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
		VG		NVG			

[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*

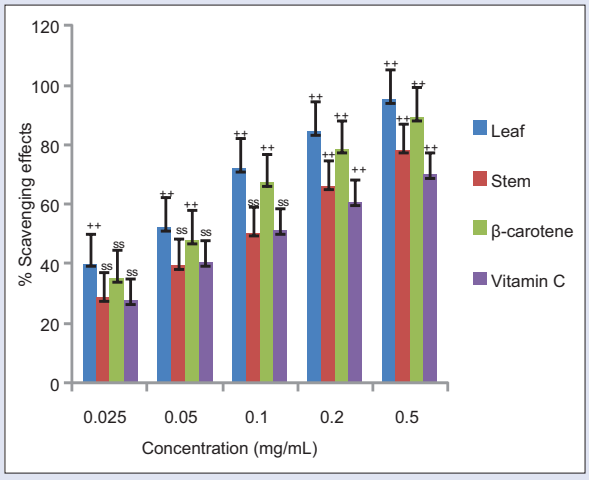

Figure 2: Radical scavenging effects of *Peperomia pellucida* essential oil and reference compounds on 2,2-diphenyl-1-picrylhydrazyl radicals

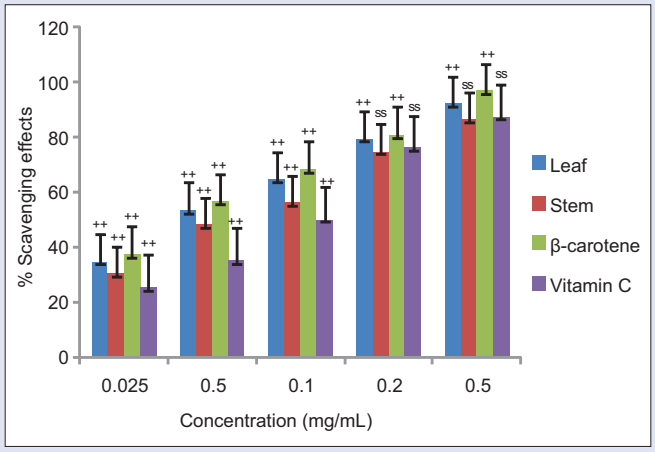

Figure 3: Radical scavenging effects of *Peperomia pellucida* essential oil and reference compounds on 2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt radicals

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

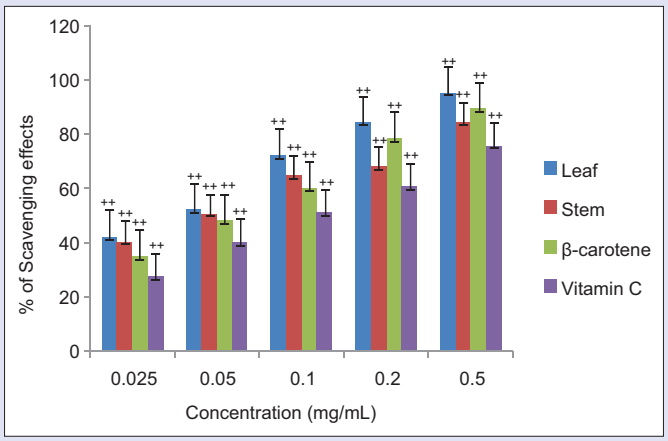

Figure 4: Antiradical effects of *Peperomia pellucida* extracts and reference compounds on lipid peroxyl radicals

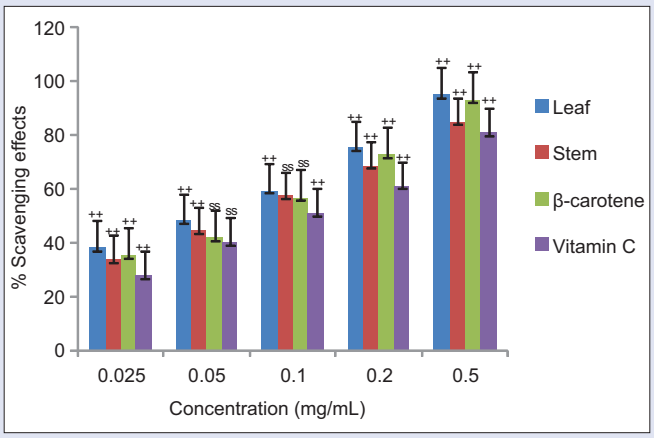

Figure 5: Radical scavenging effects of *Peperomia pellucida* essential oil and reference compounds on nitric oxide radicals

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.

