C was more active (Figure 4) than the UPVO, while the RSVO exhibited the best NO inhibition, followed by β-carotene.

The constituents of extracted volatile oils:
The compositions of the four VOs as revealed by the GC-MS analysis are presented in Table 4. Twenty-nine and 27 constituents were quantified from the RPVO and UPVO, representing 90.40 and 84.51 % of the total VO content respectively. The unripe seed VO (USVO) contained thirty-three, while the RSVO had the highest number (36) of constituents, accounting for 96.70 and 98.72 % of the total oil content respectively. The result revealed that there are more terpenoids (terpinen-4-ol and geraniol), polyunsaturated fatty acids (PUFA) and phenolic (thymol) contents in both ripe peel and seed VOs than in the unripe seed and peel VOs, which corroborates Adebayo et al.[31]report, that ripening of pepper fruit brings about increased total content. Similarly, the total terpenoids content (37.46 %) in unripe peel oil UPVO, increased to 53.45 % in the ripe peel oil (RPVO). This might have enhanced its radical inhibitory effects of RPVO compared to UPVO. Another major differences in the composition of the unripe and seed ripe oils is the presence more quantity of bioactive phytol[32], thymol, phenyl ethyl alcohol, α-terpinene[28],and caryophyllene oxide[33] in the latter oil, while the major components 2-methyl phenylacetate (MPA) and longipinene in the VOs were domicile in the unripe seed.

Table 1: Minimum inhibitory concentrations (mg/mL) of *D. tripetala* volatile oils and controls

Test bacterial	Volatile oils in *D. tripetala*				Controls	
	UPVO[a]	USVO[b]	RPVO[c]	RSVO[d]	Ciprofloxacin (positive)	DMSO (negative)
L.ivanovii (ATCC19119)	0.81±0.02	0.10± 0.04	1.62±0.00	0.10 ±0.02	0.05±0.00	500µL
S. aureus (NCIB50080)	1.62±0.00	0.20±0.01	3.25±0.02	0.20±0.00	0.05±0.03	500µL
M. smegmatis (ATCC700084)	0.40±0.02	0.10±0.02	0.81± 0.02	0.10±0.02	0.10±0.00	500µL
E. faecium (ATCC19434)	0.81±0.00	0.20±0.00	1.62± 0.00	0.20±0.02	0.05±0.01	500µL
V. parahea-molyticues *	1.62± 0.01	0.40±0.03	3.25±0.00	0.81±0.00	0.05±0.02	500µL
E. cloacae (ATCC13047)	0.81±0.01	0.20±0.00	3.25±0. 02	0.20±0.02	0.05±0.02	500µL
E. coli 057 (ATCC700728)	3.25±0.03	0.40±0.00	1.62±0.03	0.81±0.04	0.10±0.00	500µL

[a]unripe peel volatile oil, [b] unripe seed volatile oil, [c] ripe peel volatile oil,[d] ripe seed volatile oil, * laboratory confirmed resistant isolate to antibiotics.

Table 2: Minimum bactericidal concentrations (mg/mL) of *D. tripetala* volatile oils and controls

Test bacterial	Volatile oils of *D. tripetala*				Controls	
	UPVO	USVO	RPVO	RSVO	Ciprofloxacin (positive)	DMSO (negative)
L.ivanovii ATCC19119	Bacteriostatic at 0.81±0.02VG	Bactericidal at 0.20±0.02 WVG	Bacteriostatic at 1.62 ±0.04 VG	Bactericidal at 0.40±0.04 WVG	Bactericidal at 0.05±0.01 WVG	500µL VG
S. aureus NCIB50080	Bacteriostatic at 1.62±0.02VG	Bacteriostatic at 0.20± 0.02 VG	Bactericidal at 3.25±0.04WVG	Bacteriostatic at 0.40±0.00 VG	Bactericidal at 0.05±0.01 WVG	500µL VG
M. smegmatis ATCC700084	Bacteriostatic at 0.40 ±0.00VG	Bactericidal at 0.20± 0.00 WVG	Bacteriostatic at 0.81±0.00 VG	Bactericidal at 0.20± 0.04WVG	Bactericidal at 0.01±0.00 WVG	500Ml VG
E. faecium (ATCC19434	Bacteriostatic at 0.81±0.01 VG	Bactericidal at 0.20±0.04 WVG	Bacteriostatic at 1.62±000VG	Bactericidal at 0.40±0.04 WVG	Bactericidal at 0.05±0.01 WVG	500µL VG
V. parahea-molyticues *	Bacteriostatic at 1.62 ±0.03VG	Bactericidal at 0.40 ±0.04 WVG	Bacteriostatic at 3.25± 0.03 VG	Bactericidal at 0.81±0.04 WVG	Bactericidal at 0.05±0.01 WVG	500 µL VG
E. cloacae ATCC13047	Bacteriostatic at 0.81 ±0.04VG	Bactericidal at 0.20± 0.00 WVG	Bacteriostatic at 3.25 ±0.00 VG	Bacteriostatic at 0.40 ± 0.00 VG	Bactericidal at 0.05±0.01 WVG	ND
E. coli 057 ATCC700728	Bacteriostatic at 3.25±0.00VG	Bacteriostatic at 0.40±0.04 VG	Bacteriostatic at 1.62 ±0.00 VG	Bacteriostatic at 0.81 ±0.03 VG	Bactericidal at 0.10±0.00 WVG	ND

