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Research Article

PHYTOCHEMICAL PROFILE AND ANTIOXIDANT ACTIVITY OF THE ESSENTIAL OIL FROM *BLUMEA ERIANTHA* DC

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ABSTRACT

Objective: The present study was designed to evaluate the phytochemical composition and antioxidant activity of essential oil from *Blumea eriantha* DC collected from Seawoods, Navi Mumbai (Maharashtra, India).

Methods: The essential oil was extracted by hydrodistillation using Clevenger type apparatus and subjected to GC-FID, GC-MS and HPTLC analysis. Furthermore antioxidant activity of essential oil was investigated by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay.

Results: Total 72 compounds were identified representing 96.83% of the oil. The main components of the essential oil are Ocim-(4E,6Z)-ene <allo-> (13.72%), Caryophyllene <(E)-> (9.71%), Caryophyllene oxide (5.76%), Carvotanacetone (5.36%), Pinene <alpha-> (3.90%), Eudesmol <7-epi-alpha-> (3.74%). HPTLC fingerprint of essential oil was also developed in order to carry out easy and fast identification of essential oil constituents. The oil showed activity as a radical scavenger at 437.92 ± 4.22 µg/ml.

Conclusion: The present study describes the phytochemical profile and antioxidant activity of essential oil from *Blumea eriantha* DC. These findings will be helpful in further application of this plant in cosmetics as well as traditional medicines.

Keywords: *Blumea eriantha* DC, GC-MS analysis, HPTLC analysis, Antioxidant activity, DPPH assay

INTRODUCTION

The ability to utilize oxygen has provided humans with the benefit of metabolizing carbohydrates, fats and proteins for energy; however, it does not come without a cost. A paradox in metabolism is that, while the vast majority of complex life on Earth requires oxygen for its existence, oxygen is a highly reactive molecule that can damage living organisms by producing reactive oxygen species. Oxygen is a highly reactive atom that is capable of becoming part of potentially damaging molecules commonly called "free radicals" [1]. Free radicals are capable of attacking the healthy cells of the body, causing them to lose their structure and function [1]. Cell damage caused by free radicals appears to be a major contributor to aging and to degenerative diseases of aging such as cancer, cardiovascular diseases, cataracts, immune system decline, and brain dysfunction [2]. Overall, free radicals have been implicated in the pathogenesis of at least 50 diseases [3, 4]. Fortunately, free radical formation is controlled naturally by various beneficial compounds known as antioxidants. Therefore there has been a growing and considerable interest to identify new sources of safe and inexpensive antioxidants. The antioxidant activity of essential oils is another a biological property of great interest because they may preserve foods from the toxic effects of oxidants. Plant essential oils as antioxidants were researched in detail with the view to investigating their protective role for highly unsaturated lipids in animal tissues [5]. The oils have shown their action as hepatoprotective agents in ageing mammals and these studies described the beneficial impact of volatile oils upon the PUFA's, in particular the long chain C20 and C22 acids [6]. Moreover, essential oils being also able to scavenge free radicals may play an important role in some disease prevention such as brain dysfunction, cancer, heart disease and immune system decline [7].

Blumea is a genus of shrubs and small trees which comprises of about 80 species distributed in tropical and subtropical Asia, Africa, and Oceania [8]. This genus includes some important medicinal plants largely used in traditional medicine. For example, the essential oil from *Blumea mollis* [9], *Blumea perrottetiana* [10] have shown notable insecticidal activities. *Blumea membranacea* shows significant antifungal activity [11] Also the essential oil from *Blumea membranacea* produces a marked and long-lasting fall in the blood pressure of anaesthetized dogs, exerts a direct depressant action on frog hearts, and a spasmolytic effect on rabbit ilea [11]. *Blumea eriantha* DC (*B. eriantha*) is also one of the medicinally important species belongs to this genus. In a recent paper, we reported antimicrobial efficacy of essential oil from *B. eriantha* [12]. The oil showed significant antimicrobial activity against skin pathogens namely, *Propionibacterium acnes, Staphylococcus epidermidis, Staphylococcus aureus* and *Streptococcus pyogenes.* In present study we have further investigated the composition of essential oil from *B. eriantha* by Gas Chromatography-Mass Spectrometry (GC-MS) along with its antioxidant potential.

It is crucial to develop a suitable and reliable identification method to confirm the quality of extracts and herbal drugs. Separation and detection of different constituents in plants have been always complicated. While conventional research mainly focuses on determination of the active components, fingerprinting can offer characterization of a complex system with a degree of quantitative reliability, so it has gained increasing attention as a quality control tool over the past few years [13]. Chromatographic methods including TLC, HPLC, GC and electromigration techniques such as capillary electrophoresis are mainly used for fingerprinting [14-16]. Different methods to analyse essential oils are available, of which GC is usually-used because of the volatile components of essential oils. GC analysis gives information about the individual components of an essential oil and its relative amounts. But GC is an expensive and time-consuming method which takes 45-60 minutes per run for analysis of single sample. High Performance Thin Layer Chromatography (HPTLC) is a common rapid and cost-efficient method used for fingerprinting of plant extracts. Moreover several samples can be chromatographed simultaneously on a single plate and complicated instrumentation is not necessary [15, 17]. With the introduction of automated equipment chromatograms can be well documented. Also methods of HPTLC fulfill GMP guidelines. Hence a HPTLC method was used to develop a fingerprint pattern for the preliminary identification of the essential oil from *B. eriantha.*

MATERIALS AND METHODS

Plant Material

The entire upper portion including aerial part, Stem and leaves of *B. eriantha* were collected from Seawoods, Navi Mumbai, Maharashtra, India between the months of December to March. The authentication of plant was carried out at Agharkar Research Institute, Pune,

Maharashtra, India and the voucher specimen was deposited with the institute.

Extraction of Essential Oil

The fresh plant material including aerial part, stem and leaves of *B. eriantha* were chopped into small pieces. 750g of fresh plant material was subjected to hydrodistillation using Clevenger type apparatus of capacity 5 Liters. 3 liters of water was added to the material. The mixture was heated on heating mantle at 85^{0}C. The distillation was continued for three hours. The essential oil obtained was dried over anhydrous sodium sulphate and stored at 4^{0}C in sealed vials until analysis.

