JOPAT Vol 20(2) 714– 727, July – Dec. 2021 Edition.

ISSN2636 – 5448 https://dx.doi.org/10.4314/jopat.v20i2.7

Chemical Constituents, Antimicrobial and Antioxidant Activities of the Essential Oil of Leaves of *Dombeya buettneri* K. Schum. (Sterculiaceae)

Deborah O. Idowu[1], Philip A. Idowu [2*] and Olapeju O. Aiyelaagbe[1]

ABSTRACT

The essential oil of the leaves of *Dombeya buettneri* was investigated for chemical composition, antioxidant and antimicrobial activities. The oil was separated by Gas Chromatography (GC) and analysed by Gas Chromatography/Mass Spectrometry (GC-MS) methods. Antimicrobial activity was evaluated by broth dilution method to determine the minimum inhibitory concentration (MIC) on *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922 and *Candida albicans* ATCC 18804 while antioxidant activity was determined by three methods (DPPH, FRAP and ABTS).

The GC-MS detected a total of 23 compounds (93.9% identification) which were dominated by Apocarotenes (64.9%) and Non-terpene derivatives (20.2%), the main compounds identified being hexahydrofarnesylacetone (32.5%), dihydroedulan IIA (9.6%), *(E)* –geranylacetone (7.8%), β-selinene (5.4%) and 2-pentyl furan (4.6%). The oil showed a good antimicrobial activity with respective MIC and MBC of 6.25 and 12.50%v/v on *Staph. aureus*, 12.5 and 25.0 %v/v on *E. coli*; MIC and MFC on *Candida albicans* were 12.5 and 25.0%v/v respectively. The free radical antioxidant activity (DPPH expressed as % inhibition) at 25, 50 and 100 mg/mL were 8.0993±0.827, 14.7657±0.8151 and 19.449±0.6678 respectively. The antioxidant potential (FRAP) was 0.076713±0.012275 mg/$FeSO_4$.eq/g. The result of 2, 2-azobis-3-ethylbenzothiazoline-6-sulfonate radical scavenging ability (ABTS) showed the oil to possess free radical scavenging ability of 0.37759±0.0025 mg/AA Equiv./g.

The leaves of *Dombeya buetnneri* possess essential oils that are rich in phytochemicals with good antimicrobial and antioxidant activities; which can be of significant application in food, drug and cosmetic industries.

Word count: 233

Keywords: *Dombeya buettneri* leaves, Essential oil, Gas Chromatography, antimicrobial activity, antioxidant activity.

Corresponding author: * P.A. Idowu. Email: igboyega@yahoo.com. Phone: +234 8033524399.

Introduction

Medicinal plants are found to produce interesting and biologically active secondary metabolites which include essential oils. Essential oils are odoriferous, natural products containing complex mixtures of volatile constituents. Apart from the fragrance nature of essential oils that makes them attractive, many have biological activities useful in cosmetic and pharmaceutical industries. Testing essential oils for bioactivity has led to the discovery of many therapeutic chemical compounds with antibacterial, antifungal, antiviral, antiplasmodial, antioxidant, antitumour and anti-inflammatory activities. Many of such compounds are monoterpenes, diterpenes and sesquiterpenes in nature and can as well be oxygenated to form alcohols, ketones, aldehydes, oxides and phenolics.

Evidence from previous studies have indicated that essential oils may be potent antibacterial agents and as such could combat pathogenic bacterial species [1], as well as treat diseases associated with free radicals [2]. Components of essential oils are abundant and a vast number have been shown to possess antimicrobial activity, mainly; aromatic hydrocarbons, phenylpropenes, aliphatic and cyclic terpenoids [3]. Considering the mechanism of antimicrobial activity, essential oils are known to diffuse quickly through bacterial cell membranes resulting in increased cell membrane permeability [4], leading to subsequent leakage of vital intracellular constituents [5] and eventual cell apoptosis. Cell membrane, intracellular proteins, enzymes and nucleic acids are important target sites for drug design and some essential oils constituents have been implicated to target these locations [6-7]. Enzymatic endogenous antioxidants including catalase, superoxide dismutase, glutathione peroxidase attempt to get rid of oxidants in a physiological process, however, the constellation of radicals generated including lipid peroxyl ($LP^{\bullet}$), superoxide ($O_2^{\bullet}$), nitric oxide ($NO^{\bullet}$), hydroxyl ($HO^{\bullet}$), consequent to metabolic activities and environmentally induced stress factors, overwhelms the naturally produced antioxidants. In recent years, phytochemical studies have reported that secondary metabolites including alkaloids, flavonoids, and phenols from plants and their essential oils exhibit strong antioxidant activity [5-8]. Essential oils may serve as a plausible alternative to synthetic antibiotics and antioxidants because they are readily available and well tolerated by human bodily systems [9]. Essential oil constituents including carvacrol, carvone, caryophyllene, limonene and thymol have been reported to possess antimicrobial and antioxidant properties [7, 10-11].