[a]unripe peel volatile oil, [b] unripe seed volatile oil, [c] ripe peel volatile oil,[d] ripe seed volatile oil, * laboratory confirmed resistant isolate toantibiotics, VG = visible growth, WVG = Without visible growth, ND = not determined

Table 3:Antioxidant capacity of *D.tripetala* volatile oils and reference compounds (IC_{50}mg/mL)

Activity	Volatile oils of *D. tripetala*				Reference compounds*	
	UPVO	USVO	RPVO	RSVO	β-Carotene	Vitamin C
DPPH •	2.24 ± 0.02	1.48± 0.04	1.86±0.01	1.03±0.03	0.35 ±0.04	3.03±0.02
ABTS • +	3.18± 0.02	2.50± 0.01	2.70±0.03	1.50±0.04	0.73±0.01	2.65±0.02
LP •	3.80± 0.03	2.03± 0.04	2.30±0.01	1.42±0.00	1.70±0.01	3.64±0.04
NO •	2.94± 0.02	2.23± 0.03	2.50±0.01	1.70±0.02	1.90±0.03	2.52±0.04

* positive controls (commercial antioxidant). •+ cation radical; values are mean ±SD, n=3, significant difference was considered at a level of $P < 0.05$; UPVO: unripe fruit peel volatile oil; USVO: unripe seed volatile oil; RPVO: ripe fruit peel volatile oil; RSVO: ripe seed volatile oil