Acknowledgement

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Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Dickson RA, Houghton PJ, Hylands PJ, Gibbons S. Antimicrobial, resistance-modifying effects, antioxidant and free radical scavenging activities of *Mezoneuron benthamianum* baill. *Securinega virosa* roxb. & Wlld. and *Microglossa pyrifolia* lam. Phytother Res 2006;20:41-5.
2. Aly M, Balkhy HH. The prevalence of antimicrobial resistance in clinical isolates from gulf corporation council countries. Antimicrob Resist Infect Control 2012;1:26.
3. Van VS, Kamatou GP, Viljoen AM. Volatile composition and antimicrobial activity of twenty commercial frankincense essential oil samples. S Af J Bot 2010;76:686-91.
4. Prakash D, Gupta C, Sharma G. Importance of phytochemicals in nutraceuticals. J Chin Med Res Dev 2011;1:70-8.
5. Valente J, Zuzarte M, Gonçalves MJ, Lopes MC, Cavaleiro C, Salgueiro L, *et al.* Antifungal, antioxidant and anti-inflammatory activities of *Oenanthe crocata* L. essential oil. Food Chem Toxicol 2013;62:349-54.
6. Rammanee K, Hongpattarakere T. Effects of tropical citrus essential oils on growth, afflatoxin production and ultrastructure alterations of *Aspergillus flavus* and *Aspergillus parasiticus*. Food Bioproc Tech 2011;4:1050-9.
7. Bakkali F, Averbeck S, Averbeck D, Idaomar M. Biological effects of essential oils – A review. Food Chem Toxicol 2008;46:446-75.
8. Helander IM, Akakomi H, Latva-Kala K, Mattila-Sandholm T, Pol I, Eddy J. Characterization of the action of selected essential oil components on gram-negative bacteria. J Agric Food Chem 1998;46:3590-5.
9. Hyldgaard M, Mygind T, Meyer RL. Essential oils in food preservation: Mode of action, synergies, and interactions with food matrix components. Front Microbiol 2012;3:12.
10. Murray AP, Rodriguez S, Frontera MA, Tomas MA, Mulet MC. Antioxidant metabolites from *Limonium brasiliense* (Boiss.) Kuntze. Z Naturforsch C 2004;59:477-80.
11. Nagendrappa CG. An appreciation of free radical chemistry-3, free radicals in diseases and health. Resona 2005;10:65-73.
12. Saikat S, Chakraborty R, Sridhar CY, Reddy SR, Biplab D. Free radicals, antioxidants diseases and phytomedicine: Current status and future prospect. Int J Pharm Sci Rev Res 2010;3:91-100.
13. Wang W, Wu N, Zu YG, Fu YJ. Antioxidative activity of *Rosmarinus officinalis* L. essential oil compared to its main components. Food Chem 2008;108:1019-22.
14. Nweze EI, Okafor JI. Activities of a wide range of medicinal plants and Essential oil vs. *Scedospaorium isolates*. Am Eur J Res 2010;5:161-9.
15. Kotamballi N, Chidambara M, Jayaprakasha GK, Bhimanagouda SP. Antiinflammatory mediated applications of monoterpenes found in fruits. Am Chem Soc 2013;10:121-31.
16. Penoel MD, Penoel R. Natural Health Care Using Essential Oils. Abundant Health. New Zealand; 2012. Available from: http://www.abundanthealth4u.com.
17. Habsah M, Yosie A, Baker K, Siang CC, Syamsumir DF, Alias A, *et al.* Effect of drying method on antimicrobial, antioxidant and isolation of bioactive from *P. pellucida*. J Chem Pharm Res 2015;7:578-84.
