

Chemical composition and anti-inflammatory activity of *Boswellia ovalifoliolata* essential oils from leaf and bark

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Abstract We isolated the essential oils from *Boswellia ovalifoliolata* N.P. Balakr and A.N. Henry using hydrodistillation, identified the obtained compounds using gas chromatography–mass spectrometry (GC–MS) and Fourier transform-infrared spectroscopy (FT-IR), and studied the photophysical and electrochemical properties using UV–visible and fluorescence spectroscopy and cyclic voltammetry. On the basis of GC–MS spectra, 38 compounds were identified in the essential oil from leaves and 26 in the oil from bark. The bark oil contained 13.44% β -Farnesene (sesquiterpene), 10.45% caryophyllene oxide (sesquiterpene) and 6.6% spathulenol, (2Z,6E)-(sesquiterpene alcohol); the leaf oil contained 11.1% spathulenol (sesquiterpene alcohol), 9.0% caryophyllene oxide (sesquiterpenoids) and 6.3% decyl acetate (ester). FT-IR spectra confirmed the presence of aliphatic aldehydes and ketones, carboxylic acid, alcohols, esters and ethers in both oil types. UV–visible absorption spectra showed maximum absorbance at 245, 290 and 402 nm for bark oil, and 250, 285 and 325 nm for leaf. Bark oil showed strong emission with maximum emission wavelength at 456 nm was higher than that of leaf oil at 414 nm. The essential oil had significant anti-inflammatory activity, and the reduction potential of the leaf oil was -0.44 and -0.56 eV for bark.

Keywords Essential oil · *Boswellia ovalifoliolata* · Gas chromatography–mass spectrometry · Fourier transform-infrared spectroscopy · Cyclic voltammetry fluorescence spectroscopy

Introduction

In India, plant parts of species of *Boswellia* are used to treat arthritis, pain, and respiratory ailments. Boswellic acid, the main compound in *Boswellia* species is used to treat several inflammatory conditions of the skin, eye, and other organs, and the gum is used to treat respiratory disorders such as asthma, bronchitis and laryngitis (Anderson and Davis 2007). *Boswellia ovalifoliolata* (BO) is an endangered medicinal plant of the family Burseraceae that grows in the Seshachalam hill range of the Eastern Ghats of India (Anitha and Sudarsanam 2013; Prabhakar et al. 2013). Ten compounds and three new compounds with anti-inflammatory activity were isolated from the oleo-gum resin of the plant (Chib et al. 2014). Crude extracts of *B. ovalifoliolata* have traditionally been used for their antimicrobial, antiadipogenesis, and antihyperlipidemic properties, and for their cardiotoxicity, antidiabetic and hepatoprotective activities (Anitha and Sudarsanam 2013; Prabhakar et al. 2013; Mahesh et al. 2014; Marella et al. 2014). Essential oils of *B. elongata*, *B. socotrana*, *B. dioscorides*, *B. sacra* and *B. carterii* have been reported. The essential oil of *B. elongata*, *B. socotrana* and *B. dioscorides* were shown to have antimicrobial activity and weak antioxidant activity (Mothana et al. 2011). The essential oil of *B. sacra* and *B. carterii* is 68.2 and 37.3% α -pinene, respectively (Woolley et al. 2012). Al-Harrasi and Al-Saidi (2008) reported the essential oil of *B. sacra* contains β -Ocimene and limonene as major constituents. Because they contain terpenoids,

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Boswellia species have commonly been used to treat inflammatory conditions; however, the anti-inflammatory activity of the essential oil from *B. ovalifoliolata* had previously not been studied.

Essential oil is a complex mixture of a low molecular weight compound extracted from plants mainly using steam distillation, hydrodistillation, solvents, or supercritical fluid extraction. Distillation methods are appropriate for powdered material, which remains loose in boiling water, whereas in the case of steam distillation it forms lumps that cannot penetrate the plant material. The advantages of hydrodistillation are as follows: the setup is inexpensive, easy to construct, and suitable for field operations. Modern supercritical fluid extraction allows extraction at high speed and efficiency, without harmful organic solvents. The use of CO₂ in supercritical fluid extraction is relatively nontoxic, nonflammable, and available at high purity and low cost. Disadvantages of conventional methods are low yield, loss of volatile compounds, long extraction time, and degradation of the unsaturated compounds (Sodeifian et al. 2016a, b), whereas for supercritical fluid extraction the primary disadvantage is that the extraction must be operated at 1000–5000 psi, to maintain the solvent in a supercritical state, which results in high costs. A modifier can be used to separate the polar analyte from the nonpolar analyte, which is comparatively difficult to achieve without the use of modifier. An essential oil (or volatile or ethereal oil) is a lipophilic liquid comprising the volatile aroma compounds of plants, which comprise the “essence” of the fragrance of a particular plant. They are used in food products, perfumeries, drinks, pharmaceuticals, and cosmetics as flavoring agents. An essential oil comprises terpenoids and phenylpropanoids as major constituents, and a few aromatic and aliphatic components are also present. Chemical entities such as monoterpenes, sesquiterpenes, and oxygenated derivatives are also present in the essential oil. The chemical compound of essential oil consists of hydrogen and carbon as the building blocks, which are isoprene in structure. Essential oil may also possess various biological activities such as antimicrobial, antiviral, anti-mutagenic, anticancer, antioxidant, anti-inflammatory, immunomodulatory, and antiprotozoal activities (Raut and Karuppayil 2014; El Asbahani et al. 2015).

