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 parahaemolyticus*) 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 IC50 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) diaminonium salt, lipid peroxide 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, 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) diaminonium 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
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.\textsuperscript{[3-5]} Some plants' volatile oils (EOs) have been shown to speedily diffuse cell membrane of bacteria owing to their permeability properties across biological lipid barriers.\textsuperscript{[6,7]} 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.\textsuperscript{[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.\textsuperscript{[10]} However, the scavenging of oxidants generated including superoxide radical, lipid peroxyl radical (LP\textsuperscript{-}), nitric oxide radical (NO\textsuperscript{-}), and hydroxyl (HO\textsuperscript{-}) produced during metabolic activities and environmentally induced radicals overwhelms the naturally produced antiradicals.\textsuperscript{[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.\textsuperscript{[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.\textsuperscript{[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.\textsuperscript{[9,15,16]}

\textit{Peperomia pellucida} (shiny bush, silver bush) of the family \textit{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.\textsuperscript{[17,18]} Ethnomedical reports of \textit{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.\textsuperscript{[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.\textsuperscript{[17,20]} The decoction of the whole plant in India served as potent medication in rheumatism, renal disorders, breast cancer, boils, and small pox.\textsuperscript{[14,21,22]} 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.\textsuperscript{[19]} Another study by Xu \textit{et al.}\textsuperscript{[23]} of the solvents leaves crude extracts indicated alkaloids, sterols, flavonoids, and styrene as dominant bioactive compounds of \textit{P. pellucida}. Previous investigation of the leaf essential oil (LEO) revealed apiol and β-caryophyllene as the major volatile compounds.\textsuperscript{[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 \textit{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 \textit{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

\textit{P. pellucida} was collected in August 2016 from Southwest Nigeria at the Forest Research Institute of Nigeria, Ibadan. A 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.\textsuperscript{[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 Zebrom-5MS (ZBMS 30 m × 0.25 mm × 0.25 μm) (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\textsuperscript{[26,27]} were used for the antibacterial test. The reference and laboratory bacterial strains consist of four Gram-positive bacteria: \textit{Staphylococcus aureus} (NCINB 50080), \textit{Listeria ivanovii} (ATCC 19119), \textit{Mycobacterium smegmatis} (ATCC 19420), \textit{Streptococcus uberis} (ATCC 29213) and three Gram-negative bacteria: \textit{Enterobacter cloacae} (ATCC 13047), \textit{E. coli} 180, and \textit{Vibrio parahaemolyticus}. All the test strains were confirmed to be resistant to ampicillin, cefuroxime, tetracycline, nalidixic, cephalaxin, sulfamethoxazole, and streptomycin.\textsuperscript{[27]} They 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 × 10⁶ 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.[30] The experiment was carried out in parallel triplicate and average value was recorded.

Antiradical assays

DPPH, ABTS⁺, NO⁻, and LP⁺ 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 was measured using a microplate reader. The result was expressed as percentage inhibition of DPPH radical scavenging per concentration of the EO or RC.

The equation (a) described in DPPH radical test. The assay was carried out in parallel triplicate and mean value calculated.

\[
\text{IC}_{50} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100
\]

Measurement of the inhibition of nitric oxide radicals assay

The radical scavenging effect of the EO on NO⁻ was investigated according to the modified method described by Makhija et al.[31] Sodium nitroprusside molecule in aqueous solution at physiological pH (7.2) decomposed to produce NO⁻ 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 triplicate 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 IC₅₀ 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
The radical scavenging activities of the EOs (LEO and SEO) were studied in vitro in four different oxidants (DPPH, ABTS, 