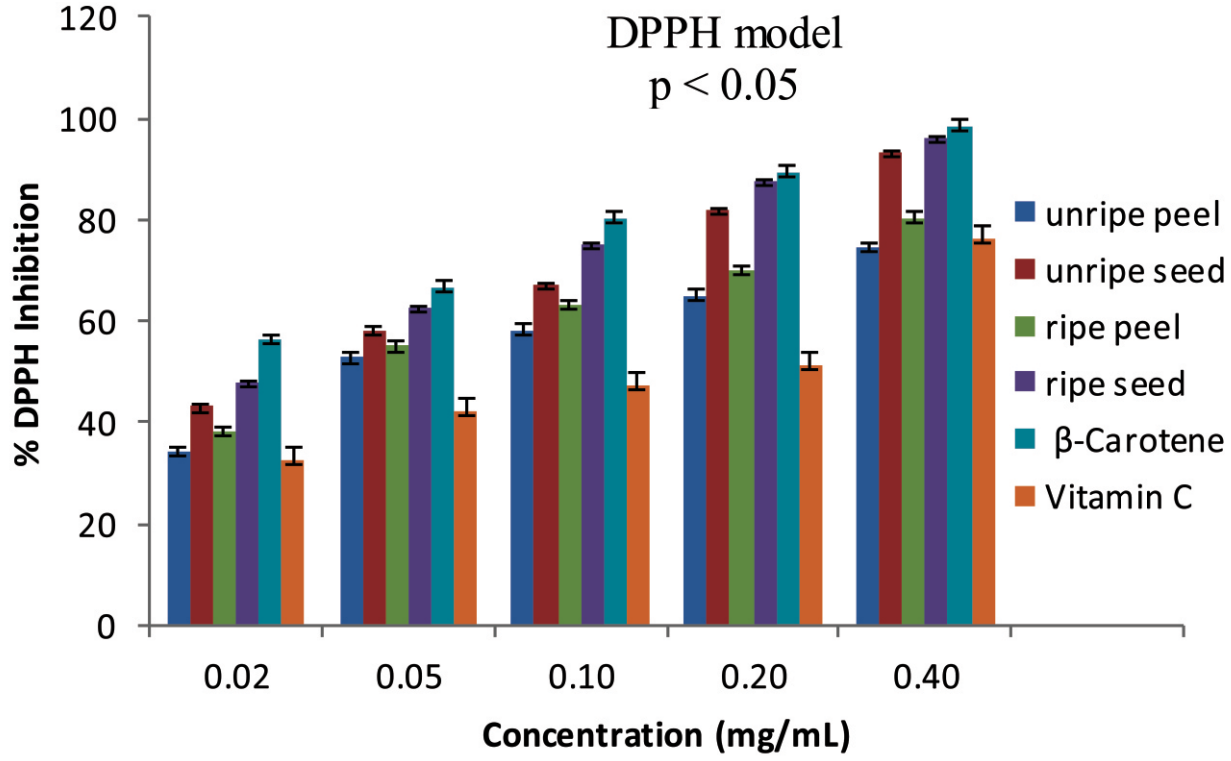

Figure 1: Inhibitory effects of *D. tripetala* volatile oils and reference compounds on DPPH radical

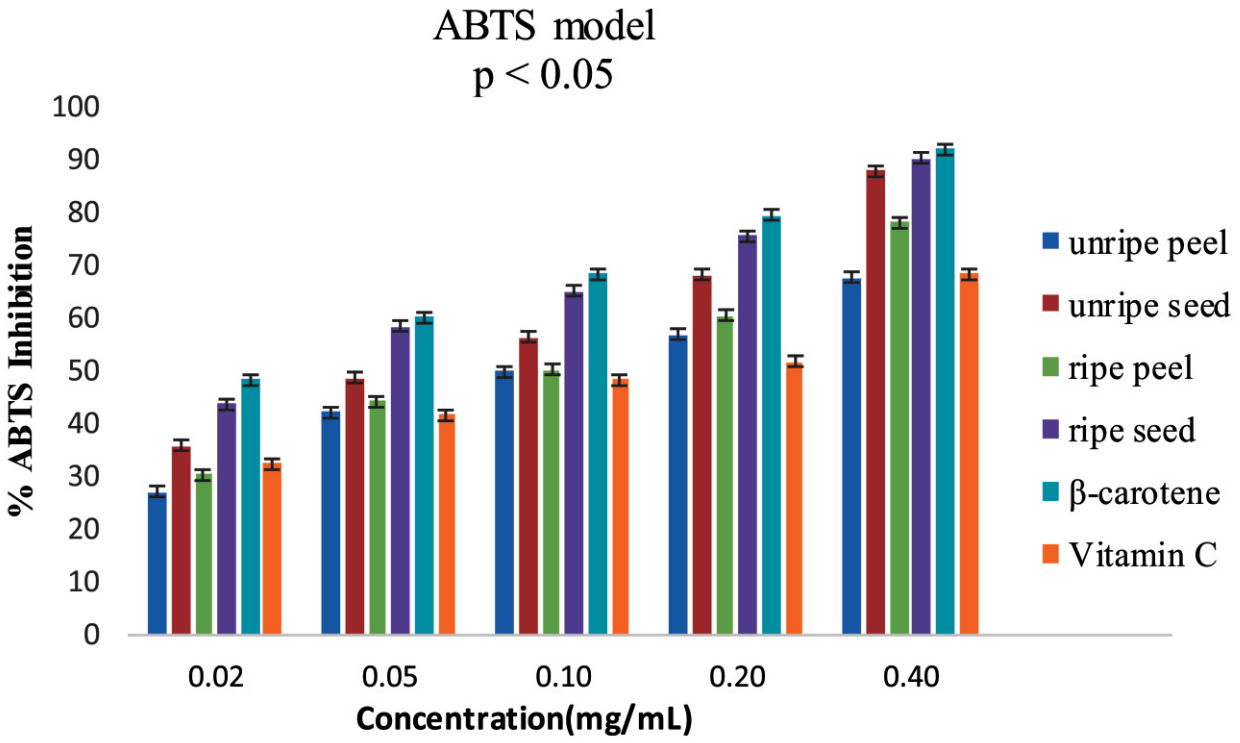

Figure 2: Inhibitory effects of *D. tripetala* volatile oils and reference compounds on ABTS radical