Gas Chromatography (GC) Analysis

Initially GC was used for development of chromatographic method for the selected plant essential oil. The GC analysis was accomplished using Shimadzu GC-2014 gas chromatograph equipped with FID and Rtx®-5 capillary column (0.25mm X 30m X 0.25 µm film thickness). Following temperature program was optimized for analysis (See **Table 1**).

Table 1: The optimized temperature program for column oven

Rise in Temperature per minute (^{0}C)	Temperature (^{0}C)	Hold time (minute)
0	50	0
5	65	2
2	75	5
8	99	0
5	140	8
Total Program Time	**34.20 minutes**	

Injector temperature was 200^{0}C while detector temperature was 225^{0}C. Nitrogen was used as a carrier gas, at a flow rate 1.53 cm^3/ min. Split ratio was 1:200.

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The GC method was then transferred to GC-MS with slight modifications for identification of various phytoconstituents of selected plant essential oil. The oil was analyzed by Shimadzu GCMS QP-2010 Ultra system. The system was equipped with fused silica Rtx-5 Sil MS silarylene capillary column with dimensions 30m X 0.25mm X 0.25µm. Helium (0.93 ml/min) was used as a carrier gas. The program used for GC oven temperature was 1 minute isothermal at 50^{0}C, followed by 50-220^{0}C at a rate of 5^{0}C/min, then held at 220^{0}C for 1minute, followed by 220 - 260^{0}C at a rate of 20^{0}C/min, then again held at 260^{0}C for 15 minutes. The injection port temperature was 266^{0}C. The ionization of sample components was performed in the E.I. mode (70eV). The Linear Retention Indices (LRI) for all the compounds was determined by co-injection of the sample with a solution containing the homologous series of C_8-C_{29} *n*-alkanes. Individual constituents were identified by referring to compounds known in the literature data and also by comparing their mass spectra with known compounds and NIST Mass Spectral Library (NIST 05), Flavor and Fragrance Natural and Synthetic Compounds mass spectral library database and LRI.

High Performance Thin Layer Chromatographic (HPTLC) Analysis

A densitometric HPTLC analysis was performed for the development of characteristic finger printing profile. HPTLC was performed on 5 x 10 cm silica gel 60 F_{254} aluminum TLC plate (Merck, Germany). The essential oil was diluted with toluene in the ratio 1:5. The diluted essential oil was applied to the TLC plates using Camag Linomat IV Sample Applicator equipped with Hamilton syringe of 100µl capacity. 5 µl and 10 µl of the diluted essential oil was applied on two separate tracks of 8mm band width. For the separation of essential oils toluene-ethyl acetate (93:7) is a recommended mobile phase [18]. The same mobile phase was used for the analysis. The plates were developed in twin-trough chamber previously saturated with the mobile phase up to a migration distance of 90mm. The developed plate was dried using hot air to evaporate solvents from the plate. The plate was kept in UV cabinet and observed at 254nm and 366 nm. The plate was sprayed with vanillin sulphuric acid reagent and observed for colouration in visible light. Finally, the plate was fixed in scanner stage in Camag TLC Scanner III and scanned at 254nm. The Peak table, Peak display and Peak densitogram were identified.

Antioxidant Activity

Free Radical Scavenging Activity by 2,2-diphenyl-1-picrylhydrazyl (DPPH) Assay

The free radical scavenging activity of *B. eriatha* essential oil was determined using the DPPH assay. 0.00035gm of DPPH (Sigma Aldrich, USA) was dissolved in 10ml of methanol (Qualigens) to give concentration of 9 x 10-5 M. α –Tocopherol (Sigma Aldrich, USA) was used as standard for the assy. 1 mg/ml stock solution of α - Tocopherol was prepared in methanol and serially diluted in methanol to give concentration 1, 5, 25 µg/ml.

Test stock solution (1 mg/ml) for oil sample was prepared in methanol and serially diluted in methanol to give concentration 5, 10, 25, 50, 75, 100, 150, 250, 500, 1000 µg/ml. 500 µl of each test concentration was taken in the test tube. To this added equal amount of 9 X 10^{-5}M of methanolic solution of DPPH and allowed to react in dark for 30minutes at room temperature. Appropriate control and color blank samples were run. The decrease in absorbance was measured at 519nm using Cary-50, Varian spectrophotometer. All the test samples and reference standard samples were run in triplicates and the experiment was repeated thrice. The free radical scavenging activity was calculated by using following formula. These average values were further used to prepare the IC_{50} graphs.

Calculation:

$$\% \text{ DPPH reduction} = \frac{(\text{Absorbance of control} - \text{absorbance of test sample})}{(\text{Absorbance of control})} \times 100$$

RESULTS

The essential oil from *B. eriantha* was subjected to GC and GC-MS analysis. Total 72 compounds were identified in the essential oil, accounting for 96.83% of the oil composition. The components identified, their linear retention indices and their percentage area are summarized in **Table 2,** and are arranged in their order of elution on DB-5 column. **Fig. 1 & 2** show the total ion chromatogram for essential oil of *B. eriantha* and n-alkane standards. The essential oil from *B. eriantha* showed diverse composition. The chemical composition of essential oil was dominated by monoterpene and sesquiterpene hydrocarbons. The most representative class of compounds in the oil was monoterpenes (37.45%), followed by sesquiterpenes (30.73%), epoxides (5.76%), Alcohol (3.33%), aldehydes (3.08%) and small amount of ketones (0.69%), ethers (0.62%). The major components identified were: Ocim-(4E,6Z)-ene <allo-> (13.72%), Caryophyllene <(E)-> (9.71%), Caryophyllene oxide (5.76%), Carvotanacetone (5.36%), Pinene <alpha-> (3.90%), Eudesmol <7-epi-alpha-> (3.74%). The oil showed presence of important monoterpenes such as α- pinene and β-pinene, Myrcene, Mentha-2,8-dien-1-ol <trans-, para->, Thujol Limonene, α- and γ- terpinene, α-thujene, Sabinene as well as the aromatic hydrocarbon *p*-cymene and its hydroxylated derivatives Carvacrol. Also the oil showed presence of variety of sesquiterpenes which include Muurolol <alpha->, tricyclic sesquiterpenes Santalol, Nerolidol <(E)->, Kessane, Humulene**,** Cubebene <beta->, Gurjunene <alpha->, Selinene <alpha-> etc.

Table 2: Chemical composition of essential oil from *Blumea eriantha* DC.