The aim of this work was to study the chemical composition and variability between the essential oils from leaves and bark of *B. ovalifoliolata* grown in Seshachalam hill range of India using gas chromatography-mass spectrometry, UV-visible and fluorescence spectroscopy and cyclic voltammetry.

Materials and methods

Collection of plant material

The plant materials of leaves and bark were collected from the Seshachalam hills of Andhra Pradesh during February 2015. Species identification was confirmed by Prof. Jayaraman, Presidency College, Chennai. The plant parts were thoroughly washed with distilled water to remove contaminants and then shade dried and ground into fine powder using a mechanical grinder. The powder was stored in an airtight container for future analyses.

Extraction of essential oils

Twenty-five grams of finely powdered plant material was mixed with 250 mL of distilled water for hydrodistillation at 100 °C for 5–6 h using a Clevenger type apparatus. The extracted essential oils were separated from the water phase using chloroform in a separating funnel. Essential oils were dried using anhydrous magnesium sulfate and then stored at 4 °C in the dark. The percentage yield of essential oils was calculated (Li et al. 2013). All reagents were analytical grade from SD-fine chem, Mumbai, India.

Isolation and identification of essential oil

Essential oils were analyzed using a GC-2010 Plus High-end gas chromatograph (GC; Shimadzu, Kyoto, Japan) with a flame ionization detector (FID) and an Rtx-1 cross bond 100% dimethyl polysiloxane column (30 m × 0.32 mm) (Restek, Bellefonte, PA, USA); film thickness was 0.5 µm. The flow rate was 1 mL/min using helium as the carrier gas. The injector temperature was 250 °C and the oven temperature was maintained at 300 °C for 6 min. The oven temperature was increased from 50 to 270 °C (4 min) at a rate of 3 °C/min and held for 6 min. The GC-MS was carried out using a Clarus 680 GC coupled with a Clarus 600 (EI) mass spectrometer (Perkin Elmer, Waltham, MA, USA) and the same conditions as for the GC. The compounds were identified by calculating the retention index of each peak and comparing the mass spectra fragmentation with those from the National Institute of Standards and Technology (NIST) Library (Babushok and Zenkevich 2009; Babushok et al. 2011).

FT-IR analysis

Infrared (IR) spectra were obtained using a Shimadzu (Japan) Infrared spectrophotometer (400–4000 cm⁻¹) with resolution IV. The sample was dissolved in dilute chloroform solution for the analysis.

