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ORIGINAL ARTICLE

Bioactive Constituents, Radical Scavenging, and Antibacterial Properties of the Leaves and Stem Essential Oils from *Peperomia pellucida* (L.) Kunth

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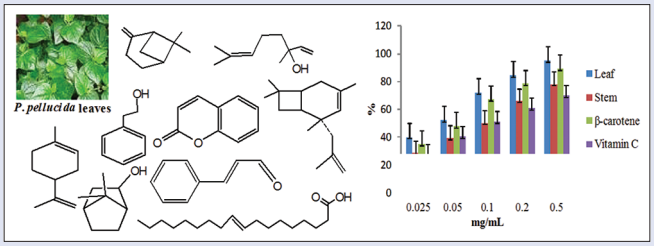
ABSTRACT

Background: *Peperomia pellucida* is an annual herbaceous ethnomedicinal plant used in the treatment of a variety of communicable and noncommunicable diseases in the Amazon region. **Objective:** The study aimed at profiling the bioactive constituents of the leaves and stem essential oils (LEO and SEO) of *P. pellucida*, their *in vitro* antibacterial and radical scavenging properties as probable lead constituents in the management of oxidative stress and infectious diseases. Materials and **Methods:** The EOs were obtained from the leaves and stem *P. pellucida* using modified Clevenger apparatus and characterized by a high-resolution gas chromatography-mass spectrometry, while the radicals scavenging and antibacterial effects on four oxidants and six reference bacteria strains were examined by spectrophotometric and agar diffusion techniques, respectively. **Results:** The EOs exhibited strong antibacterial activities against six bacteria (*Escherichia coli* [180], *Enterobacter cloacae, Mycobacterium smegmatis, Listeria ivanovii, Staphylococcus aureus, Streptococcus uberis*, and *Vibrio paraheamolyticus*) strains. The SEO antibacterial activities were not significantly different ($P < 0.05$) from the LEO against most of the test bacteria with minimum inhibitory concentration ranging between 0.15 and 0.20 mg/mL for both EOs. The two oils were bactericidal at 0.20 mg/mL against *S. aureus* while the minimum bactericidal concentration (0.15 mg/mL) of LEO against *L. ivanovii* was lower than of SEO (0.20 mg/mL) after 24 h. The LEO IC_{50} value (1.67 mg/mL) revealed more radical scavenging activity than the SEO (2.83 mg/mL) and reference compounds against 2,2-diphenyl-1-picrylhydrazyl radical. The EOs also scavenged three other different radicals (2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt radical , lipid peroxyl radical, and nitric oxide radical) in concentration-dependent manner. **Conclusion:** Our results suggest that apart from the indigenous uses of the plant extracts, the EO contains strong bioactive compounds with antibacterial and radicals scavenging properties and may be good alternative candidates in the search for novel potent antibiotics in this present era of increasing multidrug-resistant bacterial strains as well as effective antioxidants agents.

Key words: β-caryophyllene, antibacterial, limonene, radicals scavenging, *Peperomia pellucida*

SUMMARY

- Established gas chromatography-mass spectrometry technique was applied to quantitatively and qualitatively analyze the volatile constituents in *Peperomia pellucida* essential oil (EO)
- The Clinical and Laboratory Standards Institute (2014) guidelines were employed to evaluate the antibacterial effects of the EOs
- Among the known prominent bioactive terpenoids, linalool 17.09%, limonene 14.25%, β-caryophyllene 12.52%, and linalyl acetate 10.15% were the main constituents of the EOs in this current study
- The leaf and stem EOs were bactericidal at a concentration below 0.23 mg/mL against three multidrug-resistant bacteria and significantly scavenged known free radicals reported to be associated with contagious and oxidative stress-related disorders.



Abbreviations used: GC-MS: Gas chromatography-mass spectrometry, DPPH: 2,2-diphenyl-1-picrylhydrazyl, ABTS: 2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt, DMSO: Dimethyl sulfoxide, LP•: Lipid peroxide radical, NO•: Nitric oxide radical, LEO: Leaf essential oil, SEO: Stem essential oil, RC: Reference compound, TBARS: Thiobarbituric acid

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INTRODUCTION

Infectious and noncommunicable diseases, particularly those due to multidrug-resistant microorganisms such as *Staphylococcus*, *Escherichia coli*, *Enterococcus* species, and reactive oxygen species, are almost impossible to combat.[1] The resistant rate of pathogens to vast synthetic antimicrobial agents coupled with rising side effects of antibiotics deserves novel therapies for efficient public health care.[2] Accordingly, some articles on bioactive phytochemical including alkaloids, polyphenol, flavonoids, and essential oil (EO) constituents have been suggested in recent years as possible

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option.[3-5] Evidence from the previous studies suggest that EO has therapeutic properties and could stand as alternative of antibiotics against certain pathogenic bacteria species, besides filamentous fungi and yeasts.[5,6] Some plants' volatile oils (EOs) have been shown to speedily diffuse cell membrane of bacteria owing to their permeability properties across biological lipid barriers.[4,6] This interaction can lead to membrane instability and consequently leakage of the bacterial important intracellular components and ultimate cell death. Cell wall, cell membrane, intracellular proteins, nucleic acids or enzymes, and few others are vital target sites for drug design, and some volatile oil compounds have these specialized sites of the cell as their target.[7-9]

Enzymatic antiradical defense systems made up of glutathione peroxidase, catalase, superoxide dismutase, as well as other endogenous antiradical molecules, especially glutathione, do scavenge oxygen-derived-free radicals produced in physiological and pathological processes.[10] However, the scavenging of oxidants generated including superoxide radical, lipid peroxyl radical (LP•), nitric oxide radical (NO•), and hydroxyl (HO•) produced during metabolic activities and environmentally induced radicals overwhelms the naturally produced antiradicals.[11] Man has used spices, fruits, vegetables, and plant's decoction now acknowledged containing potent secondary metabolites against diseases for more than 20 decades. In recent time, some studies have shown secondary metabolites including phenols, flavonoids, and alkaloids from plants and their EOs are potent antiradicals.[12-14] EO could function as a credible option to synthetic antibiotics due to its property to penetrate the cell membrane as well as radical scavenger.[15] The European Commission has approved EO constituents including limonene, linalool, menthol, and caryophyllene that possess such properties as food flavors and additives in cosmetics products.[9,15,16]