No.	Relative Retention Indices	Relative concentration of components in Area Percentage (%)	Name of compound
1	849	0.20	Hex-(2E)-enal
2	857	0.28	Hex-(3Z)-enol
3	870	0.31	Hexanol <n->
4	883	0.34	Acetylvaleryl
5	920	0.06	Tricyclene
6	926	0.28	Thujene <alpha->
7	933	3.90	Pinene <alpha->
8	942	0.08	Butyrate <2-methyl-, propyl->
9	946	0.35	Fenchene <alpha->
10	951	0.06	Thuja-2,4(10)-diene
11	971	1.05	Sabinene
12	974	0.20	Pinene <beta->
13	982	0.32	Vinyl amyl carbinol
14	992	1.47	Myrcene
15	1016	0.07	Terpinene <alpha->
16	1023	0.96	Cymene <para->
17	1027	0.62	Limonene
18	1038	0.11	Ocimene <(Z)-, beta->
19	1040	0.28	Furan <tetrahydro-, 2,2-dimethyl-, 5-(1-methylpropenyl)->
20	1057	0.12	Terpinene <gamma->
21	1113	2.35	Phenethyl alcohol
22	1117	1.89	Mentha-2,8-dien-1-ol <trans-, para->
23	1122	0.35	Isophorone
24	1132	13.72	Ocim-(4E,6Z)-ene <allo->
25	1138	0.43	Terpin-3-en-1-ol
26	1142	0.07	trans-pinocarveol
27	1145	1.06	Verbenol <trans->
28	1156	0.07	Nerol oxide
29	1165	0.05	Menthofuran
30	1167	1.16	Thujol <(-)->
31	1172	0.11	Borneol
32	1179	2.29	Terpinen-4-ol
33	1192	0.65	Myrtenal
34	1196	2.68	Dec-(4Z)-enal
35	1210	0.04	Verbenone
36	1221	0.62	Coahuilensol <methyl-> ether
37	1234	0.34	Nerol
38	1251	5.36	Carvotanacetone
39	1260	0.23	Geraniol DB5-1018
40	1273	1.23	Neryl formate
41	1278	0.14	Perillaldehyde
42	1303	0.31	Carvacrol
43	1320	0.20	Deca-(2E,4E)-dienal
44	1326	0.25	Myrtenyl acetate
45	1342	0.26	Nona-1,6-dien-3-ol <3,7-dimethyl-> acetate
46	1390	0.08	Cubebene <beta->
47	1392	0.16	Elemene <beta->
48	1401	3.35	Gurjunene <alpha->
49	1423	9.71	Caryophyllene <(E)->
50	1428	2.53	Nopyl acetate
51	1439	0.07	Benzoate <isopentyl->
52	1454	1.67	Humulene <alpha->
53	1481	2.46	Muurolene <gamma->
54	1486	1.63	Ionone <(E)-, beta->
55	1499	0.18	Selinene <alpha->
56	1518	0.09	Sesquiphellandrene <beta->
57	1538	0.59	Kessane
58	1559	0.27	Nerolidol <(E)->
59	1579	2.67	Cedrene <alpha-, epoxy->
60	1586	5.76	Caryophyllene oxide
61	1601	0.44	Fokienol
62	1613	3.11	Dodecyl acetate
63	1639	1.47	Bicyclo[7.2.0]undecan-3-ol <11,11-dimethyl-, 4,8-bis(methylene)->
64	1643	3.66	Naphthalen-1-ol <1,2,3,4,4a,7,8,8a-octahydro-, 2,4a,5,8atetramethyl-> formate
65	1647	2.75	Muurolol <alpha->
66	1657	0.78	Eudesmol <beta->(Bicyclic)
67	1662	3.74	Eudesmol <7-epi-alpha->
68	1665	0.71	Nerolidyl acetate <(Z)->
69	1675	0.40	Tetradeca-(9Z,12E)-dien-1-ol
70	1679	1.21	Santalol <alpha->
71	1684	0.11	Bisabolone oxide A <alpha->
72	1715	0.34	Bicyclo[10.1.0]trideca-4,8-diene <13-oxa-, trimethyl->

The HPTLC profile of the essential oil from *B. eriantha* was recorded. TLC plate under UV light (long and short) and after chemical treatment (in the day light) is shown in **Fig. 3**. The separated components of essential oil appeared as dark zones under UV light. Approximately ten such dark bands were seen under UV at 254 while no characteristic fluorescence was observed at 366nm. Various colored bands were seen in day light after derivetaization of the chromatogram with vanillin-sulphuric acid reagent. The colored bands include blue, Purple, light brown, yellow colors. The densitogram of the essential oil of *B. eriantha* showed twelve separated peaks (**Fig. 4**). The retention times, area under curve and area % of the twelve peaks are represented in **Table 3.**

The values of % DPPH reduction and IC_{50} from the three experiments along with their mean and standard deviation values for both sample and standard are represented in **Table 4 and 5**. The % DPPH reduction curves for sample and standard are shown in **Fig. 5 and 6.** The essential oil of *B. eriantha* showed 50 % radical scavenging activity at 437.92 ± 4.22 µg/ml, which is much higher than the IC_{50} value of standard α-tocopherol i.e. <1 µg/ml.