Fig. 1 Essential oils from bark and leaves viewed in daylight and UV light

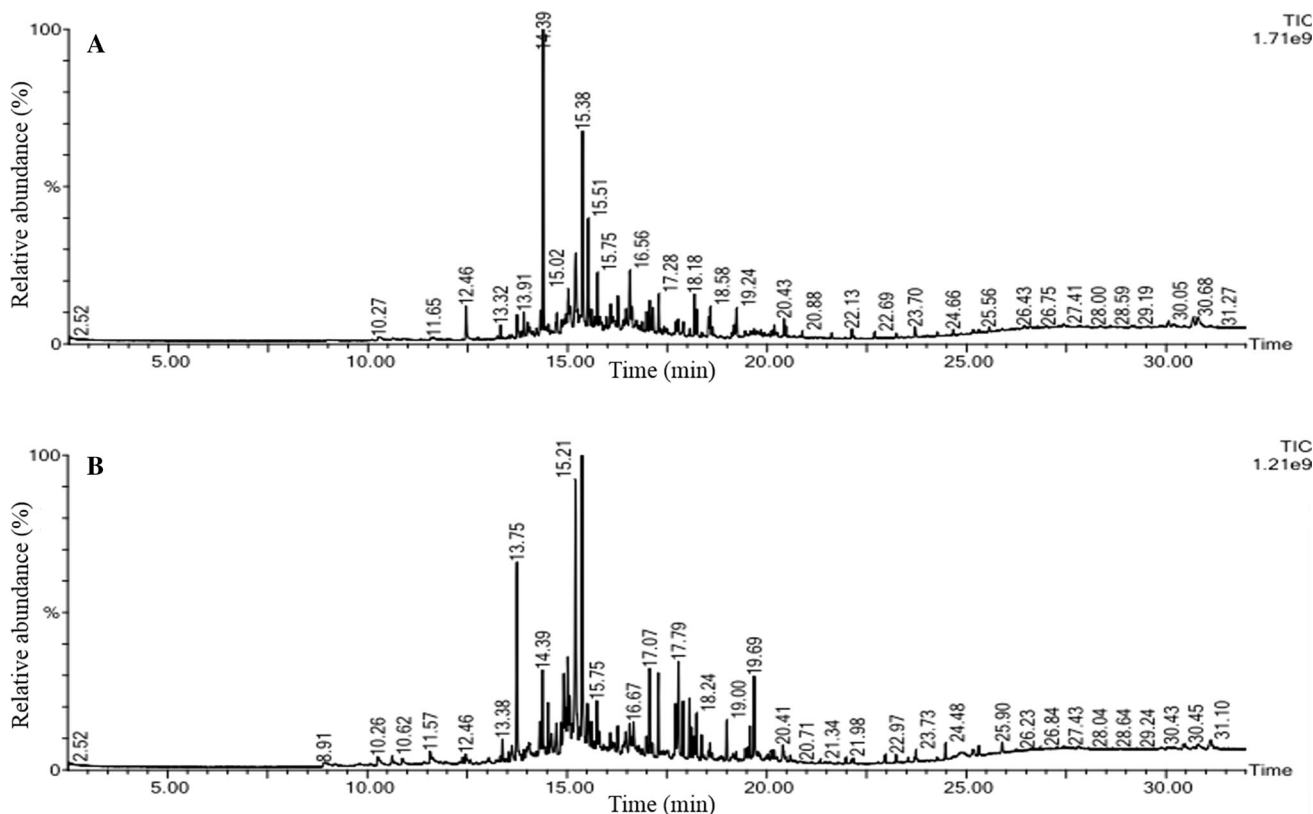
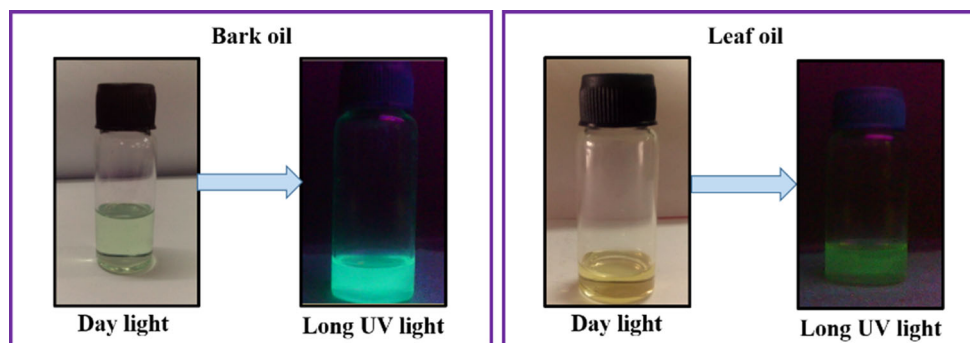


Fig. 2 GC-MS chromatogram of essential oils of *Boswellia ovalifoliolata*. **a** Bark. **b** Leaf

UV-visible spectroscopy analysis

The UV-visible absorption spectra were determined using a JASCO (Great Dunmow, UK) V-670 spectrophotometer in the absorption wavelength of 200–800 nm at room temperature.

Fluorescence spectroscopy analysis

Fluorescence was measured using Hitachi (Tokyo, Japan) F-7000 FL fluorescence spectrophotometer. The samples were dissolved in chloroform solution for the analysis.