Dombeya buettneri leaves essential oil has not been previously reported for antimicrobial and antioxidant activities. Hence, the aim of the present study is to analyze and report for the first time the chemical compounds in *Dombeya buettneri* leaves and subject the oil to antimicrobial and antioxidant studies.

Materials and Methods

Sample Collection and Essential Oil Isolation

Leaf samples of the plant were collected fresh from Olokemeji Forest Reserve in Ogun State, Nigeria in August, 2020, the plant was authenticated at the herbarium section of Forestry Research Institute of Nigeria (FRIN), Ibadan where voucher specimens (FHI Number 112907) were deposited.

Distillation of Essential Oil

Aliquots of 200 g of air-dried and pulverized leaves were subjected to hydrodistillation for 4 h at normal pressure in a glass Clevenger-type apparatus according to the British Pharmacopoeia specifications [12].

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis and Identification of Components.

Gas Chromatographic (GC) analyses of the essential oils were performed on a HP-5890 Gas chromatograph equipped with a HP-Wax and HP-5 capillary columns (30 m x 0.25 mm, film thickness of 0.25 mm). The GC oven temperature which was programmed at 60 °C was held for 10 min and heated to 220 °C at 5 °C/min. The temperature for both the injector and the detector was maintained at 250 °C. The carrier gas used was Helium at a flow rate of 2 mL/min. The Gas

Chromatographic-Mass Spectrometry (GC-MS) analyses were carried out on a Varian CP-3800 gas chromatograph interfaced to a Varian Saturn 2000 ion trap Mass Detector operated at 70 eV. The injector and transfer line temperatures were 220 °C and 240 °C, respectively. The GC oven temperature was programmed from 60-240 °C at 3 °C/min. Helium was used as a carrier gas at a flow rate of 1 mL/min.

Identification of the Components

The constituents of the oils were identified on the basis of comparison of the retention times with those of the reference standard samples, comparing their retention indices relative to the series of n-hydrocarbons, and by comparison of their mass spectra with published spectra and those of reference compounds [13]. The relative concentration of each constituent was calculated by integration of gas chromatography peak areas.

Determination of Antimicrobial Activity

Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) of the essential oil was determined using broth microdilution technique [14]. Suspensions of the test organisms in 0.85% normal saline with turbidity equivalent to that of 0.5 McFarland Standard (1.5×10^8 cfu/mL) were inoculated into broth microdilution plates already containing different dilutions of the oil in 100 µL of Tryptone Soy Broth (TSB). The plates were incubated at 37 ^{o}C for 24 hours. The plates were examined for growth by the addition of tetrazolium salt as colour indicator. A change in

colour to red or pink is indicative of growth of the test organisms. The lowest concentration of the oil showing absence of growth is taken as the MIC. Two columns of the titre plate containing broth and gentamicin (10 µg/mL) and broth alone were used as positive and negative control respectively. All tests were carried out in triplicates.

Determination of Minimum Bactericidal/Fungicidal Concentration (MBC/MFC)

The MBC (on *Staph aureus* and *E. coli*) and MFC (on *Candida albicans*) were determined as a subsequent step to MIC determination above. Wells representing the MIC and two more of higher concentrations that showed no growth were used, the antimicrobial agent (oil) was neutralised in phosphate buffer pH 7-0. Aliquots were inoculated into 5mls sterile Nutrient Broth (for bacteria) and Tryptic Soy Broth (for fungi) and were incubated at 37 ^{o}C (25 ^{o}C for fungi) for 24 hours. The tubes were clearly labelled according to the concentrations of the oil present in the titre wells. The minimum concentration with no visible growth was taken as the MBC/MFC [15].

Determination of antioxidant activity

Determination of 1, 1-diphenyl-2-picrylhydrazyl scavenging activity (DPPH)

DPPH radical scavenging activity of the samples was done using the [16] method with slight modification. Appropriate dilutions of 1.0 mL (0.1 - 0.4 mg/mL) sample were added to 4 mL of DPPH solution (30 mg/l) prepared in methanol. The samples were mixed thoroughly and left in the dark for 30 minutes. The absorbance was read at 520 nm. The inhibition percentage was calculated as:

$$\%\ inhibition\ of\ DPPH = \frac{Abs_{control} - Abs_{sample}}{A_{control}} \times 100$$

DPPH solution without sample served as control.