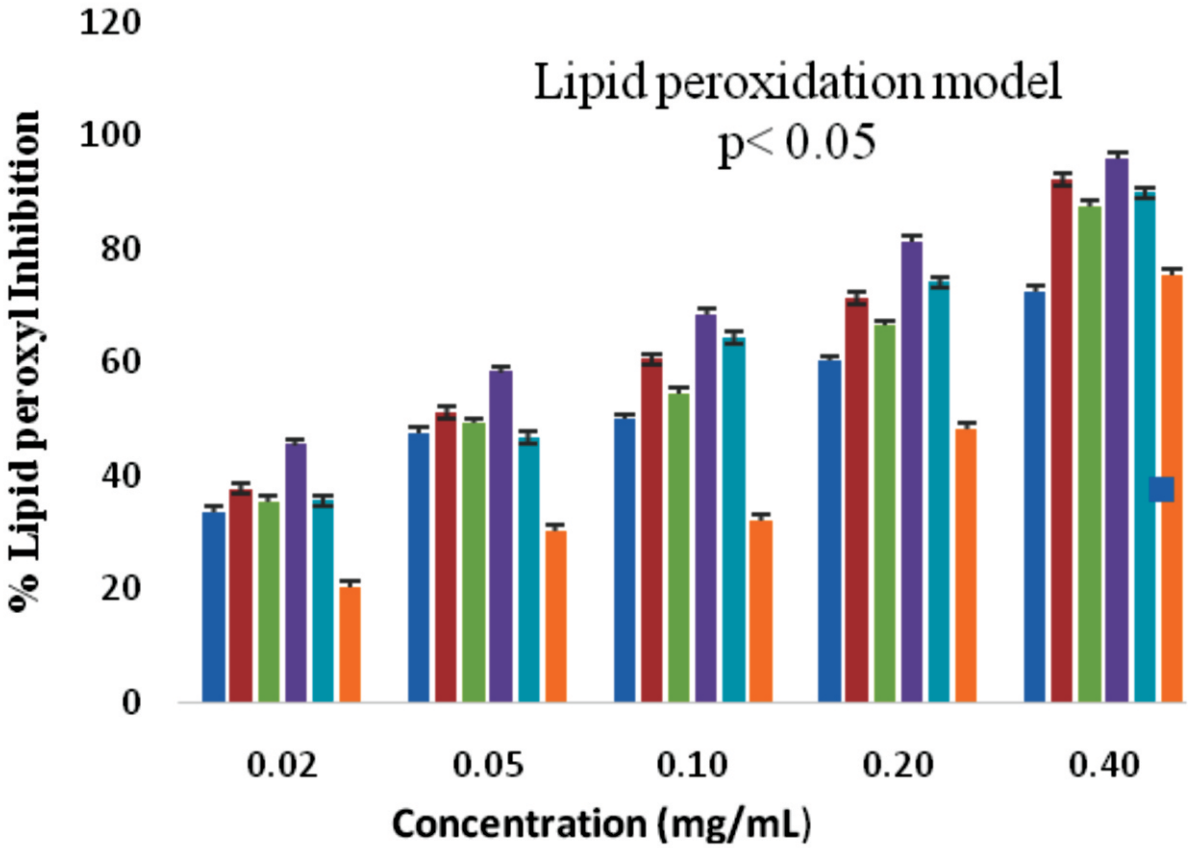

Figure 4: Inhibitory effects of *D. tripetala* volatile oils and reference compounds on nitric oxide radical

Constituent [a]	KI [b]	% Composition of Volatile Oils	QA [c]	MF [d]	MOI [e]

Table 4: Chemical profile of volatile oils extracted from *D. tripetala* seed and fruit peel.