Table 1: Essential oils constituents of Peperomia pellucida

| Constituent* | KI§ | Molecular formulae | Percentage composition | Methods of identification | MSD* | QA# |
|--------------|-----|--------------------|------------------------|----------------------------|------|-----|
|              |     |                    | LEO                    |                            |      |     |
| Phenylethyl alcohol | 856 | C14H20O | 3.81 | 5.22 | RI, MSD | 81, 69, 55, 108 | 94 |
| Coumarin      | 879 | C13H10O2        | 3.46 | 0.42 | RI, MSD | 161, 69, 25, 141 | 98 |
| 3-phenylpropanoic acid | 897 | C10H14O       | 3.15 | -    | RI, MSD | 81, 69, 55, 136 | 98 |
| α-pinene      | 927 | C10H16          | 0.46 | 0.07 | RI, MSD | 93, 79, 41, 136 | 99 |
| Camphene      | 940 | C10H16          | 0.43 | 1.40 | RI, MSD | 93, 69, 41, 77 | 99 |
| d-limonene    | 950 | C10H16          | 14.25 | 10.73 | RI, MSD | 93, 68, 136, 79 | 95 |
| (-)-4-carene  | 985 | C10H16          | t   | 4.84 | RI, MSD | 145, 41, 135, 128 | 90 |
| Linalool      | 990 | C10H16          | 17.09 | 12.60 | RI, MSD | 113, 71, 44, 29 | 93 |
| α-terpineol   | 992 | C10H16          | 2.49 | 10.57 | RI, MSD | 41, 71, 93, 111 | 99 |
| Borneol       | 1116 | C15H22O2      | t   | 6.45 | RI, MSD | 43, 95, 41, 105 | 99 |
| (+)-terpinen-4-ol | 1128 | C10H16O     | -   | 0.25 | RI, MSD | 71, 93, 111, 41 | 96 |
| 2,6-dimethyl-7-octen-2-ol | 1145 | C13H22O        | 6.55 | 3.80 | RI, MSD | 73, 44, 113, 28 | 96 |
| Citronellol   | 1220 | C15H22O2      | 3.40 | 0.07 | RI, MSD | 43, 41, 77, 55, 78 | 98 |
| Linalyl acetate | 1372 | C13H22O2     | 11.67 | 4.86 | RI, MSD | 71, 43, 68, 109 | 91 |
| Phenylmethylen octane | 1412 | C15H22       | 0.46 | 4.63 | RI, MSD | 14, 29, 57, 129 | 93 |
| Ui            | 1413 | -               | -   | 0.54 | RI, MSD | 14, 57, 91, 111 | 34 |
| β-caryophyllene | 1415 | C20H30        | 12.52 | 11.47 | RI, MSD | 41, 93, 133, 79 | 99 |
| 9-octadecenoic acid | 1925 | C19H38O3    | t   | 0.24 | RI, MSD | 209, 253, 344, 44 | 93 |
| Phyto1       | 2045 | C15H22O2      | t   | 3.41 | RI, MSD | 71, 57, 41, 123 | 93 |
| Total oil content (%) | 80.36 | -               | 86.02 | -     | -     | -     | -     |
| Yield (% w/w) | 0.51 | -               | 0.32  | -     | -     | -     | -     |

*Constituent elution order in column HB-5; §KI: Some of the m/z for most abundant peaks in the mass spectrum; *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, <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

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

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 IC50 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 IC50 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                     | Ciprofloxacin*            | DMSO*                     |
|                            | Essential oil                  | MIC (mg/mL) | MBC (mg/mL) | 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 | 0.05±0.01 Bactericidal at 0.05±0.01 | 0.05±0.01 Bactericidal at 0.05±0.01 |
| *S. aureus (NCINB50080)*   | 0.20±0.01 Bactericidal at 0.20±0.01 | 0.20±0.00 Bacteriostatic at 0.20±0.00 | 0.20±0.01 Bactericidal at 0.05±0.01 | 0.05±0.01 Bactericidal at 0.05±0.01 | 0.05±0.01 Bactericidal at 0.05±0.01 |
| *M. smegmatis* (ATCC19420) | 0.20±0.00 Bacteriostatic at 0.20±0.00 | 0.20±0.02 Bacteriostatic at 0.20±0.02 | 0.05±0.02 Bactericidal at 0.05±0.02 | 0.05±0.02 Bactericidal at 0.05±0.02 | 0.05±0.02 Bactericidal at 0.05±0.02 |
| *E. coli 180*              | 0.20±0.03 Bactericidal at 0.20±0.03 | 0.20±0.02 Bacteriostatic at 0.20±0.02 | 0.20±0.01 Bacteriostatic at 0.20±0.00 | 0.05±0.01 Bactericidal at 0.05±0.01 | 0.05±0.01 Bactericidal at 0.05±0.01 |
| *V. paraheamolyticus*      | 0.20±0.01 Bacteriostatic at 0.20±0.01 | 0.20±0.00 Bacteriostatic at 0.20±0.00 | 0.20±0.01 Bacteriostatic at 0.05±0.03 | 0.05±0.01 Bactericidal at 0.05±0.01 | 0.05±0.01 Bactericidal at 0.05±0.01 |
| *E. cloacae* (ATCC 13047)  | 0.20±0.00 Bacteriostatic at 0.20±0.00 | 0.20±0.02 Bacteriostatic at 0.20±0.02 | 0.20±0.01 Bacteriostatic at 0.20±0.00 | 0.05±0.01 Bactericidal at 0.05±0.01 | 0.05±0.01 Bactericidal at 0.05±0.01 |
| *S. uberis* (ATCC 29213)   | 0.20±0.00 Bacteriostatic at 0.20±0.00 | 0.20±0.02 Bacteriostatic at 0.20±0.01 | 0.20±0.01 Bacteriostatic at 0.20±0.01 | ND | ND | ND |

*Positive control, *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

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’+) 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’ and ABTS’+) 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’ 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’, while β-carotene and LEO demonstrated higher effects than SEO and Vitamin C. Interestingly, the assessed IC_{50} values from the regression equation...
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 H₂O₂, thus reducing it to 2H₂O.

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 IC₅₀ 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 IC₅₀ for SEO (2.40 mg/mL) and β-carotene does not differ significantly (SS), P < 0.05 [Table 3].

**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,[35] camphene,[41] 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. 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 synergistic 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.[32] The result in this current study agrees with other reports that have implicated aliphatic terpene with radical scavenging properties, while effect of sesquiterpene (C₅), for example, β-caryophyllene (11.47%–12.52%), found in SEO and LEO, is similar to the property of phenolic compounds or alpha tocopherol.[7,13,15,43] 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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Conflicts of interest
There are no conflicts of interest.

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