Determination of Ferric Reducing Antioxidant Potential (FRAP)

FRAP of the oil samples was done using the method reported by Benzie and strain [17]. The FRAP working reagent was freshly prepared by mixing solutions of 25 mL acetate buffer, 2.5 mL TPTZ solution, and ferric chloride in ratio 10:1:1 and warmed at 37 °C before use. Samples (0.2 mL) were mixed with 2.80 mL of the FRAP reagent and the mixtures were kept in the dark for 30 min at room temperature. The absorbance was read at 593 nm and FRAP was evaluated from ferrous sulphate standard curve and expressed as (mg Fe^{2+}E/g)

Determination of Radical Scavenging Ability Using 2, 2-azobis-3-ethylbenzothiazoline-6-sulfonate (ABTS) Method

The ABTS scavenging ability of the extracts were determined as described by [18]. ABTS was generated by reacting ABTS aqueous solution (7 mM) with $K_2S_2O_8$ (2.45 mM) in darkness for 16 hr and adjusting the absorbance 734 nm to 1.320 with ethanol. About 0.2 mL of the extract was added to 2.0 mL ABTS solution and the absorbance was measured at 734 nm using a spectrophotometer after 15 min. Ascorbic acid was used as standard. Then Ascorbic acid equivalent was subsequently calculated and reported as mg AAE/g.

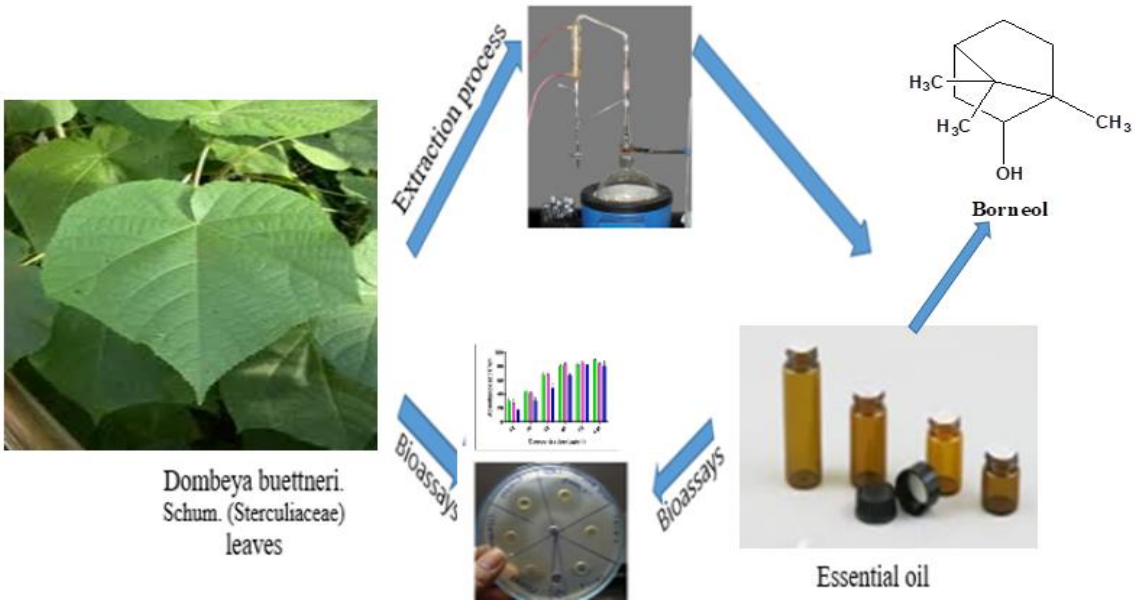

Fig. 1.0: Graphical representation of methodology.

RESULTS AND DISCUSSION

Essential oil composition of *Dombeya buettneri* leaves

Thirty-two essential oil components detected in the leaves of *Dombeya buettneri* are listed in Table 1.0 and they include the following: Benzaldehyde (1.0%), 1-octen-3-ol (0.7%), 2-methyl-3-octanone (2.0%), 6-methyl-5-hepten-2-one (3.1%), 2-pentyl furan (4.6%), Myrcene (2.3%), Nonanal (2.8%), *(E)* -2-nonenal (0.9%), Borneol (1.1%), Safranal (1.5%), β-cyclocitral (1.2%), dihydroedulan IIA (9.6%), dihydroedulan IA (2.2%), theaspirane I (1.6%), theaspirane II (2.2%), *(E)* –β-damascenone (3.3%), ϒ-dihydroionone (1.9%), 2-dodecanone (3.2%), *(E)* –geranylacetone (7.8%), dehydro-β-ionone (1.1%), β-selinene (5.4%), pentadecanal (1.9%) and hexahydrofarnesylacetone (32.5%) (Table 1.0)