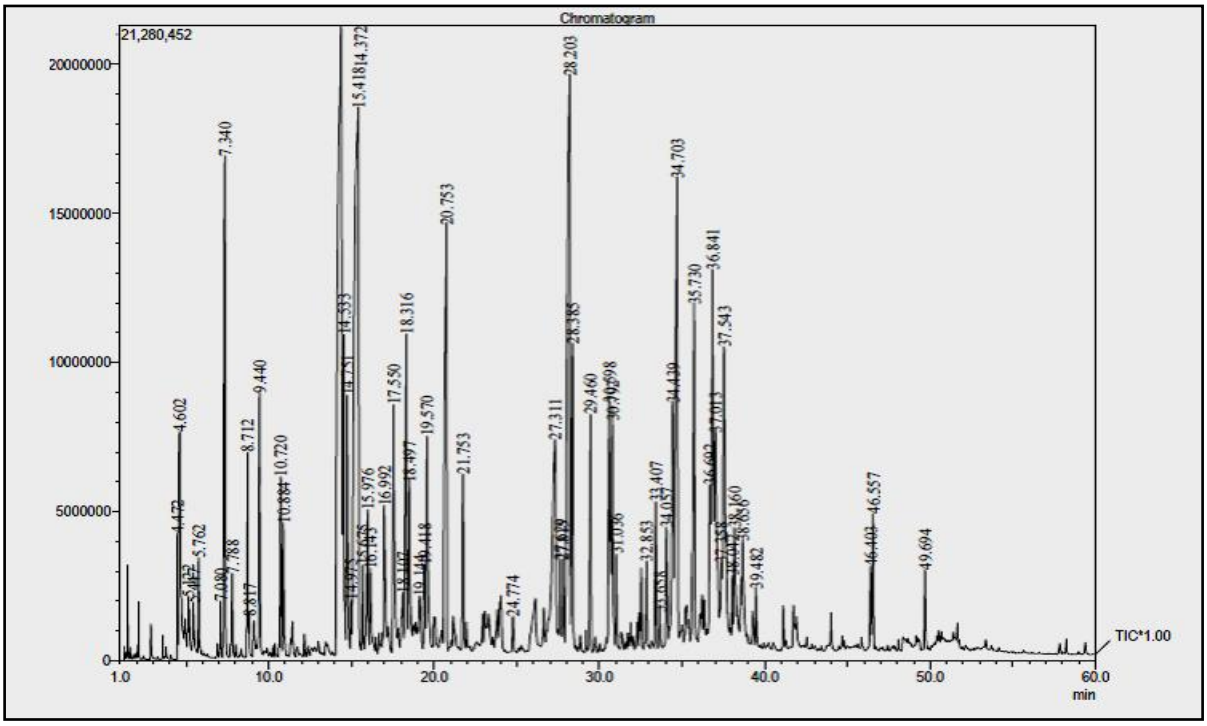


Fig. 1: Total ion chromatogram of *Blumea eriantha* DC essential oil

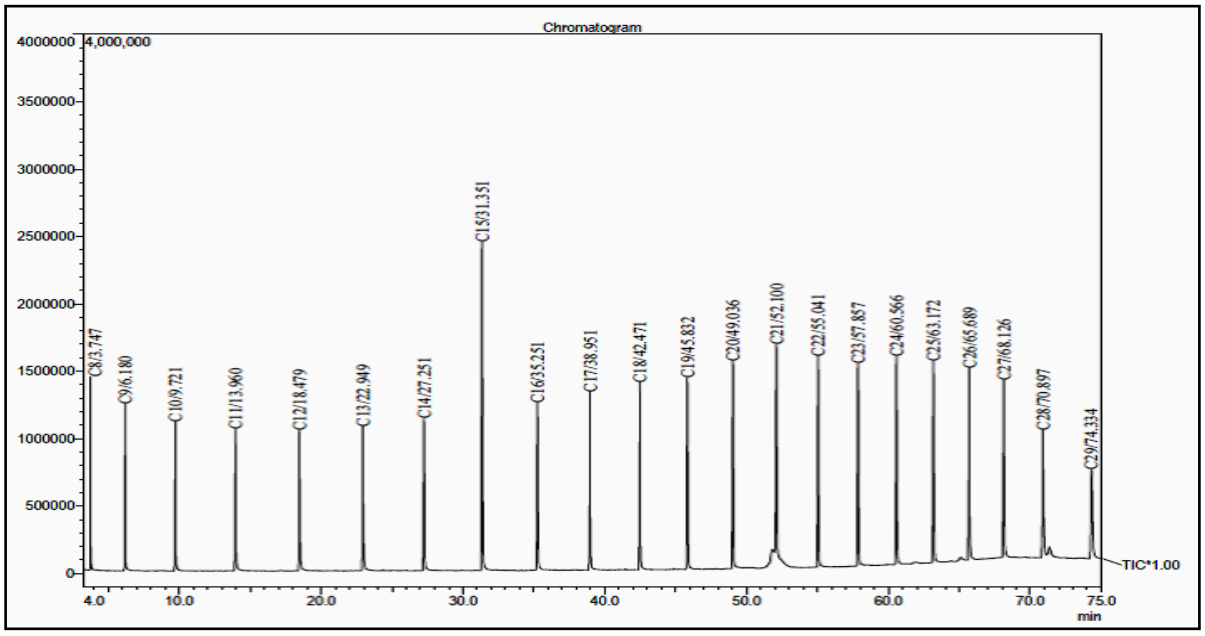
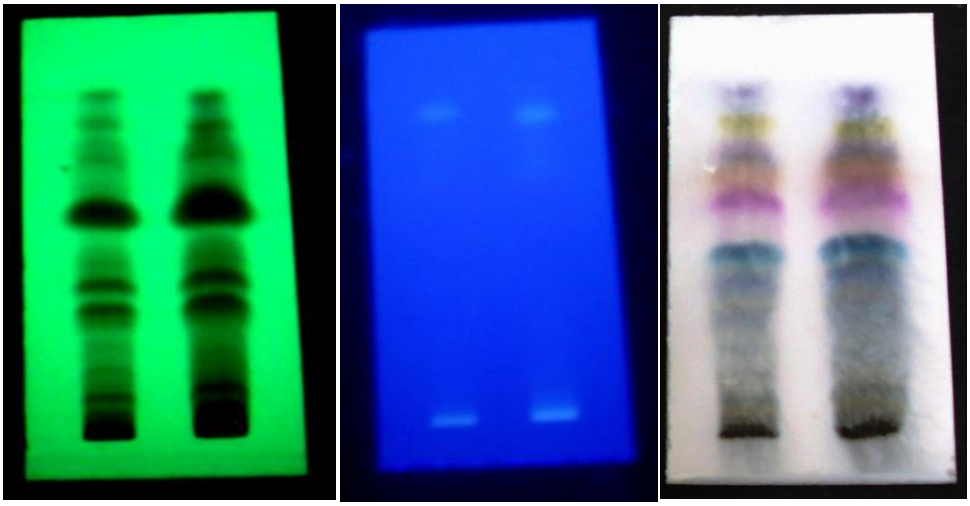


Fig. 2: Total ion chromatogram of n-alkanes standard.



(A) (B) (C)

Fig. 3: (A)TLC plate under UV 254nm (Short UV), (B) TLC plate under UV 366nm (long UV), (C) TLC plate after chemical treatment with Vaniline Sulphuric Acid under day light.