Peperomia pellucida (shiny bush, silver bush) of the family *Piperaceae* is an annual herbaceous plant. It grows in rainy (often in the spring) season to height of 15–46 cm in humid loose soil, especially under the trees. It is commonly found in West African rainforest belt including Southeast and Southwest Nigeria and many tropical Asian and South American countries.[17,18] Ethnomedical reports of *P. pellucida* shows that the leaf uses vary depending on the region where it is found. In the Amazon region, it has been used to reduce cholesterol level, as a diuretic, dementia disorder, and in treating cardiac arrhythmia.[18,19] In Ayurvedic records, the leaves and stem aqueous mixture is used in treating hemorrhage, fever, headache, abdominal pain, wounds dressing and as cough suppressant.[17,20] The decoction of the whole plant in India served as potent medication in rheumatism, renal disorders, breast cancer, boils, and small pox.[18,21,22] Previous pharmacological studies revealed that the solvents' crude extracts exhibit significant analgesics, antimicrobial, anti-inflammatory, and anti-protozoa activities and cytotoxic to breast cancer cell lines.[19] Another study by Xu *et al.*[23] of the solvents leaves crude extracts indicated alkaloids, sterols, flavonoids, and styrene as dominant bioactive compounds of *P. pellucida*. Previous investigation of the leaf essential oil (LEO) revealed apiole and β-caryophyllene as the major volatile compounds.[24] Nevertheless, information on radical scavenging effects on a variety of free radicals and antibacterial activity on multidrug-resistant bacteria strains is scanty, while comparative studies on the LEO and stem EO (SEO) constituents of *P. pellucida* are lacking. This information is imperative for thorough understanding of the plant bioactive value and economic evaluation. We aimed in this present study to characterize the bioactive constituents and to evaluate the radical scavenging and antibacterial effects of the LEO and SEO of *P. pellucida*.

MATERIALS AND METHODS

Analytical reagents

The chemicals and reagents used included the following: Mueller-Hinton agar from Oxford Ltd (Hampshire, England), dimethyl sulfoxide (DMSO) from Fluka Chemicals (Buchs, Switzerland). 2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), and 2,2-diphenyl-1-picrylhydrazyl (DPPH) from Sigma-Aldrich (St. Louis, USA). All chemicals and reagents were of analytical grade.

Plant material

P. pellucida was collected in August 2016 from Southwest Nigeria at the Forest Research Institute of Nigeria, Ibadan. A plant taxonomist authenticated the plant, and the sample was kept in the Lagos University Herbarium (LUH) with voucher specimen number LUH 6956. The leaves were left to air-dry at an ambient room temperature for 5 days, while the stem was cut into smaller pieces and air-dried for 7 days. They were pulverized and the EO extracted for 3 h from each (200 g) using modified Clevenger-type apparatus as previously described.[25] The hydrodistillation experiment was carried out thrice on the leaf and stem separately to obtain enough oil for bioactivity assays. The two EOs were dried with anhydrous sodium sulfate and stored in tinted vials at 4°C. The EO yield was then computed per gram (w/w%) of the plant sample.

Characterization of the essential oils

We utilized a gas chromatography/mass spectrometry (GC/MS) to analyze and identify the EO constituents. The analysis was carried out on Agilent 5977A mass spectra data (MSD) and 7890 GC system, Chemetrix (Pty) Ltd, Agilent Technologies, DE (Germany), with a Zebron-5MS (ZBMS 30 m × 0.25 mm × 0.25 um) (5% - phenyl methyl polysiloxane). The temperature and column conditions were applied: the injector, source, and oven temperature set at 280°C, 280°C, and 70°C, respectively. GC grade helium at a flow rate of 2 mL/min and splitless 1 mL injection was used. The ramp settings were 15°C/min to 120°C, then 10°C/min to 180°C, then 20°C/min to 270°C, and held to for 3 min. Subsequently, identification of each constituent was ascertained using agreement of their MSD with the reference held in the computer library (Wiley 275, New York). Furthermore, matching the retention index of each compound with those in literature was also employed in identifying the compounds. The peak areas were used to obtain total percentage composition of oil.

Antibacterial activity

Bacteria suspensions test

Four multidrug-resistant reference bacterial strains and two bacteria isolates from our laboratory stock culture confirmed to be multidrug-resistant bacteria[26,27] were used for the antibacterial test. The reference and laboratory bacterial strains consist of four Gram-positive bacteria: *Staphylococcus aureus* (NCINB 50080), *Listeria ivanovii* (ATCC 19119), *Mycobacterium smegmatis* (ATCC 19420), *Streptococcus uberis* (ATCC 29213) and three Gram-negative bacteria: *Enterobacter cloacae* (ATCC 13047), *E. coli* 180, and *Vibrio paraheamolyticus*. All the test strains were confirmed to be resistant to ampicillin, cefuroxime, tetracycline, nalidixic, cephalexin, sulfamethoxazole, and streptomycin[27] were tested against the oils and ciprofloxacin following Clinical and Laboratory Standards Institute (2014) guidelines. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) potentials of the EOs and controls were determined.