Table 1.0: Chemical compounds of the essential oil of *Dombeya buetnneri* leaves

No	Compounds	LRI	Abundance (%)
1	Benzaldehyde	962	1.0
2	1-octen-3-ol	981	0.7
3	2-methyl-3-octanone	985	2.0
4	6-methyl-5-hepten-2-one	987	3.1
5	2-pentyl furan	992	4.6
6	Myrcene	993	2.3
7	Nonanal	1102	2.8
8	*(E)* -2-nonenal	1163	0.9
9	Borneol	1168	1.1
10	Safranal	1199	1.5
11	β-cyclocitral	1222	1.2
12	dihydroedulan IIA	1285	9.6
13	dihydroedulan IA	1291	2.2
14	theaspirane I	1298	1.6
15	theaspirane II	1315	2.2
16	*(E)* –β-damascenone	1386	3.3
17	ϒ-dihydroionone	1396	1.9
18	2-dodecanone	1397	3.2
19	*(E)* –geranylacetone	1456	7.8
20	dehydro-β-ionone	1484	1.1
21	β-selinene	1485	5.4
22	Pentadecanal	1712	1.9
23	hexahydrofarnesylacetone	1845	32.5
	Monoterpene hydrocarbons		2.3
	Oxygenated monoterpenes		1.1
	Sesquiterpene hydrocarbons		5.4
	Oxygenated sesquiterpenes		0.0
	Apocarotenes		64.9
	Non-terpene derivatives		20.2
	Total identified		93.9

Key: LRI = Retention index, 1-23 = number of compounds.

The oil showed good antimicrobial activity with an MIC and MBC activities of 6.25 and 12.50 %v/v respectively against *Staph. aureus*, 12.5 and 25.0 %v/v respectively against *E. coli*. and MIC/MFC of 12.5/25.0 %v/v on *Candida albicans* (Table 2.0).

Table 2.0: Results of Antimicrobial Activity of Essential Oil of *Dombeya buettneri* Leaves

Antimicrobial parameter	*Staphylococcus aureus*	*Escherichia coli*	*Candida albicans*
MIC %v/v	6.25	12.5	12.5
MBC %v/v	12.50	25.0	-
MFC %v/v	-	-	25.0

Key:

MIC = Minimum Inhibitory Concentration

MBC = Minimum Bactericidal Concentration

MFC = Minimum Fungicidal Concentration

The 1, 1-diphenyl-2-picrylhydrazyl (DPPH) scavenging activity of the oils of *Dombeya buettneri* leaves showed the percentage inhibitions at different concentration (much lower than the ascorbic acid standard) as follows. The activity of the oil at the lowest concentration of 25 mg/mL was 8.0993±0.827 expressed as % inhibition. The activity at 50 mg/mL was 14.7657±0.8151 while the activity at 100 mg/mL was 19.449±0.6678 as shown in Fig. 3.0.

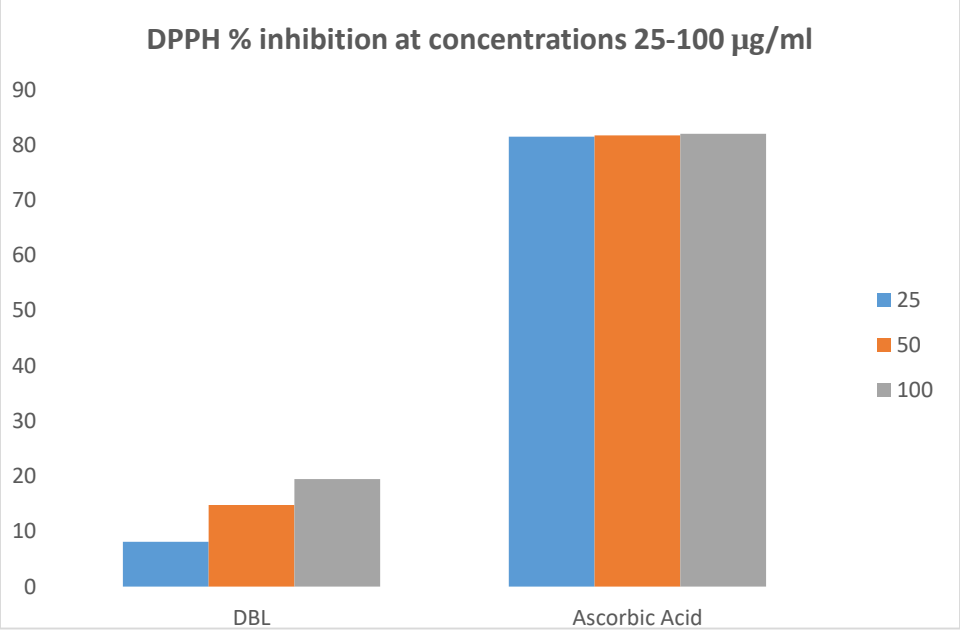

Fig. 2.0: DPPH Antioxidant result of *Dombeya buettneri* leaves.