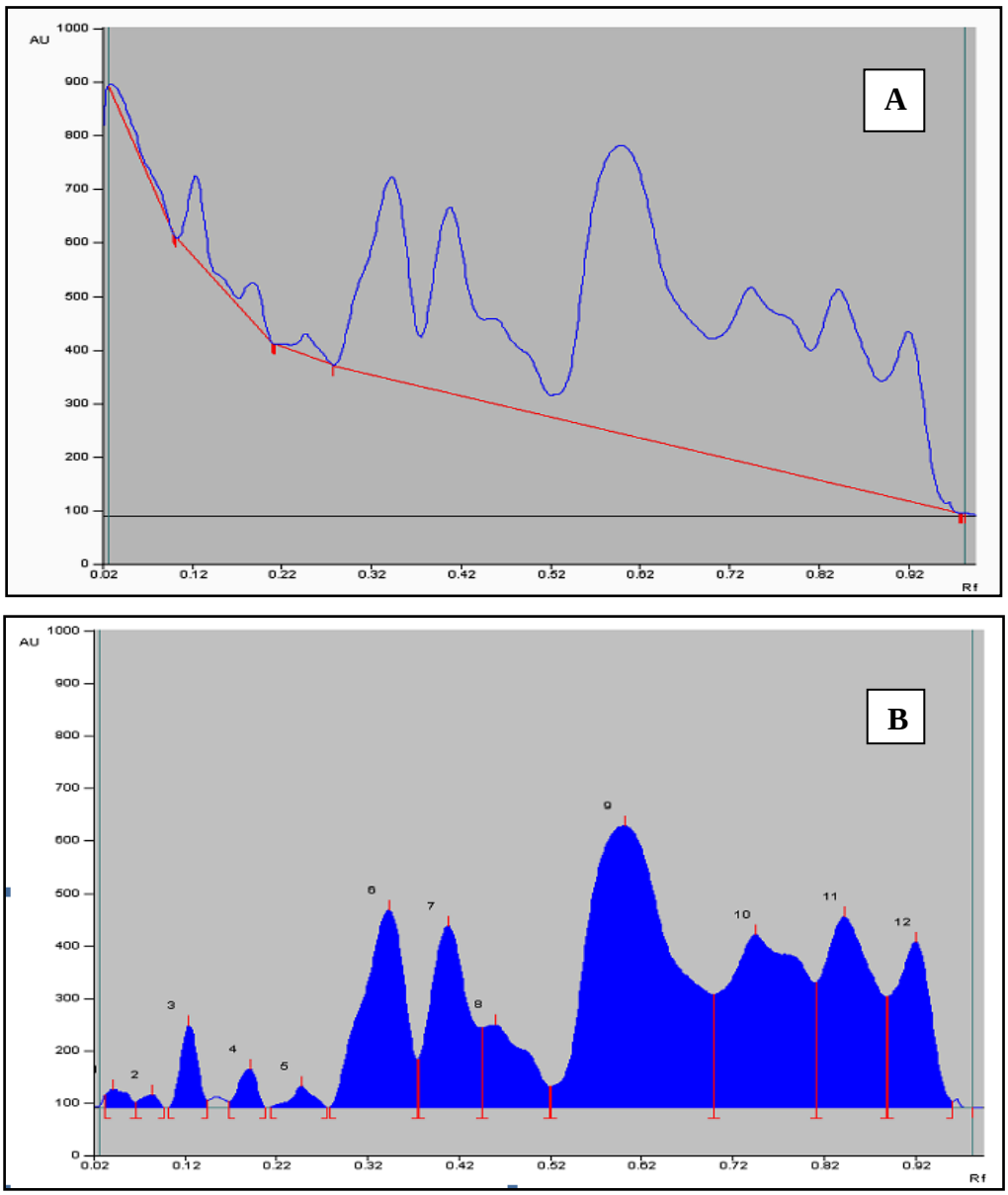


Fig. 4: Densitogram of *Blumea eriantha* DC Essential Oil. (A) Outline of Densitogram, (B) Densitogram with Marked Peak Area.

Table 3: Peak table for HPTLC analysis of essential oil from *Blumea eriantha* DC with R_f values, area under curve and area %.

Peak no.	R_f values	Area under curve (AUC) in AU.	Area %
1	0.05	756.8	0.52
2	0.09	431.3	0.30
3	0.13	2652.4	1.82
4	0.20	1371.5	0.94
5	0.25	890.7	0.61
6	0.35	16201.6	11.11
7	0.41	13323.5	9.14
8	0.46	6982.7	4.79
9	0.61	47630	32.66
10	0.75	25507.7	17.49
11	0.85	18584.5	12.74
12	0.92	11506.8	7.89

Table 4: Percentage DPPH scavenging activity for the essential oil from *Blumea eriantha* DC.

Concentration (µg/ml)	DPPH Scavenging Activity (%)			Mean DPPH Scavenging Activity (%) ± SD
	Experiment 1	Experiment 2	Experiment 3	
5	8.57	13.34	12.78	11.56 ± 2.61
10	9.26	14.77	15.22	13.08 ± 3.32
25	9.47	12.06	15.97	12.50 ± 3.27
50	10.95	17.59	15.33	14.62 ± 3.38
75	15.87	18.15	17.70	17.24 ± 1.21
100	21.30	22.54	23.88	22.57 ± 1.29
150	33.22	18.82	27.86	26.63 ± 7.28
250	41.26	42.55	29.19	37.67 ± 7.37
500	76.98	56.79	53.04	62.27 ± 12.88
1000	84.61	90.14	88.76	87.84 ± 2.88
IC_{50} Value (µg/ml)	433.17	441.24	439.35	437.92 ± 4.22

Table 5: Percentage DPPH scavenging activity for α-Tocopherol.