Minimum inhibitory concentration and minimum bactericidal concentration evaluation

The microdilution technique was used to evaluate the minimum inhibitory concentrations (MICs). Eight hundred, 900, 950, 975, and 987.5 µL of Mueller-Hinton Broth (MHB) were added into each Eppendorf tube. Five hundred milligrams of both SEO and LEO stocks after evaporation of n-hexane was separately dissolved in DMSO (500 µL) and each solution vortexed. Thereafter, aliquots of 200, 100, 50, 25, and 12.5 µL were added, respectively, into each tube containing MHB to bring the final volume in each to 1 mL, and the mixtures were properly vortexed. The inoculum suspension (20 µL) of each test bacterial isolate (0.5 McFarland, ~1×10^8 CFU/mL) was added subsequently, vortexed to permit adequate mixing of the EO and broth. Ciprofloxacin and DMSO served as the positive and negative controls, respectively. The tubes were then subjected to incubation for 24 h at 37°C. The lowest concentration without visible growth was reported as the MIC. MBC was examined by pour plate method of all tube content without visible growth in the MIC technique above onto fresh nutrient agar plates; thereafter, plates were incubated at 37°C for 24 h. The lowest amount of concentration of EO that does not yield any culture growth on the solid medium at the end of incubation period was recorded as MBC.[28] The experiment was carried out in parallel triplicate and average value was recorded.

Antiradical assays

DPPH$^{\bullet}$, ABTS$^{\bullet}$, NO$^{\bullet}$, and LP$^{\bullet}$ inhibiting tests were performed to determine the antiradical effects of the two EOs.

2,2-diphenyl-1-picrylhydrazyl assay

The DPPH test was performed as described by Liyana-Pathirana and Shahidi[29] with a slight modification (DMSO used instead of methanol). Concisely, a solution of DPPH (2.7 mM) in DMSO was prepared; afterward, 1 mL of it was added to the EO (1 mL) dissolved in DMSO which holds double-fold concentration (0.025–0.50 mg/mL) of the EO as well as the reference standard (RC). All mixtures were then vortexed and reacting solutions were then incubated in a dark chamber at ambient temperature for 30 min. Thereafter, absorbance of the reaction solution was read against a reference blank containing DMSO at 517 nm. EO's potency to reduce DPPH$^{\bullet}$ to neutral molecule was computed as radical scavenging percentage using the following formula:

$$\%\ \text{radical scavenging of DPPH}^{\bullet}\ \text{by EO or RC} = \{(\text{Absc}_{\text{control}} - \text{Absc}_{\text{sample}})\}/(\text{Absc}_{\text{control}}) \times 100 \quad \text{(a)}$$

Where $\text{Absc}_{\text{control}}$ is the absorbance of the DPPH radical + DMSO and $\text{Absc}_{\text{sample}}$ is the absorbance of DPPH radicals + essential oil/RC. The assay was carried out in parallel triplicate.

The IC_{50} is that concentration of the EO or reference compound (RC) (positive control) required reducing 50% of the DPPH radicals. This precise value was obtained from the regression equation generated from standard curve produced with increasing concentrations against inhibitions and results compared to that of RC.

2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt assay

We used the cation radicals scavenging (ABTS$^{\bullet+}$) procedure of Re *et al.*[30] with some modification according to Witayapan *et al.*[31] by mixing 1:1 volumes of ABTS 7.00 mM and 4.90 mM of $K_2S_2O_8$ solution. The mixed solution was kept in a dark chamber at ambient temperature for 12 h. Thereafter, the generated ABTS$^{\bullet+}$ was equilibrated at 734 nm with DMSO to its absorbance (0.705 ± 0.001). To carry out the assay, 1 mL of 0.025–0.50 mg/mL solutions of the test samples (SEO and LEO) in DMSO was mixed with 1 mL ABT$^{\bullet+}$ solution, bringing final volume of each mixture to 2 mL. The reaction solution was kept in dark chamber for 7 min. Subsequently, its absorbance was measured spectrophotometrically at 760 nm. The EO as well as RC radical scavenging effects on ABTS$^{\bullet+}$ was expressed in terms of percentage (%) inhibition of ABTS$^{\bullet+}$ using equation (a) described in DPPH radical test. The test was performed in parallel triplicates and average value calculated.

Thiobarbituric acid assay

The EOs and RCs radical scavenging effects on LP$^{\bullet}$ were measured using the technique as presented by Badmus *et al.*[32] utilizing egg yolk as lipid-rich source. The test samples (0.1 mL) at increasing concentration (0.025, 0.05, 0.10, 0.20, and 0.5 mg/mL in DMSO) were added to 10% egg yolk homogenate (0.5 mL) and the reaction mixture made up to 1 mL. Thereafter, 50 µL of 0.07 M iron (11) sulfate heptahydrate was added to induce lipid peroxidation and the solution incubated for 30 min at ambient temperature. Thereafter, 1.5 mL of acetic acid (10%) (pH 3.50) and 1.5 mL of 0.08% 2-thiobarbituric acid, plus 20% trichloroacetic acid, and 1.1% sodium dodecyl sulfate were added and the mixture was heated at 65°C for 1 h. Upon cooling, the solution was vortexed and n-butanol (0.5 mL) was added to the solution. The solution was then centrifuged at 3000 rpm for 10 min. The resultant upper layer was then aspirated and its absorbance at 532 nm read. The equation (a) described in DPPH radical test was thereafter used to calculate the scavenging effects (%) of the EO and RC on the LP generated. The test was carried out in parallel triplicate and average calculated.

Measurement of the inhibition of nitric oxide radicals assay

The radical scavenging effect of the EO on NO$^{\bullet}$ was investigated according to the modified method described by Makhija *et al.*[33] Sodium nitroprusside molecule in aqueous solution at physiological pH (7.2) decomposed to produce NO$^{\bullet}$ radicals. In aerobic conditions, the radical reacts with oxygen molecule producing nitrite and nitrate as stable molecules and applying Griess reagent these resultant molecules are measured.[34] To 1.0 mL of the EO at increasing doses (0.025, 0.05, 0.10, 0.20, and 0.5 mg/mL prepared in DMSO) was added to 1.0 mL (10 mM) of sodium nitroprusside solution. The solutions were incubated for 110 min at ambient temperature. Thereafter, 1 mL of the aliquot was added to Griess solution (1%, sulfanilamide, 1% N-naphthyl-ethylenediamine hydrochloride in 2% orthophosphoric acid). Subsequently, absorbance of the color developed was then measured spectrophotometrically at 546 nm against the reagent blank. The scavenging effect (%) was then obtained using equation (a) described in DPPH radical test. The assay was carried out in parallel triplicates and mean value calculated.