18. Oloyede KG, Onocha AP, Olaniran BB. Phytochemical, toxicity, antimicrobial and antioxidant screening of leaf extracts of *P. pellucida*. Adv Environ Biol 2011;5:3700-9.
19. Wei LS, Wee W, Siong JY, Syamsumir DF. Characterization of anticancer, antimicrobial, antioxidant properties and chemical compositions of *Peperomia pellucida* leaf extract. Acta Med Iran 2011;49:670-4.
20. Aziba PI, Adedeji A, Ekor M, Adeyemi O. Analgesic activity of *Peperomia pellucida* aerial parts in mice. Fitoterapia 2001;72:57-8.
21. Khan A, Rahman M, Islam MS. Neuropharmacological effects of *Peperomia pellucida* leaves in mice. DARU 2008;16:35-40.
22. de Fátima Arrigoni-Blank M, Dmitrieva EG, Franzotti EM, Antoniolli AR, Andrade MR, Marchioro M, *et al.* Anti-inflammatory and analgesic activity of *Peperomia pellucida* (L.) HBK (*Piperaceae*). J Ethnopharmacol 2004;91:215-8.
23. Xu S, Li N, Ning MM, Zhou CH, Yang QR, Wang MW, *et al.* Bioactive compounds from *Peperomia pellucida*. J Nat Prod 2006;69:247-50.
24. Helio LS, Maria GB, Eloisa HA. Andrade, JGS. The essential oils of *Peperomia pellucida* Kunth and *P. circinnata* Link. Flavour Fragr J 1999;14:312-4.
25. Okoh SO, Asekun OT, Familoni OB, Afolayan AJ. Composition and antioxidant activities of leaf and root volatile oils of *Morinda lucida*. Nat Prod Commun 2011;6:1537-41.
26. Iweriebor BC, Iwu CJ, Obi LC, Nwodo UU, Okoh AI. Multiple antibiotic resistances among Shiga toxin producing *Escherichia coli* O157 in feces of dairy cattle farms in Eastern cape of South Africa. BMC Microbiol 2015;15:213.
27. Iwu CJ, Iweriebor BC, Obi LC, Basson AK, Okoh AI. Multidrug-resistant *Salmonella* isolates from swine in the Eastern Cape Province, South Africa. J Food Prot 2016;79:1234-9.
28. Ramalivhana JN, Iweriebor BC, Obi CL, Samie A, Uaboi-Egbenni P, Idiaghe JE, *et al.* Antimicrobial activity of honey and medicinal plant extracts against Gram negative microorganisms. Afr J Biotechnol 2014;13:616-25.
29. Liyana-Pathirana CM, Shahidi F. Antioxidant properties of the essential oils from lemon, grape, coriander, clove and their mixtures. J Sci Food Agric 2006;86:477.
30. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C, *et al.* Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic Biol Med 1999;26:1231-7.
31. Witayapan N, Sombat C, Siriporn O. Antioxidant and antimicrobial activities of *Hyptis suaveolens* essential oil. Sci Pharm 2007;75:35-46.
32. Badmus JA, Odunola OA, Obuotor E. Phytochemical and *in vitro* antioxidant potentials of *Holarrhena floribunda* leaf. Afr J Biotechnol 2010;9:340-6.
33. Makhija IK, Aswatha RH, Shreedhara CS, Vijay KS, Devkar R. *In-vitro* antioxidant studies of Sitopaladi churna, a polyherbal ayurvedic formulation. Free Radic Antioxid 2011;1:37-41.
34. Giustarini D, Rossi R, Milzani A, Dalle-Donne I. Nitrite and nitrate measurement by Griess reagent in human plasma: Evaluation of interferences and standardization. Methods Enzymol 2008;440:361-9.
35. Okoh OO, Afolayan AJ. The effects of hydrodistillation and solvent free microwave extraction methods on the chemical composition and toxicity of essential oils from the leaves of *Mentha longifolia* L. Afr J Pharm Pharm 2011;5:2474-8.