Concentration (µg/ml)	DPPH Scavenging Activity (%)			DPPH Scavenging Activity (%) ± SD	IC_{50} Value (µg/ml)
	Experiment 1	Experiment 2	Experiment 3		
1	95.00	94.69	95.52	95.07± 0.42	<1µg/ml
5	98.08	98.05	98.13	98.09±0.04	
25	99.96	99.84	99.92	99.90±0.06	

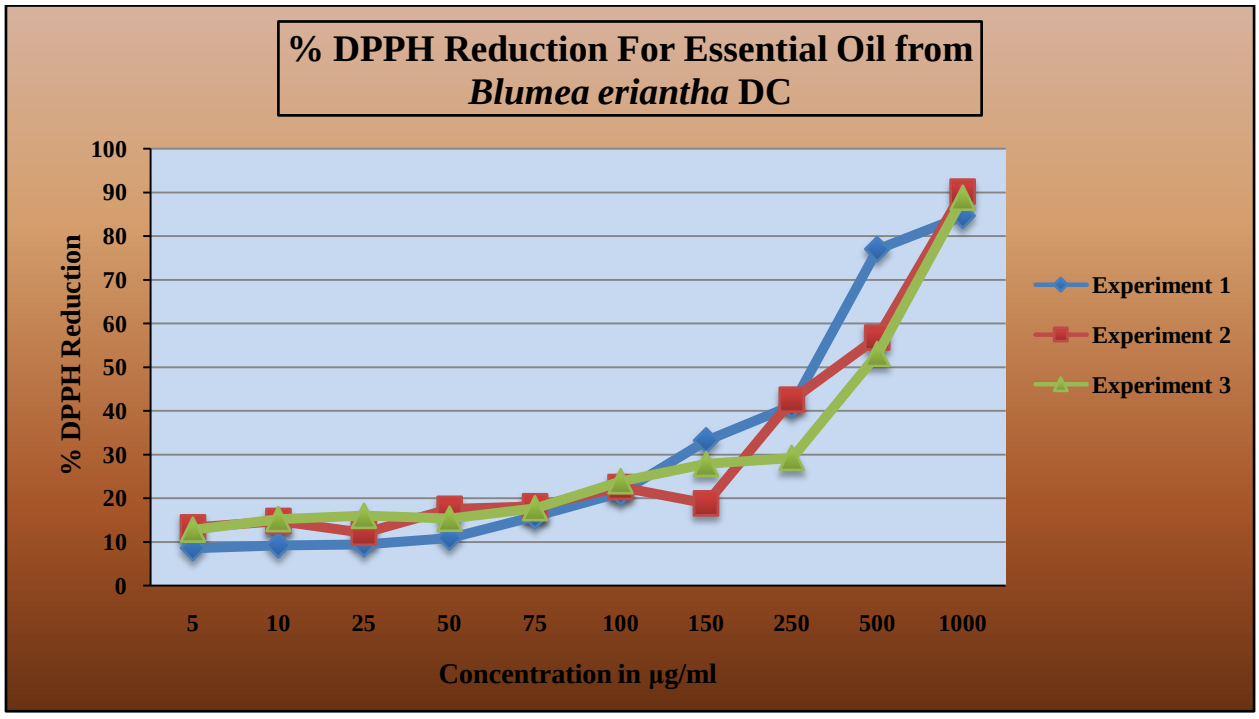


Fig. 5: % DPPH Reduction Curves for *Blumea eriantha* DC Essential oil

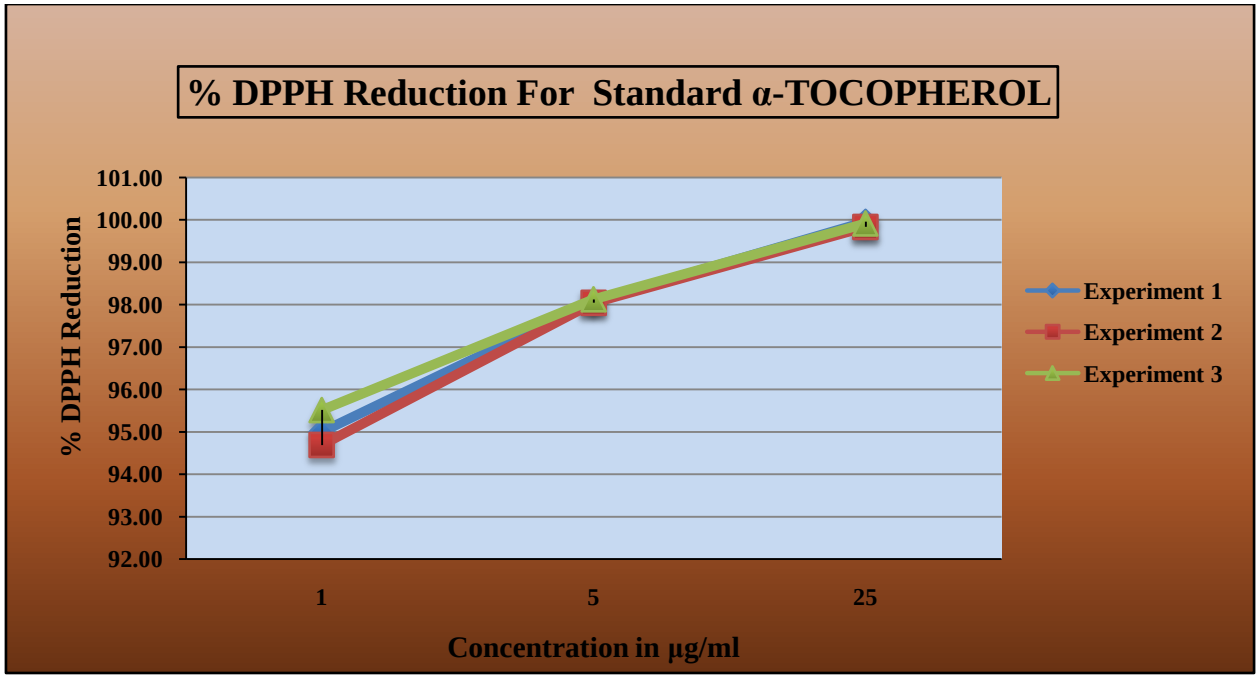


Fig. 6: % DPPH Reduction Curves for α-TOCOPHEROL

DISCUSSION

Comparing the chemical composition of essential oils from *Blumea* species they share similarity in many constituents, although concentration of these constituents is different. For example, essential oil from the aerial parts of *Blumea perrottetiana* was dominated by 2, 5-dimethoxy-p-cymene (30.0 %) and 1,8-cineole (11.0 %) with lesser amounts of sabinene (8.1 %), delta cadinene (5.3 %), and (E)-caryophyllene (3.9 %) [10]. The dominant components in the essential oil of *Blumea balsamifera* leaves were borneol (33.22 %), caryophyllene (8.24 %), ledol (7.12 %), tetracyclo[6,3,2,0,(2.5).0(1,8)tridecan-9-ol,4,4-dimethyl](5.18 %), phytol(4.63 %), caryophyllene oxide (4.07 %), guaiol (3.44 %), thujopsene-13 (4.42 %), dimethoxy-durene (3.59 %), and γ-eudesmol (3.18 %) [19]. The major chemical compounds of the essential oil of *Blumea mollis* leaves were identified as linalool (19.43 %), γ-elemene (12.19 %), copaene, (10.93 %), estragole (10.81 %), alloocimene (10.03 %), γ-terpinene (8.28 %), and allo-aromadendrene (7.44 %) [9]. The main constituents of the essential oil of *Blumea lacera* leaves were thymoquinol di-mether, β-caryophyllene, α-humulene, and E-β-farnesene [20]. The main constituents of the oil from fresh aerial parts of *Blumea megacephala* were borneol (13.6 %), β-caryophyllene (9.56 %), germacrene D (9.09 %), sabinenev(6.37 %), and α-humulene (4.78 %) [8]. These results indicate that the essential oil of *B. eriantha* shares some relatively similar components with other the species of *Blumea which* can be serve as chemosystematic markers of *B. eriantha*. However, the chemical composition of the oil found in the present study, seems to be quite different from that reported by Geda in 2003, where the major components were the terpenoids nonadecane, n-heptacosane, delta-cadinene, 2,3-dimethoxy-p-cymene,apha-pinene, 1:8-cineole, carvotanacetone and caryophyllene-oxide [21]. This result confirms that the chemical composition of the oil of species of *Blumea* can vary depending on where plants are grown, or from genetic differences in distinct populations. In our results we found monoterpene Ocim-(4E, 6Z)-ene <allo-> is the major constituent of the essential oil from *B. eriantha*. Followed by that the bicyclic sesquiterpene Caryophyllene and epoxide Caryophyllene oxide are next major constituents in the essential oils and, are also common constituents in many essential oils from many *Blumea* species. Oxygenated monoterpene carvotanacetone is also one of the major constituent obtained in our study. This compound is also reported by Geda and in other reference [22] as common constituent for *B. eriantha* essential oil. The essential oil from *B. eriantha* also showed presence of norterpene, Ionone < (E)-, beta->. Norterpenes (norterpenoids or norisoprenoids) are C13 products occur when carotenoids are cleaved at the 9–10 position. These are important minor components of some essential oils, contributing particularly to aroma and flavor [23-25]. Many norterpenes that occur in essential oils have been shown to suppress the proliferation of cancer cell lines *in vitro*, includes β-ionone [26].