Statistical analysis

All experiments involving quantitative test were performed in parallel triplicate ($n = 3$). All results expressed as means ± standard deviation. Percentage scavenging of radical was concentration-dependent and regression equation generated from the standard curve for each radical scavenger was used to calculate its IC_{50} value. *t*-test correlation analysis was employed to test significant differences between the concentrations versus percentage of radical scavenging effect, carried out using SPSS 15.0 for windows (IBM SPSS Inc., OLRAC SPS registration number 2012/1786646/07). At $P < 0.05$ confidence level, result was considered being significantly different.

RESULTS

Constituents of the essential oils

The yields, constituents of the EOs extracted, molecular formula as well as of methods of identification each constituent from the leaves and stem

oils of *P. pellucida*, are presented in Table 1. The LEO yield of 0.51% was significantly ($P < 0.05$) higher than the SEO 0.32%, while the color and aroma of the two oils were similar. Compared to the yields (0.05%–0.58%) of EOs extracted by hydrodistillation from China and elsewhere,[13,35] the LEO of *P. pellucida* plant could be considered as rich in EO. The identified bioactive constituents of the plant's EO predominantly monoterpenoid alcohols, sesquiterpenes, aromatic and aliphatic aldehydes [Figure 1] might be responsible for bioactivity of the EOs. Eighteen constituents were found in the SEO with one unidentified constituents, while the LEO contained 16 constituents representing 86.02% and 80.36% of the total oil content, respectively [Table 1]. In the SEO, monoterpenoids and monoterpenes content accounted for 60.27%, followed by sesquiterpenoids 11.71%, while the diterpenoid content was phytol 3.41%. Among the dominant monoterpenoids constituents, linalool (12.60%) was the highest, followed by d-limonene (10.7l%) and α-terpineol (10.57%), while β-caryophyllene (11.47%) was major sesquiterpene. In the LEO, d-limonene (14.25%) and β-caryophyllene (12.52%) were the major constituents. In addition to similar monoterpenoids (35.04%) identified in the SEO, the quantity of aldehydes (18.90%) constituents found in the LEO was higher than those in the SEO. The chemical profiles of the two oils significantly differed; there were traces of borneol and phytol in LEO, while 9-octadecenoic acid and terpen-4-ol were not among the constituents LEO. Conversely, these components were present in significant amount in the SEO of *P. pellucida*. The percentage content of caryophyllene and limonene was higher in LEO than in the SEO. Among the previously reported bioactive compounds in this study are borneol, (+)-4-carene, camphene, β-caryophyllene, 9-octadecenoic acid, phytol, and pinene.[19,34,36] In this present study, more constituents (16) were found in EO compared to those from *P. pellucida* leaf methanol extract in Wei *et al.*'s[19] study. However, some of the constituents reported by Rajendra *et al.*[37] in EO of *P. pellucida* from India including carotol, apiol, and camphor were not among those we identified. The discrepancies observed in *P. pellucida* EO constituents grown in Nigeria, China, and elsewhere may be due to climatic, seasonal, and environmental variation. Other factors including the age of the plant, humidity of the harvested plant material, and the existence of chemotype, may also influenced EO's constituents.[13,38] To the best of our knowledge, this is the first report of comparative investigation of the bioactive constituents and bioactive properties in the LEO and SEO of *P. pellucida* and HMOS was reported in Kumaradevan *et al.*[39] and Parmar *et al.*[40] studies as a strong antimicrobial phytochemical compound.

Antibacterial activity of essential oils

The leaves and stem oils of *P. pellucida* effectively exhibited inhibitory activity against the three reference strains bacteria (*S. aureus, E. cloacae*, and *L. ivanovii,* as well as two isolates – *E. coli* 180 and *V. paraheamolyticus*) confirmed to be multidrug-resistant bacteria from our laboratory stock culture.[26] The SEO antibacterial activities were not significantly different from the LEO against most of the test bacteria with MIC ranging between 0.15 and 0.20 mg/mL for EOs. The LEO and SEO were bactericidal at 0.15 and 0.20 mg/mL against *L. ivanovii*, respectively, while the MBCs against *S. aureus* for both EOs were similar (0.20 mg/mL) after 24 h. The LEO also exhibited bactericidal effect against *E. coli* 180 at 0.20 mg/mL [Table 2]. However, the two oils displayed more bacteriostatic activity at higher dose (>0.20 mg/mL) against the Gram-negative test bacteria (*V. paraheamolyticus* and *E. cloacae*) except *E. coli* 180 and exhibited lower antibacterial activity than the positive control drug (ciprofloxacin). The differences in antibacterial activity may be linked to net repulsion of the complex outer membrane in Gram-negative bacteria. This has been shown in the previous studies to contain hydrophilic lipopolysaccharide (a two-lipid bilayer).[8,41] Consequently, higher tolerance is created toward hydrophobic antibacterial terpene molecules common in EOs.[8] The activity of the stem and leaves oils of *P. pellucida* against the bacteria also differed; the variation observed in the constituent's profiles of two oils [Table 1] may account for their varied bioactivity in this present study.