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Constituent [a]	KI [b]	% Composition of Volatile Oils				QA[c]	MF [d]	MOI [e]
		UPVO	USVO	RPVO	RSVO			
Azulene	925	0.29	0.22	t	0.53	83	$C_{10}H_8$	KI, MSD
p-Cymene	928	1.05	-	0.05	-	95	$C_{10}H_{14}$	KI, MSD
α-Pinene	934	0.63	0.65	1.52	2.52	89	$C_{10}H_{16}$	KI, MSD
β-Pinene	937	0.76	0.92	5.73	0.54	98	$C_{10}H_{16}$	KI, MSD
β-Myrcene	944	0.17	0.28	0.10	0.32	97	$C_{10}H_{16}$	KI, MSD
α- Phellandrene	953	1.66	-	0.12	-	95	$C_{10}H_{16}$	KI, MSD
(+) - 4 - Carene	963	-	1.92	0.12	2.02	90	$C_{10}H_{16}$	KI, MSD
β-Ocimene	968	t	0.08	0.07	1.01	94	$C_{10}H_{16}$	KI, MSD
Ui	985	5.16	-	4.02	-	46		
3,4-dimethyl 2,4,6 octatriene	995	-	-	-	0.45	90	$C_{10}H_{16}$	KI, MSD
α-Fenchene	1140	14.30	0.71	26.20	0.54	90	$C_{10}H_{16}$	KI, MSD
Thymol	1004	0.14	2.34	0.20	4.91	90	$C_{10}H_{14}O$	KI, MSD
Linalool	1011	3.60	0.06	3.58	3.41	97	$C_{10}H_{18}O$	KI, MSD
α-Terpinene	1028	-	7.17	-	9.66	96	$C_{10}H_{18}$	KI, MSD
Phenylethyl alcohol	1043	8.10	3.93	7.14	4.35	90	$C_{10}H_8O$	KI, MSD
Terpinen-4-ol	1128	-	1.20	-	2.35	96	$C_{10}H1_8O$	KI,MSD
α-Terpineol	1131	1.60	0.36	1.59	1.88	99	$C_{10}H_{18}O$	KI, MSD
Geraniol	1131	-	0.13	-	0.64	98	$C_{10}H_{18}O$	KI, MSD
Methyl phenyl acetate	1356	7.65	35.82	5.83	27.02	89	$C_9H_{10}O_2$	KI, MSD
α-Calacorene	1461	-	t	-	0.57	90	$C_{15}H_{20}$	KI, MSD
Caryophyllene	1486	7.33	0.24	6.38	t	99	$C_{15}H_{24}$	KI, MSD
Trans-Bergamotene	1490	-	0.77	t	0.03	90	$C_{15}H_{24}$	KI, MSD
Alloaromadendrene	1497	8.59	0.07	0.05	1.48	98	$C_{15}H_{24}$	KI, MSD
β-longipinene	1503	1.59	-	2.20	-	90	$C_{15}H_{24}$	KI, MSD
Humulene	1517	-	4.38	-	5.05	90	$C_{15}H_{24}$	KI, MSD
α-Cubebene	1523	2.91	-	5.37	t	91	$C_{15}H_{24}$	KI, MSD
Ui	1531	-	t	-	0.80	62		
α -Farnesene	1537	0.57	4. 30	0.96	4.56	95	$C_{15}H_{24}$	KI, MSD
Longifolene	1543	2.08	12.44	6.82	10.75	83	$C_{15}H_{24}$	KI, MSD

Copaene	1550	2.09	1.40	3.11	0.72	95	$C_{15}H_{24}$	KI, MSD
5-Azulenemethanol	1556	-	0.11	-	1.32	98	$C_{15}H_{26}O$	KI, MSD
Spathulenol	1568	0.31	-	0.04	-	91	$C_{15}H_{24}O$	KI, MSD
Caryophyllene oxide	1579	0.09	2.53	0.20	4.16	94	$C_{15}H_{24}O$	KI, MSD
Guaiol	1589	1.69	3.82	6.04	2.03	90	$C_{15}H_{26}O$	KI, MSD
Cadinol	1810	-	-	-	0.05	90	$C_{15}H_{26}O$	KI, MSD
Pentadecanoic acid	1590	-	0.26	-	3.04	96	$C_{15}H_{30}O_2$	KI, MSD
Hexadecanoic acid	1620	7.05	2.13	2.00	1.24	98	$C_{16}H_{32}O_2$	KI, MSD
Linoleic acid	1925	-	3.33	-	3.58	89	$C_{18}H_{34}O_2$	KI, MSD
Phytol	2045	5.67	1.82	1.13	6.40	95	$C_{20}H_{40}O$	KI, MSD
Heneicosane	2102	-	0.51	-	0.02	90	$C_{21}H_{44}$	KI, MSD
Squalene	2322	-	0.12	-	0.05	99	$C_{30}H_{50}$	KI, MSD
Total oil content (%)		**84.51**	**96.70**	**90.40**	**98.72**			
Yield (% w/w)		**0.40**	**0.70**	**0.36**	**0.82**			

Ui =Unidentified, MSD = mass spectra data, KI =retention index relative to C_9-C_{23} on the column HB-5, t=less than 0.02%, a = Constituents elution order in HB-5column;b =Kovat's index, c =% of library quality assurance, d= Molecular formula, e= methods of identification, UPVO: unripe fruit peel volatile oil; USVO: unripe seed volatile oil; RPVO: ripe fruit peel volatile oil; RSVO: ripe seed volatile oil

DISCUSSION:

The inhibitory effects of the DT seeds and peels VOs against LPand NOassociated with cells inflammation, as well as DPPH, ABTS radicals and eight pathogens were studied. The esteemed inhibitory property of RSVO against nitric oxide radical in this study confirms Oyemitan et al.[34]report of significant anti-inflammatory effects of the plant's fruit EO on rodents. Studies have shown that these reactive oxygen species (ROS) are among free radicals involved in variety of different cellular processes including apoptosis, necrosis, cell proliferation and carcinogenesis[35,36].