As stated earlier we have investigated the antimicrobial potential of essential oil from *B. eriantha* against skin pathogens. While considering the antimicrobial activity of essential oil with respect to chemical composition, it showed presence of many antimicrobial components for which antimicrobial activity have been tested in past. In general, the aldehydes, phenols and alcohols found to be the most active antimicrobial component groups and the terpene hydrocarbons and methyl esters appear to be the least active [27, 28]. The activity of the aldehydes may be due to the reactivity of the carbonyl group, which may react to form several antimicrobial moieties such as alcohols or acids [26].

The essential oil of *B. eriantha* contains 3.08% of aldehydes such as Perillaldehyde, Dec-(4Z)-enal and Deca-(2E,4E)-dienal. Perillaldehyde which is a monoterpenoid containing an aldehyde as functional group, has shown broad spectrum antimicrobial activity against range of gram negative pathogenic bacteria [26]. While decenal is one of the aldehydes present in Coriander leaf oil. The oil is having MICs in the range of 108 to 217 mg/ml (10.8 to 21.7%) for pathogenic bacteria including *Escherichia coli*, *Staphylococcus aureus* and *Klebsiella pneumoniae* and 163 mg/ml (16.3%) for *Candida albicans* [29]. The terpene alcohols include some of the most antimicrobially active essential-oil components. Their activity may be attributed to the alcohol moiety, which has intrinsic antimicrobial activity and enhances the solubility of these components in both aqueous laboratory media and microbial membranes [26]. The essential oil of *B. eriantha* contains alcohols such as geraniol (0.23%), terpinen-4-ol (2.29%), Nerolidol (0.27%). Geraniol, which is terpene alcohol, it has been tested against range of pathogenic microorganisms such as *Bacillus cereus*, *Staphylococcus aureus* and *Escherichia coli* etc. [26]. Terpinen-4-ol is a monoterpene cyclic alcohol is a major component of tea-tree oil has been extensively studied in the past for its antimicrobial activity. It has broad spectrum antimicrobial activity against many bacteria and fungi including dermatophytes such as *Trichophyton, Epidermophyton and*

Microsporon [26, 30]. Hydrophobicity of phenolics is mainly responsible for the antibacterial activity [31]. The phenolic component carvacrol present in the essential oil of *B. eriantha* also showed activity against various pathogenic bacteria including *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis* [26]. Verbenone, a ketone present in essential oil of *B. eiriantha* has MICs between 1.25 to 1.85 mg/ml (0.12 to 0.18%) for organisms including *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus* and *Candida albicans*, and 2.25 mg/ml (0.22%) for *Aspergillus niger* [26]. The essential oil of *B. eiriantha* constitute variety of terpene hydrocarbons such as limonene, α and β pinene, γ-terpinene which are studied extensively for their antimicrobial activity. Also compounds like alpha and beta pinene, sabinene, para-cymene, limonene, alpha-terpinene, terpin-4-ol, verbenone are also present in other essential oils such as Tea tree oil, lime, Citronella, Mint, Ginger, Lavender, Thyme and Clove, Cinnamon, Rose Chamomile, Grapefruit etc. [32, 33]. These oils are some of the important essential oils which showed significant activity against *Propionibacterium acnes.* Hence presence of these constituent may have important role for overall antimicrobial activity of the essential oil of *B. eriantha*, which also needs to be confirmed by in further studies with the individual constituents against the selected test pathogens.