Essential oils radical scavenging activities

The radical scavenging activities of the *P. pellucida* EOs (LEO and SEO) were studied *in vitro* in four different oxidants (DPPH$^{\bullet}$, ABTS$^{\bullet+}$,

Table 1: Essential oils constituents of *Peperomia pellucida*

Constituent[a]	KI[b]	Molecular formulae	Percentage composition		Methods of identification	MSD[c]	QA[d]
			LEO	SEO			
Phenylethyl alcohol	856	$C_8H_{10}O$	3.81	5.22	RI, MSD	81, 69, 55, 108	94
Coumarin	879	$C_9H_8O_2$	3.46	0.42	RI, MSD	161, 69, 25, 141	98
3-phenylpropanoic acid	897	$C_9H_{10}O_2$	3.15	-	RI, MSD	81, 69, 55, 136	98
α-pinene	927	$C_{10}H_{16}$	0.46	0.07	RI, MSD	93, 79, 41, 136	99
Camphene	940	$C_{10}H_{16}$	0.43	1.40	RI, MSD	93, 69, 41, 77	99
d-limonene	950	$C_{10}H_{16}$	14.25	10.73	RI, MSD	93, 68, 136, 79	95
(+)-4-carene	985	$C_{10}H_{16}$	*t*	4.84	RI, MSD	145, 41, 135, 128	90
Linalool	990	$C_{10}H_{18}O$	17.09	12.60	RI, MSD	113, 71, 44, 29	93
α-terpineol	992	$C_{10}H_{18}O$	2.49	10.57	RI, MSD	41, 71, 93, 111	99
Borneol	1116	$C_{10}H_{18}O$	*t*	6.45	RI, MSD	43, 95, 41, 105	99
(+)-terpinen-4-ol	1128	$C_{10}H_{18}O$	-	0.25	RI, MSD	71, 93, 111, 41	96
2,6-dimethyl-7-octen-2-ol	1145	$C_{10}H_{20}O$	6.55	3.80	RI, MSD	73, 44, 113, 28	96
Citronellol	1220	$C_{10}H_{20}O$	3.40	0.07	RI, MSD	43, 41, 77, 55, 78	98
Linalyl acetate	1372	$C_{12}H_{20}O_2$	11.67	4.86	RI, MSD	71, 43, 68, 109	91
Phenylmethylene octane	1412	$C_{14}H_{22}$	0.46	4.63	RI, MSD	14, 29, 57, 129	93
Ui	1413		-	0.54		14, 57, 91, 111	34
β-caryophyllene	1415	$C_{15}H_{24}$	12.52	11.47	RI, MSD	41, 93, 133, 79	99
9-octadecenoic acid	1925	$C_{18}H_{34}O_2$	-	0.24	RI, MSD	209, 253, 344, 44	93
Phytol	2045	$C_{20}H_{40}O$	*t*	3.41	RI, MSD	71, 57, 41, 123	
Total oil content (%)			80.36	86.02			
Yield (% w/w)			0.51	0.32			

[a]Constituent elution order in column HB-5; [b]KI; [c]Some of the m/z for most abundant peaks in the mass spectrum, [d]Percentage of GC/MS library quality assurance of constituent in SEO/LEO. MSD: Mass spectra data; RI: Retention index relative to carbon 9 - carbon 23 on HB-5 column; Ui: Unidentified constituent, *t*<0.05%. LEO: Leaves essential oil; SEO: Stem essential oil; GC-MS: Gas chromatography-mass spectrometry; KI: Kovat's index; HB: Hemoglobin, QA: Quality assurance