The ferric chloride reducing antioxidant potential (FRAP) of the essential oil of *Dombeya buettneri* leaves showed a good reducing antioxidant potential of 0.076713±0.012275 mg/$FeSO_4$.equiva./g (Table 3.0).

Table 3.0: Results of Ferric reducing antioxidant potential (FRAP) of *Dombeya buettneri* leaf oil

Absorbance			mg/$FeSO_4$Equiv./g		
0.152	0.110	0.132	0.08879	0.06425	0.07710

The result of 2, 2-azobis-3-ethylbenzothiazoline-6-sulfonate radical scavenging ability determination (ABTS) of *Dombeya buettneri* leaf oil showed the oil to possess free radical scavenging ability. This is presented as mean of the triplicate values with their standard deviations; 0.37759±0.0025 mg/AA Equiv./g (Table 4.0).

Table 4.0: Results 2, 2-azobis-3-ethylbenzothiazoline-6-sulfonate radical scavenging ability determination (ABTS) of *Dombeya buettneri* leaves

Absorbance			mg AA Equiv./g		
0.421	0.426	0.417	0.37778	0.37500	0.38000

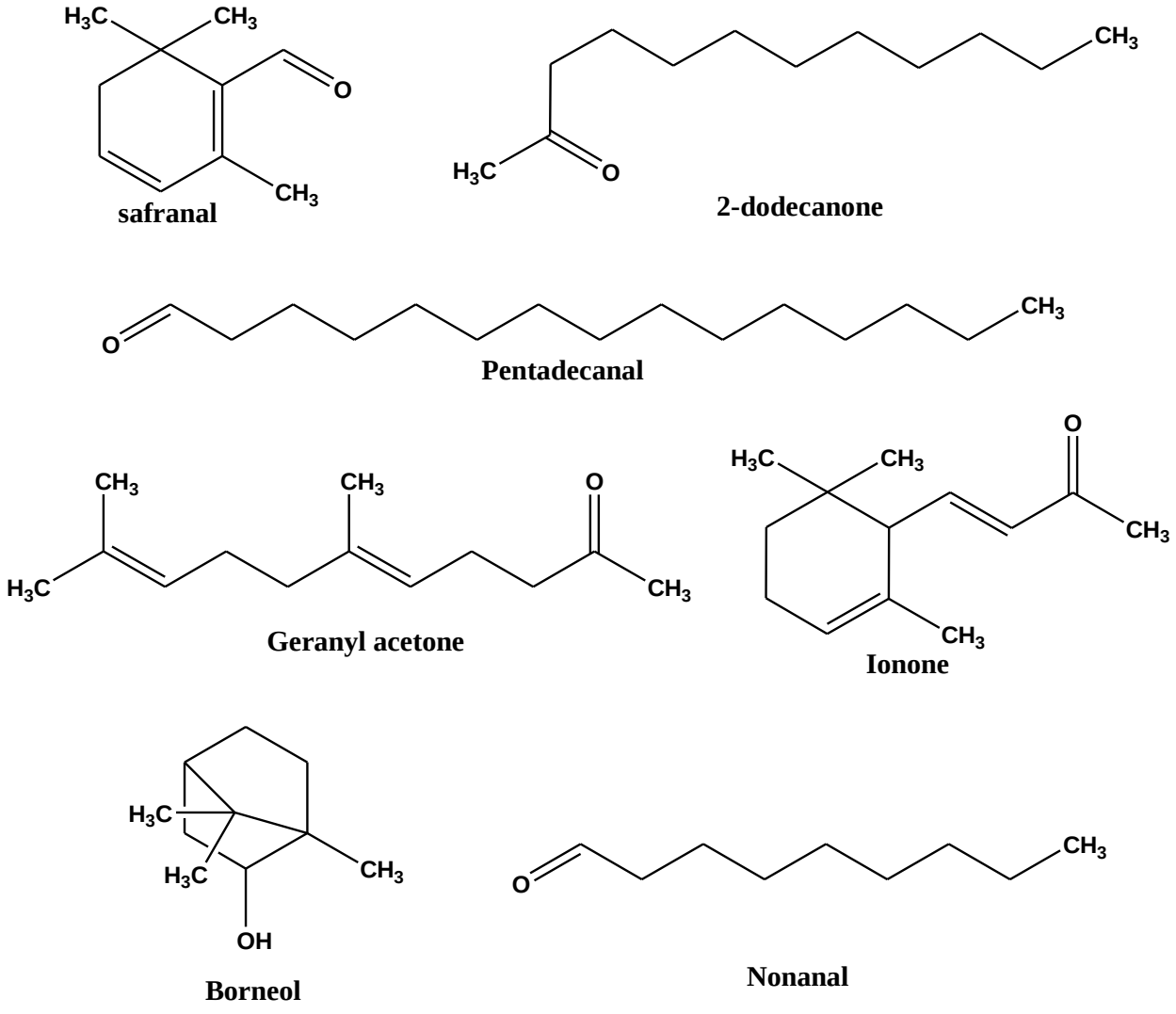

Fig. 3.0: Antimicrobial and antioxidant compounds detected in *Dombeya buettneri* leaves

The extraction yield of essential oil was 1.07% (v/w), calculated on a dry weight basis. The essential oil sample was found to be colourless.