36. Potzernheim MC, Bizzo HR, Silva JP, Vieira RF. Chemical characterization of essential oil constituents of four populations of *P. aduncum* L. from Distrito Federal, Brazil. Biochem Syst Ecol 2012;42:25-31.
37. Rajendra CP, Prakash G, Amit C. Essential oil composition of *Peperomia pellucida* (L.) Kunth from India. J Essent Oil Res 2015;27:89-95.
38. Valero D. The addition of essential oils to MAP as a tool to maintain the overall quality of fruits. Trends Food Sci Tech 2008;19:464-71.
39. Perussi JR. Photodynamic inactivation of microorganisms. Quim Nova 2007;30:988-94.
40. Guerrini A, Sacchetti G, Rossi D, Paganetto G, Muzzoli M, Andreotti E, *et al.* Bioactivities of *Piper aduncum* L. and *Piper obliquum* ruiz & Pavon (*Piperaceae*) essential oils from Eastern Ecuador. Environ Toxicol Pharmacol 2009;27:39-48.
41. Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J, *et al.* Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol 2007;39:44-84.
42. Vallianou I, Peroulis N, Pantazis P, Hadzopoulou-Cladaras M. Camphene, a plant-derived monoterpene, reduces plasma cholesterol and triglycerides in hyperlipidemic rats independently of HMG-CoA reductase activity. PLoS One 2011;6:e20516.
43. Elanur A, Hasan T, Fatime G. Antioxidative, anticancer and genotoxic properties of α-pinene on N2a neuroblastoma cells. Biologia 2013;68:1004-9.
44. Horvathova E, Katarina K, Srancikova A, Hunakova L, Galova E, Sevcovicova A, Slamenova D. Borneol administration protects primary rat hepatocytes against exogenous oxidative DNA damage. Oxf J Life Sci 2012;27:581-8.
45. Ganesan P, Phaiphan A, Mourugan Y, Baharin B. Comparative study of bioactive compounds in curry and coriander leaves – An update. J Chem Pharm Res 2013;5:590-4.
46. Duke JA. Phytochemical and Ethno-Botanical Databases. Florida USA: CRS Press, Inc.; 2008.
47. Santos CC, Salvadori MS, Mota VG, Costa LM, de Almeida AA, de Oliveira GA, *et al.* Antinociceptive and antioxidant activities of phytol *in vivo* and *in vitro* models. Neurosci J 2013;2013:949452.
48. Trombetta D, Castelli F, Sarpietro MG, Venuti V, Cristani M, Daniele C, *et al.* Mechanisms of

antibacterial action of three monoterpenes. Antimicrob Agents Chemother 2005;49:2474-8.

49. Probst IS, Sforcin JM, Rall VL, Fernandes AA, Fernandes A. Antimicrobial activity of propolis and essential oils and synergism between these natural products. J Vernom Anim Toxins Incl Trop Dis 2011;17:157-69.
50. Naito Y, Uchiyama K, Yoshikawa T. Oxidative stress involvement in diabetic nephropathy and its prevention by astaxanthin. Oxid Stress Disord 2006;21:235-42.
51. Foti MC, Amorati R. Non-phenolic radical-trapping antioxidants. J Pharm Pharmacol 2009;61:1435-48.
52. Paz-Elizur T, Sevilya Z, Leitner-Dagan Y, Elinger D, Roisman LC, Livneh Z. DNA repair of oxidative DNA damage in human carcinogenesis: Potential application for cancer risk assessment and prevention. Cancer Lett 2008;266:60-2.
53. Fini MA, Johnson RJ, Stenmark KR, Wright RM. Hypertension, nitrite, xanthine oxidoreductase catalyzed nitric oxide generation: Pros and cons. Hypertension 62, e9. *floribunda* leaf. Afr J Biotech 2013;9:340-6.