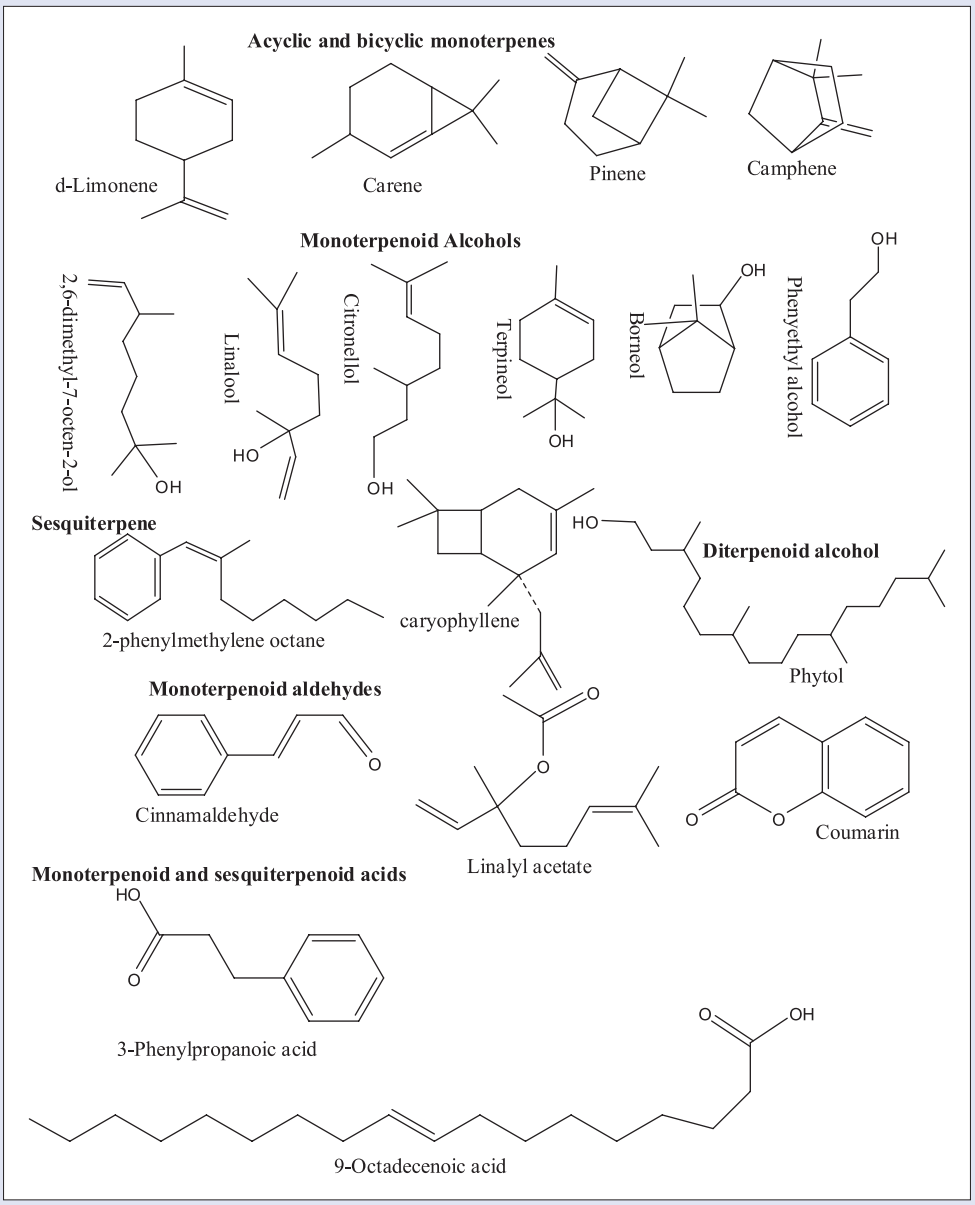

Figure 1: Structures of some major bioactive constituents in *Peperomia pellucida* essential oil

LP•, and NO•) models. The scavenging effect of both EOs and RCs (Vitamin C and β-carotene) on the test radicals were concentration dependent (0.025–0.5 mg/mL). The radical scavenging effects of LEO on DPPH• at increasing doses (0.025, 0.05, 0.10, 0.2, and 0.5 mg/mL) were significantly different ($P < 0.05$) than the SEO as well as the two RCs (++). The SEO and Vitamin C demonstrated comparable effect (ss) at low (0.025–0.10 mg/mL), while at high doses (0.2 and 0.5 mg/mL), scavenging effects of SEO on DPPH• were better (++) than Vitamin C [Figure 2]. The DPPH• scavenging protocol is based on the premise that a substance donating an atom of hydrogen or an electron is an antioxidant or radical scavenger and its property is demonstrated as DPPH• color changes (purple to yellow) in the test sample due to formation of neutral DPPH-H molecule upon receiving H atom from an antiradical.[42] However, DPPH model is not a specific radical species test but general radicals scavenging potency of an antioxidant.[43] Therefore, to evaluate the precise antiradical efficacy of LEO and SEO of *P. pellucida,* we quantitatively investigated the presumed radical scavenging effects using different specific radical (LP• and NO•) and a cation radical (ABTS•+).

In the four-radical scavenging *in vitro* assays, the LEO and SEO of *P. pellucida* showed effective radicals scavenging potencies against the different radicals, indicating that they are good electron or H atom donors to DPPH, ABTS radicals, and exhibited valuable scavenging property against lipid and NO• radicals [Figures 1-4]. Assessing the IC_{50} values from regression equations generated from standard curves as well as *t*-test analysis for significant difference of % scavenging effects versus concentrations, both oils reduced the DPPH• to a neutral DPPH-H molecule attaining 50% decrease with IC_{50} value of 1.67 ± 0.01 mg/mL for LEO and 2.82 ± 0.11 mg/mL for the SEO. The RCs radical scavenging effects on DPPH• (Vitamin C 2.86 ± 0.01 and β-carotene 2.02 ± 0.12 mg/mL) values were significantly lower than LEO ($P < 0.05$) [Table 3].

The scavenging effects on the ABTS radicals by the SEO and Vitamin C were lower compared to results obtained in DPPH assay. However, LEO and β-carotene exhibited high effects especially at low

Table 2: Antibacterial activities of the essential oils *Peperomia pellucida*

Test organism	Essential oils of *P. pellucida*				Controls		
	Leaves oil		Stem oil		Ciprofloxacin[a]		DMSO[b]
	MIC (mg/mL)	MBC (mg/mL)	MIC (mg/mL)	MBC (mg/mL)	MIC (mg/mL)	MBC (mg/mL)	
L. ivanovii (ATCC19119)	0.15±0.02	Bactericidal at 0.15±0.02	0.15±0.03	Bactericidal at 0.20±0.02	0.025±0.01	Bactericidal at 0.012±0.00	VG
S. aureus (NCINB50080)	0.20±0.0 1	NVG Bactericidal at 0.20±0.01	0.20±0.00	NVG Bactericidal at 0.20±0.00	0.05±0.01	NVG Bactericidal at 0.05±0.01	VG
M. smegmatis (ATCC19420)	0.20±0.00	NVG Bacteriostatic at 0.20±0.00	0.20±0.02	NVG Bacteriostatic at 0.20±0.02	0.05±0.02	NVG Bactericidal at 0.05±0.02	VG
E. coli 180*	0.20±0.03	VG Bactericidal at 0.20±0.03	0.20±0.02	VG Bacteriostatic at 0.20±0.02	0.05±0.01	NVG Bactericidal at 0.05±0.01	VG
*V. paraheamolyticus**	0.20±0.01	NVG Bacteriostatic at 0.20±0.01	0.20±0.00	VG Bacteriostatic at 0.20±0.00	0.05±0.01	NVG Bactericidal at 0.05±0.03	VG
E. cloacae (ATCC 13047)	0.20±0.00	VG Bacteriostatic at 0.20±0.00	0.20±0.02	VG Bactericidal at 0.20±0.02	0.025±0.01	NVG Bactericidal at 0.006±0.00	VG
S. uberis (ATCC 29213)	0.20±0.00	VG Bacteriostatic at 0.20±0.01	0.20±0.02	NVG Bacteriostatic at 0.20±0.01	ND	NVG ND	ND