A total of 23 compounds were detected by GC-MS, compounds which are dominated by Apocarotenes (64.9%) and Non-terpene derivatives (20.2%) with a total of identified components being 93.9%. The main compounds identified in the oil were hexahydrofarnesylacetone (32.5%), dihydroedulan IIA (9.6%), *(E)* –geranylacetone (7.8%), β-selinene (5.4%) and 2-pentyl furan (4.6%).

The antimicrobial activity of some of the minor and major essential oil constituents of *Dombeya buettneri* leaves have been reported. These antimicrobial and antioxidant constituents include Pentadecanal [19], Nonanal [20], Safranal [21]. Additionally, Safranal and Geranyl

acetone; major constituents of *Dombeya buettneri* leaves have been reported for their antioxidant property [22-23].

Other biological activities have been reported such as the anticancer property of Safranal [24], Geranyl acetone [25] and D-borneol [26-27]. The anti-inflammatory property of D-borneol [26] has been previously reported. *Dombeya buettneri* minor components, 2-dodecanone [28] and Nonanal [21, 29] have shown to demonstrate good insecticidal activity. The anti-tumor property of Safranal [29-31], its antihypertensive effect [32] and anticonvulsant activity [33] have been reported. D-borneol has been reported for its analgesic property [26].

The antioxidant activities of *Dombeya buettneri* leaves essential oil was examined using three different scavengers (DPPH, FRAP and ABTS). The ferric chloride reducing antioxidant potential (FRAP) of the essential oil showed good reducing antioxidant potential (Table 3.0). The result of 1, 1-diphenyl-2-picrylhydrazyl (DPPH) scavenging activity of the oils was low in comparison with the standard. The DPPH· antiradical assay is based on the premise that a donor of an electron or hydrogen is an antioxidant, the strength is demonstrated as DPPH· color changes from purple to yellow in the test sample due to formation of neutral species-DPPH-H molecule upon absorption of hydrogen or electron from a specific antioxidant, however, DPPH technique on its own is not a specific radical species test but generally used to test radicals scavenging potency of an antioxidant [33]. The result of 1, 1-diphenyl-2-picrylhydrazyl (DPPH) scavenging activity of the oils of *Dombeya buettneri* leaves was low, in comparison with ascorbic acid as shown in Fig. 2.0, while that of 2, 2-azobis-3-ethylbenzothiazoline-6-sulfonate radical scavenging ability showed the oil to possess good free radical scavenging ability Table 4.0, the observed disparity in activities of *Dombeya buettneri* leaf oil against two different oxidants ($DPPH^{\cdot}$ and $ABTS^{\cdot+}$) could be attributed to many factors which include the polarity, complexity and the selectivity of the isomer of the free radicals [35].

Antimicrobial activity of leaf essential oil against *Staph. aureus*, *E. coli*, *Candida albicans* as well as its radical scavenging properties as observed in this present study are quite significant. These observable activities may therefore suggest that leaf essential oil of *Dombeya buettneri* could possibly be a new candidate in the search for lead compounds for the management of infectious diseases and oxidative stress-related disorders such as Alzheimer's disease, cancers, diabetic nephropathy and arteriosclerosis [36-39].

Conclusion

Essential oil from *Dombeya buettneri* leaves is a rich source of bioactive compounds as obvious from the GC-MS, antioxidant and antimicrobial results. Apart from the local uses of the leaves of *Dombeya buettneri*, the essential oil contained strong bioactive phytochemicals with good

prospect as new antimicrobials for therapeutic or preservative uses; and as an alternative to the existing synthetic antioxidant agents based on further analysis.

Acknowledgment

The authors acknowledge Prof. Guido Flamini of the Department of Pharmacy, Università di Pisa, Pisa, Italy, for his assistance in the running of the GC-MS analyses of the oil samples.

Conflict of interest

There is no conflict of interest.

References

1. Witayapan, N., Sombat, C., Siriporn, O. (2007) Antioxidant and antimicrobial activities of Hyptis suaveolens essential oil. Sci. Pharm., 75: 35 – 46.