HPTLC is one of the most rapid methods for the identification of the active constituents in medicinal plant extracts. In case of non polar terpene hydrocarbons which mainly constitute the essential oil, an adsorption separation technique is recommended [34]. Also terpene hydrocarbons can be conveniently separated on layers of silica gel [34]. As revealed by the GC-MS analysis, the essential oil from *B. eriantha* contains 37.45% monoterpenes and 30.73% of sesquiterpenes. This indicates that the essential oil constitutes about 67% of the terpene hydrocarbons. Hence HPTLC was preferred as it is a rapid as well as cost effective technique. Because of the weak polar nature of terpene hydrocarbons solvents of low polarity such as toluene and ethylacetate were used as mobile phase for the separation. The essential oil components which contain at least two conjugated double bonds, quench fluorescence and appeared as dark zones against the light green fluorescent background of the TLC plate under UV 254 nm [18]. The terpenoids from essential oil do not show any characteristic fluorescence at 366nm [18] hence no bands were appeared under UV 366nm. The treatment of chromatogram with vanillin sulphuric acid reagent resulted in appearance of various colored bands. This is an "Aldehyde Acid" reaction which depends upon protonation of aromatic aldehyde vanillin [35]. The protonation leads to formation of triphenylmethane type dyes due to condensation of certain organic molecules such as essential oil components [35]. Hence chromatographic zones often appear with variety of colors with vanillin. After treatment with Vanillin Sulphuric Acid reagent the monoterpene alcohols and their esters shows blue or blue–violet coloration in visible light [18]. The chromatogram of *B. eriantha* also showed blue coloration which may be due to the presence of monoterpene alcohols such as geraniol, borneol, nerol. Gocan [34] carried out analysis of terpenoids by TLC. In his analysis he obtained purple coloration for β–Caryophyllene with Vanillin Sulphuric Acid reagent. The essential oil of *B. eriantha* contains 9.71% of Caryophyllene. The purple colored band in the HPTLC chromatogram of essential oil may be due to the presence of Caryophyllene. All the colored bands obtained should be confirmed with the help of standards. The chromatogram may also be useful for further bioautography studies for determination of active constituent for antimicrobial activity.

The antioxidant activity of essential oil *of B. eriantha* was evaluated using DPPH method, as this is most commonly used simple and highly sensitive method for evaluating the antioxidant activity. The essential oil of *B. eriantha* is not dominated by any one constituent but it is mixture of many constituents. Many authors studied carvacrol for its antioxidant activity and reported that the higher content of carvacrol is closely related to strong antioxidant activity [36-38]. The essential oil of *B. eriantha* contains not high but only 0.31% of carvacrol which may be related to lower antioxidant activity of the essential oil. Yoko *et al* [39] reported that the oils with strong singlet oxygen scavenging have low limonene content. The essential oil of *B. eriantha* contains only 0.62% of limonene which may be responsible for the antioxidant activity. Khunkitti *et al* [40] reported β-caryophyllene exhibit weak antioxidant activity which is one of the major constituent of the essential oil from *B. eriantha* and hence may be responsible for the lower antioxidant activity of the oil. In the study of the antioxidant activity of *Thymus marschallianus* Will. and *Thymus proximus* [41], the authors stressed the importance of *p*-cymene besides thymol and γ-terpinene for the antioxidant activity. The essential oil of *B. eriantha* contains only 0.96 % of *p*-cymene. The oil also contains 1.47% of myrecene which has been also studied in vivo, in rats for its antioxidant effect and found to be an antioxidant [42]. There were many comparative studies conducted in past for antioxidant activity of either between essential oils or their individual components. For example Ruberto and Baratta [43] studied about 100 pure components of essential oils for their antioxidant effectiveness. In this study, in particular some monoterpene hydrocarbons, namely, terpinolene, α- and γ-terpinene showed a significant protective action, whereas among the oxygenated components, beside the aforesaid phenols, allylic alchols manifested an appreciable activity. Sesquiterpene hydrocarbons and non isoprenoid components subjected to this study showed a low, if any, antioxidant effect. While Sacchetti *et al* [44] studied eleven different kinds of essential oils, from those with a typical monoterpene hydrocarbon pattern. In this study the 11 essential oils tested, compared to that of Thymus *vulgaris* essential oil. He reported that oils with higher monoterpenic abundance, *Eucalyptus globulus* and *Psidium guayava*, were almost ineffective. This result is in agreement with the poor performance given by oils with similar patterns and by single monoterpenic hydrocarbons studied by Ruberto & Baratta *et al,* (2000). The essential oil of *B. eriantha* contains about 37.45% monoterpenic hydrocarbons which are highest among the other components followed by 30.73% of sesquiterpenes. Thus the oil contains approximately 67% of monoterpenic hydrocarbons and sesquiterpenes. Along with that the oil has very low levels of α- and γ-terpinene i.e. 0.07% and 0.12% respectively. Thus the presence of carvacrol, *p*-cymene, myrecene, α- and γ-terpinene in lower content and higher concentrations of non antioxidant compound i.e. monoterpenic hydrocarbons and sesquiterpenes may be responsible for the lower antioxidant activity of essential oil. The essential oils of *Ageratum conyzoides* or *Amomum tsao ko* presented better ability to prevent lipid peroxidation than for scavenging free radicals [45]. *Ageratum conyzoides* methanolic extracts that had low capacity for preventing lipid oxidation, in the DPPH assay were revealed to be more effective than the essential oil. Similar results were found for *B. eriantha* by Singh *et al* [46], who studied ethanolic and aqueous root and stem extract of *B. eriantha* for in vitro antioxidant activity using DPPH, Super oxide, Hydrogen peroxide and nitric oxide methods. Thus as future scope of research the essential oil of *B. eriantha* DC should be studied by other test methods for antioxidant activity to confirm its potential as an antioxidant.

CONCLUSION

The essential oil of *Blumea eriantha* DC from Seawoods, Navi Mumbai, (Maharashtra) India contains mostly monoterpenes and sesquiterpene hydrocarbons. Along with that the essential oil also contains epoxides, alcohols, aldehydes and small amount of ketones and ethers. Nevertheless gas chromatography is the standard method to analyse essential oils; high performance thin layer chromatography may be seen as a complementary technique for easy and fast identification of essential oil of *Blumea eriantha* DC. The antioxidant activity of the volatile oil of *Blumea eriantha* DC was measured in terms of hydrogen donating ability, using the stable radicle 2,2- diphenyl-1-picrylhydrazyl (DPPH). Reduction in absorbance is due to pairing of the odd electron of the radical indicating free radical scavenging activities. The radical scavenging activity of the essential oil is still less effective than the commercially available synthetic compound like α **–** Tocopherol. As present study and our previous investigation has proven in vitro antimicrobial and antioxidant potential of essential oil from *Blumea eriantha* DC, the essential oil can be exploited in herbal medicinal preparation and cosmetics as natural antioxidant and antimicrobial agent. The toxicity of the essential oil need to be evaluated which is a major concern in case of synthetic antioxidants and antimicrobial compounds.

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