[a]Positive control, [b]Negative control. *Confirmed multidrug-resistant bacteria from our laboratory stock culture. MIC: Minimum inhibitory concentration; MBC: Minimum bactericidal concentration; VG: Visible growth; NVG: No visible growth; ND: Not determined; DMSO: Dimethyl sulfoxide; *P. pellucida*: *Peperomia pellucida*; *L. ivanovii*: *Listeria ivanovii*; *S. aureus*: *Staphylococcus aureus*; *M. smegmatis*: *Mycobacterium smegmatis*; *E. coli*: *Escherichia coli*; *V. paraheamolyticus*: *Vibrio parahaemolyticus*; *E. cloacae*: *Enterobacter cloacae*; *S. uberis*: *Streptococcus uberis*

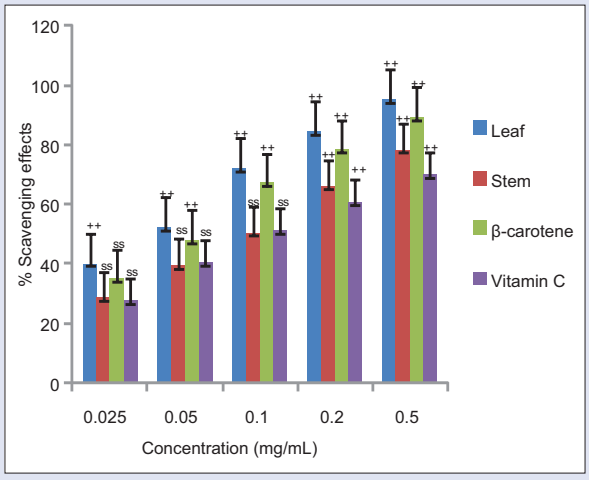

Figure 2: Radical scavenging effects of *Peperomia pellucida* essential oil and reference compounds on 2,2-diphenyl-1-picrylhydrazyl radicals

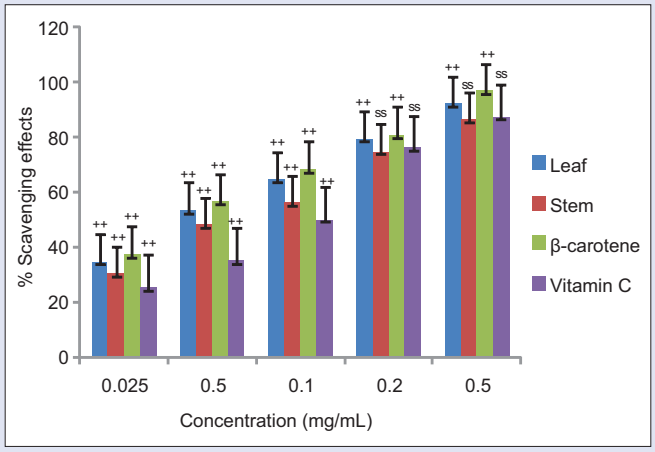

Figure 3: Radical scavenging effects of *Peperomia pellucida* essential oil and reference compounds on 2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt radicals

concentrations [Figure 3]. The LEO IC_{50} value of 1.94 ± 0.11 mg/mL further confirmed its higher radical scavenging strength over the SEO (2.34 mg/mL) and Vitamin C (2.70 mg/mL) indicated in DPPH model. However, unlike in the DPPH assay, the radical scavenger completely decolorized the blue color of the oxidant ($ABTS^{\bullet+}$) solution, turning into neutral molecules (colorless form) from the lowest to highest concentrations (0.025–0.50 mg/mL). The difference observed in activities of SEO and LEO against the two different oxidants ($DPPH^{\bullet}$ and $ABTS^{\bullet+}$) could be attributed to many factors including the complexity, polarity and isomers selectivity of the radicals. In addition, the ease at which the oils solvate the radical's medium may differ and these variables have been suggested to influence potency of volatile constituents in scavenging species of radicals.[43]

The $LP^{\bullet}$ scavenging effects of *P. pellucida* of the two EOs and the RC were concentration-dependent [Figure 4] as in DPPH and ABTS assays. Remarkably, at low concentrations (0.05–0.025 mg/mL), scavenging effects of LEO were above 40% and higher than the RC. However, as the concentration increases (0.2–0.5 mg/mL), SEO exhibited moderate scavenging effects of on $LP^{\bullet}$, while β-carotene and LEO demonstrated higher effects than SEO and Vitamin C. Interestingly, the assessed IC_{50} values from the regression equation

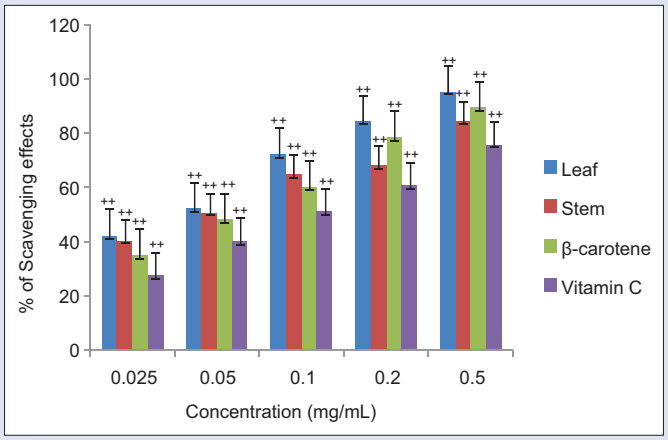

Figure 4: Antiradical effects of *Peperomia pellucida* extracts and reference compounds on lipid peroxyl radicals