2. Wang, W., Wu, N., Zu, G. and Fu, Y. (2008) Antioxidant activity of Rosmarinus officinalis essential oil compared to its main components. Food Chem., 108: 1019 – 22.

3. Penalver, P., Huerta, B., Borge, C., Astorga, R., Romero, R. and Perea, A. (2005) Antimicrobial activity of five essential oils against origin strains of the Enterobacteriaceae family. APMIS. 113: 1 – 6.

4. Nweze, E.I. and Okafor, J.I. (2010) Activities of a wide range of medicinal plants and Essential oil vs. Scedospaorium isolates. Am-Eur. J. Res, 5: 161 - 169.

5. Paul, S. (2011) Trachyspermum ammi (L.) fruit essential oil influencing on membrane permeability and surface inhibiting food –borne pathogens. Food Control, 22: 725 – 6.

6. Morten, H., Mygind, T.L. and Rikke, M. (2011) Essential Oils in Food Preservation: mode of action, synergies and interactions with food matrix components. Front Microbiol. 3: 12.

7. Helander, I.M., Akakomi, H., Latva-Kala, K., Mattila-Sandholm, T., Pol, I. and Eddy, J. (1998) Characterization of the action of selected essential oil components on gram-negative bacteria. J Agric Food Chem. 46(9): 3590 – 5.

8. Saikat, S., Chakraborty, R., Sridhar. C.Y., Reddy, S.R. and Biplab, D. (2010) Free radicals, antioxidants, diseases and phytomedicine: current status and future prospect. Int. J. Pharm. Sci. Rev. Res. 3: 91 – 100.

9. Tuttolomondo, T., La-Bella, S., Licata, M., Virga, G., Leto, C. and Saija, A., *et al*. (2013) Bimolecular characterization of wild Sicilian oregano: phytochemical screening of essential oils and extracts, and evaluation of their antioxidant activities. Chem and Biodiver. 10(3): 411 – 33.

10. Penoel, M.D. and Penoel, R. (2012) Natural Health Care Using Essential Oils. Abundant Health. New Zealand. online book. http://www.abundanthealth4u.com. Okoh *et al*. BMC Complementary and Alternative Medicine, 16:486: 11 – 12.

11. Kotamballi, N., Chidambara, M., Jayaprakasha, G.K. and Bhimanagouda, S.P. (2013) Anti- inflammatory mediated applications of monoterpenes found in fruits. Am Chem Soc. 121 – 31.

12. British Pharmacopoeia (1980). British Pharmacopoeia Commission, H.M Stationary Office, London.

13. Adams, R.P. (1989) Identification of Essential oils by Ion Trap Mass Spectroscopy. Academic Press. Inc. USA.

14. Carson, C.F, Hammer, K.A., Riley, T.V. (1995) Broth micro-dilution method for determining the susceptibility of *Escherichia coli* and *Staphylococcus aureus* to the essential oil of *Melaleuca alternifolia* (tea tree oil). Microbios, 82(332):181-185.

15. Lajubutu, B.A., R.J. Pinney, M.F. Robert, H.A. Odelola, Oso B.A. (1995) Antimicrobial activity of diosquinone and plumbagin from Diospyros mespiliformis (Hostch) (Ebenaceae). Phytother. Res, 9: 346 - 350.

16. Gyamfi, M., Yonamine, M., Aniya, Y. (1999) Free radical scavenging action of medicinal herbs from Ghana: Thonningia sanguine on experimentally induced liver injuries. General Pharmacology, 32(6): 661 - 667.

17. Benzie, I.F., Strain, J.J. (1996) The Ferric Reducing ability of plasma (FRAP) as a measure of ″Antioxidant power″: the FRAP assay. Anal. Biochem. 239: 70 -76.

18. Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., Rice-Evans. (1999) Antioxidant activity applying an improved ABTS radical cation decolorisation assay. Free Radical Biology and Medicine, 26(9–10): 1231 – 1237.

19. Ricciardelli, A., Casillo, A., Corsaro, M.M., Tutino, M.L., Parrilli, E., Van der Mei H.C. (2020) Pentadecanal and pentadecanoic acid coatings reduce biofilm formation of *Staphylococcus epidermidis* on polydimethylsiloxane (PDMS). Polym. Int. 51: 882 - 888.

20. Muroi, H., Kubo, A., Kubo, I. (1993) Antimicrobial activity of cashew apple flavor compounds. Journal of Agricultural and Food Chemistry, 41(7): 1106 - 1109.

21. Zavala-Sánchez, M.A., Pérez-Gutiérrez, S., Pérez-González, C., Sánchez-Saldivar, D., Arias-García L. (2002) Antidiarrhoeal Activity of Nonanal, an Aldehyde Isolated from Artemisia ludoviciana. Pharmaceutical Biology, 40(4): 263 – 268.