Unlike the antibacterial activities of the oils, the antioxidant efficacies (1.03±0.03 -1.42 ±0.00 mg/mL) of the ripe seed oil (RSVO) against the different test radicals (DPPH, ABTS and LP) were significantly ($P< 0.05$) better than the unripe seeds (Table 3). This corroborates our previous studies of higher bioactivity of EO from the DT ripe fruit against lipid peroxidation[16]; which implies that the best antioxidant efficacy of the DT's fruit is from the ripe seed VO. This is however contrary to Adebayo et al.[31] reports, that the physiological changes that accompany the ripening of DT fruit that leads to changes in pigment increased the total phenol content but decreased the antioxidant properties of DT fruit. Previous studies have shown the presence of phenolic compounds including thymol, phenyl ethyl alcohol, and geraniol we found in this present study to enhance antioxidant and antibacterial activities [37-40].

In addition to the phenolic and alcohol constituents, the bioactivity of the DT seeds oils may be attributed to synergy of other constituents (monoterpene, diterpene and triterpene) in the oils (Table 4). From the forty constituents we found in the seed oils,

thirteen (13) of them were not present in the two different DT peel VOs. Other known bioactive constituents such as linalool, caryophyllene, 4-carene, linoleic acid, hexadecanoic acid, phellandrene,α-pinene and β-ocimene we detected in the ripe seed volatile oil were reported as major constituents in previous studies of DT seed EO by Elekwa et al. [41] and Kumar et al.[42]

This current research is in support of previous studies suggestions, that the disparity observed in the components of EO from the same plant species grown in different or similar region are due to discrepancies of factors, including nature of soil, season, climate, moisture of the harvested plant sample, presence of chemo-types and method of oil extraction [43,44]. To the best of knowledge, this is the first report of VO from unripe and ripe pepper fruit peels and detection of thymol, geraniol, α-fenchene, trans-bergamotene, β-longipinene, neophytadiene, squalene and MPA as constituents of DT. The presence of MPA (a bioactive ester) as a major constituent in the seeds of DT is noteworthy, which might have undergone chemical or biochemical transformation to 2-methyl phenyl formate (MPF) in previous study [16], via established mechanisms[45,46]. The findings in this study, supports previous EO researchers that have evaluated terpenes, terpenoids, PUFA as well as a cyclic double bond sesquiterpenes (C_{15}), and triterpene (squalene) detected in the DT seed, that are known to exhibit bioactive properties similar to the polyphenols [47-49].

Some of the valuable antioxidant and antimicrobial constituents we detected in the VOs of *D. tripetala* in this present study have been recommended as commercial food flavors and preservatives by European Commission due to their biocompatibility, low toxicity and biodegradability[12]. The strong inhibitory efficacy displayed by unripe and ripe DT seeds VOs against these pathogens (*S. aureus*,*M. smegmatis*, *L. ivanovii*, *E. faecium*, *E. cloacae* and *V. paraheamolyticue*s) andas a good inhibitor of lipid peroxidation and nitric oxide radicals associated with communicable and non-communicable diseases respectively is worthy of note. This suggests that the plant seed's VO might be a new candidate in the hunt for principal compounds for managing infectious diseases and ailments associated with oxidative damage such as diabetic nephropathy, cancers, arthritis andarteriosclerosis[50,51].

CONCLUSION:

This current research indicates that besides the folklore uses of *D. tripetala*, the seed volatile oil from the unripe and ripe fruits contain sturdy bioactive phytochemicals. This suggests the seed oil as a novel strong antioxidant and antibacterial agent and fresh candidate in the hunt for lead phytochemicals for the synthesis of potent antibiotics in this decade of emerging multi-drug resistant microorganisms.

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AUTHOR CONTRIBUTIONS:

Sunday O.Okoh and Omobola O. Okoh designed the experiments, carried out the analysis, interpreted the results and wrote the manuscript, Anthony I. Okohsupported the preparation,proofreadingof the manuscript and coordinated the research. All authors have read and approved the final manuscript.

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