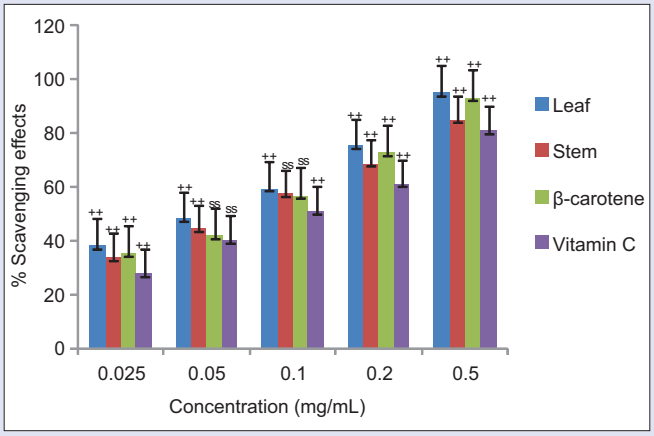

Figure 5: Radical scavenging effects of *Peperomia pellucida* essential oil and reference compounds on nitric oxide radicals

generated from each standard curve, indicated a higher scavenging strength (1.61 ± 0.02 mg/mL) for LEO than the SEO (1.88 mg/mL) as well as the RC. Notable in the lipid peroxidation model is the significant difference between the radical scavenging capacity of EOs and the Vitamin C (2.9 ± 0.00 mg/mL) [Table 3]. This may be ascribed to the bioactive constituents [Figure 1], predominantly aliphatic and aromatic alcohols that might have donated H atoms to H_2O_2, thus reducing it to $2H_2O$.

In the NO• test, the LEO was significantly more (++) effective in scavenging NO• than the SEO and RC at different doses (0.50, 0.20, 0.10, and 0.025 mg/mL) [Figure 5]. Unlike in ABTS at low doses (0.05 and 0.025 mg/mL), the two EOs and RCs demonstrated higher radical scavenging effects. The effects of LEO and SEO were significantly different (++) and superior to RC at 0.05 mg/mL. However, as the concentrations increase to 0.2 mg/mL, scavenging effect differences between the LEO, SEO, and RC were significant (++) with LEO having the highest, followed by β-carotene, then SEO, while Vitamin C had the least effect in scavenging NO• generated [Figure 5]. The LEO IC_{50} value of 2.10 ± 0.11 mg/mL indicated that it has higher radical scavenging strength over β-carotene (2.39 mg/mL) and Vitamin C, while the IC_{50} for SEO (2.40 mg/mL) and β-carotene does not differ significantly (SS) $P < 0.05$ [Table 3].

Table 3: Radical scavenging capacity of essential oils extracted from *Peperomia pellucida* IC_{50} (mg/mL)

Activity	*P. pellucida*		Reference compounds	
	Leaf oil	Stem oil	Vitamin C	β-carotene
DPPH*	1.67±0.01	2.83±0.02	2.86±0.03	2.02±0.02
ABTS*,+	1.94±0.03	2.34±0.01	2.70±0.02	1.71±0.01
LP*	1.61±0.02	1.88±0.01	2.90±0.00	2.12±0.02
NO*	2.10±0.04	2.40±0.03	2.83±0.01	2.39±0.01

*Indicated on Figure 2-5, shows at that particular concentration the compared to each other or control radical scavenging effect are similar, +Indicated on Figure 2-5, shows at that particular concentration the two EOs and controls radical scavenging effect are not similar (significantly different).The IC_{50} (mg/mL) was calculated in regression equation from standard curve for each extract and reference compound. $P<0.05$ was considered significant. Values are mean±SD (n=3). SD: Standard deviation; DPPH: 2, 2-diphenyl-1-picrylhydrazyl; ABTS: 2,2-azino-bis (3 ethylbenzothiazoline-6-sulfonic acid) diammonium salt; LP•: Lipid peroxide radical; NO•: Nitric oxide radical; *P. pellucida*: *Peperomia pellucida*

DISCUSSION

In recent years, few studies on some of the EO constituents we found in the LEO and SEO of *P. pellucida* have reported that some of them are potent bioactive secondary metabolites. For example, limonene,[16] camphene,[44] α-pinene,[45] borneol,[46] and linalool[47] are known to be strong bioactive compounds.[48] Furthermore, the presence of phytol in the LEO and SEO might have enhanced the bioactivity. Phytol, a bioactive diterpenoid alcohol, is often used as a precursor to produce synthetic forms of Vitamin E and Vitamin K_1. Santos *et al.* reported phytol to demonstrate good antioxidant effect *in vivo* as well as its high capacity to scavenge HO•, NO• and prevent the formation of LP• radicals.[49] In addition to phytol, other bioactive terpenoids, including linalyl acetate (10.15%), citronellol (3.40%), phenyl ethyl alcohol (3.18%), and phenylpropanoic acid (3.15%), found in the LEO and SEO might have enhanced the bioactivity of both EOs in this study suggesting synergetic or additive interaction of these constituents in LEO and SEO, especially in scavenging radicals and inhibitory effects on test bacteria.[50,51] Furthermore, the dominant constituent (linalool 12.60%–17.09%) identified in the SEO and LEO could have reacted with DPPH•, ABTS•, LP•, and NO• radicals through various mechanisms suggested by Foti and Amorati.[52] The result in this current study agrees with other reports that have implicated aliphatic terpene with radical scavenging properties, while effect of sesquiterpene (C_{15}), for example, β-caryophyllene (11.47%–12.52%), found in SEO and LEO, is similar to the property of phenolic compounds or alpha tocopherol.[7,13,15,53] The potential to scavenge different radicals and exhibit inhibitory activity against four reference bacterial strains and two bacteria isolates from our laboratory stock culture confirmed to be multidrug-resistant bacterial strains as observed in this current study is quite remarkable. This observation may suggest that LEO of *P. pellucida* could possibly be a new potential candidate for managing infectious diseases as well as oxidative stress-related disorders such as cancers, diabetic nephropathy, Alzheimer's disease, and arteriosclerosis.[53-55]

CONCLUSION

This present study indicates that apart from the traditional uses of *P. pellucida*, the LEO and SEO contained strong bioactive constituents; thus, they could be good candidates as new antimicrobial agents in this present era of increasing multidrug-resistant bacterial strains, also an option to synthetic antioxidant and may be used as food preservatives.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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