22. Sun, M. H. *et al*. (2016) Synthesis of geranyl acetone from myrcene. Chem. Ind. Eng. 1(11).

23. Assimopoulou, A.N., Sinakos, Z., Papageorgiou, V.P. (2005) Radical scavenging activity of Crocus sativus L. extract and its bioactive constituents. Phytother. Res. 19: 997−1000.

24. Escribano, J., Alonso, G.L., Coca-Prados, M. and Fernandez, J.A. (1996) Crocin, safranal and picrocrocin from saffron (*Crocus sativus* L.) inhibit the growth of human cancer cells in vitro. Cancer Lett. 100: 23 − 30.

25. Tatman, D., Mo, H. (2002) Volatile isoprenoid constituents of fruits, vegetables and herbs cumulatively suppress the proliferation of murine B-16 melanoma and human HL-60 leukemia cells. Cancer Lett, 75: 1 – 139.

26. Zeng, J.G. Research Progress of Natural

Borneol. *China Drug Economics*, 2011. 6: 82 –

89.

27. Xiong, Z.Y., Xiao, F.M., Xu, X., et al. (2013) Studies on Pharmacological Activity of Borneol. China Journal of Chinese Materia Medica, 38: 786 - 790.

28. Zhang, J., Zeng, L., Sun, L., Chen, S., Wang, T., Ma, S. (2017) Use of Nonanal-wax as Postharvest Fungicide of Tomato against Botrytis cinerea. Journal of Food and Nutrition Research, 5(7).

29. Kianbakht, S., Mozaffari, K. (2009) Effects of saffron and its active constituents, crocin and safranal, on prevention of indomethacin induced gastric ulcers in diabetic and nondiabetic rats. J. Med. Plants, 5: 30 - 38.

30. Pathan, S.A., Zaidi, S.M.A., Jain, G.K., Vohora, D., Ahmad, F.J., Khar, R.K. (2009) Anticonvulsant evaluation of safranal in pentylenetetrazole-induced status epilepticus in mice. Int J. Essential Oil Therapeutics, 2 (3): 106 - 108.

31. Ghoshooni, H., Daryaafzoon, M., Sadeghi-Gharjehdagi, S., Zardooz, H., Sahraei, H., Tehrani, S.P., *et al*. (2011) Saffron (Crocus sativus) ethanolic extract and its constituent, safranal, inhibits morphine-induced place preference in mice. Pak. J. Biol. Sci. 14: 939 - 944.

32. Imenshahidi, M., Hosseinzadeh, H., Javadpour, Y. (2010) Hypotensive effect of aqueous saffron extract (Crocus sativus L.) and its constituents, safranal and crocin, in normotensive and hypertensive rats. Phyter. Res., 24: 990 - 994.

33. Hosseinzadeh, H., Talebzadeh, F. (2005) Anticonvulsant evaluation of safranal and crocin from Crocus sativus in mice. Fitoterapia. 76: 722 – 724.

34. Guerrini, A., Sacchetti, G., Rossi, D. *et al*. (2009) Bioactivities of Piper aduncum L. and Piper obliquum Ruiz and Pavon (Piperaceae) essential oils from Eastern Ecuador, Environmental Toxicology and Pharmacology, 27(1): 39 – 48.

35. Valko, M., Leibfritz, D., Moncol, J., Cronin, M. T. D., Mazur, M., Telser, J. (2007) Free radicals and antioxidants in normal physiological functions and human disease. International

Journal of Biochemistry and Cell Biology. 39(1): 44 – 84.

36. Paz-Elizur, T., Sevilya, Z., Leitner-Dagan, Y., Elinger, D., Roisman, L.C., Livneh, D. (2008) DNA repair of oxidative DNA damage in human carcinogenesis: potential application for cancer risk assessment and prevention. Cancer Letters, 266(1): 60 – 72.

37. Naito, Y., Uchiyama, K., Yoshikawa, K. (2006) Oxidative stress involvement in diabetic nephropathy and its prevention by astaxanthin. Oxidative Stress and Disease, 21: 235 – 242.

38. Fini, M.A., Johnson, R.J., Stenmark, K., Wright, R.M. (2013) Hypertension, nitrite, xanthine oxidoreductase catalyzed nitric oxide generation: pros and cons. Hypertension,62, e9. Floribunda leaf, African Journal of Biotechnology, 9: 340 – 346.

39. Akande, A., Aboaba, S., Flamini, G. (2018) Constituents and Anthelmintic Activity Evaluation of Albizia Adiantifolia (Schumach) W.F. Wright Essential Oils from Nigeria. International Journal of Chemistry, 10(2).