Cyclic voltammetry analysis

The electrochemical study of leaf and bark essential oils was done by cyclic voltammetry (CV) using chloroform solution at different scan rates. A three electrode system was used for the measurement of the potential using a CHI 440B electrochemical workstation (CH Instruments, Austin, TX, USA) with a glassy carbon electrode as the working electrode (WE), a platinum counter electrode and saturated silver and silver chloride as the reference electrode (RE). A 5 μL sample was dissolved in 500 μL ethanol (stock solution) and drop casted on a pretreated

Table 1 Essential oil composition of bark and leaves from *Boswellia ovalifoliolata*

Serial. no.	Compound	RI	Bark (%)	Leaf (%)
1	2-Nonanol	1089	–	1.1
2	Carveol, cis	1208	–	1.0
3	Carvyl acetate, trans-	1325	–	0.74
4	Diphenylmethane	1380	1.74	–
5	Carvyl acetate, trans-	1313	0.59	0.74
6	α -Gurjunene	1405	–	0.91
7	Decyl acetate	1404	1.41	6.3
8	Aromadendrene	1433	1.25	–
9	Longifolene	1425	0.32	–
10	Germacrene D	1477	0.57	2.3
11	Longifolene	1425	0.96	–
12	β -Farnesene	1453	13.44	1.5
13	β -Bisabolene	1495	1.54	–
14	α -Calacorene	1530	–	3.3
15	Nerolidol,(Z)	1535	2.43	3.1
16	Spathulenol	1552	6.6	11.1
17	Caryophyllene oxide	1574	10.45	9.0
18	Caryophyllene oxide	1574	5.5	1.9
19	Caryophyllene oxide	1572	2.7	–
20	Longiborneol	1592	–	1.6
21	α -Cadinol	1640	–	0.77
22	α -Eudesmol	1646	–	1.1
23	Farnesol,(2Z,6E)-	1667	3.66	1.2
24	α -Sinensal	1740	0.965	0.65
25	2-Pentadecanone	1682	–	1.27
26	α -Selinene	1740	1.56	2.9
27	Tetradecanoic acid	1765	1.633	2.4
28	Benzyl benzoate	1758	0.35	–
29	Myrtenol	1788	1.01	3.2
30	Hexahydrofarnesylacetone	1832	1.76	–
31	Ethyl dodecanoate	1839	–	1.84
32	Pentadecanoic acid	1853	0.94	1.1
33	Hexadecyl acetate	1862	–	0.66
34	Farnesyl acetone,(5E,9E)	1913	–	0.39
35	1-Hexadecanol	1870	–	0.4
36	2-Methyl-1-nonadecene	1892	1.55	–
37	Methyl hexadecanoate	1913	–	1.3
38	Hexadecanoic acid	1965	–	0.56
39	Palustrol	1936	–	0.37
40	Eicosene	1950	0.70	–
41	Manoyl oxide	1989	–	0.94
42	13-epi-Manool	2005	–	0.27
43	Methyl eugenol	2008	–	2.7
44	Abietatriene	2039	–	0.28
45	Octadecanol	2089	0.70	–
46	Tetracosane	2424	0.63	–
47	Octanoic acid	2058	–	0.24
48	Manool	2042	–	0.27
49	Hexahydrofarnesyl acetone	2122	–	0.61
50	Undecanoic acid	2390	–	0.49

Table 1 continued

Serial. no.	Compound	RI	Bark (%)	Leaf (%)
51	Pentacosane	2490	1.06	–
Total	Essential oil identified	–	96.8%	96.09%

β -Farnesene is the main compound in the bark (13.44% of total) and caryophyllene oxide in leaf (9.0% of total)

RI retention index

glassy carbon electrode (GCE) and potential cycled at a scan rate of 50 mV/s. A cyclic voltammogram was obtained using 0.1 M phosphate buffer solution (pH 7.0.) at a potential window from +1 to –1 V versus silver/silver chloride (Basappa et al. 2015; Masek et al. 2014; Nicolson 1965; Carriedo 1988).

In vitro anti-inflammatory activity

The anti-inflammatory activity of the essential oils was determined by the albumin denaturation method according to Chandra et al. (2012) with some modifications. The reaction mixture consisted of 2 mL of different concentrations (100–1000 $\mu\text{g/mL}$) of leaf or bark oil or the nonsteroidal anti-inflammatory drug diclofenac sodium (Sun Pharmaceuticals, Mumbai, India) and 2.8 mL of phosphate-buffered saline (pH 6.4) mixed with 0.2 mL of egg albumin (from fresh hen eggs). The same volume of double-distilled water served as a control. The reaction mixture was incubated at (27 ± 2) °C for 15 min, and the test tubes were heated to induce denaturation at 70 °C in a water bath for 20 min. After the reaction mixture cooled, the absorbance was measured at 660 nm, and double-distilled water was used as the blank. The experiment was done in triplicate, and the extract values were averaged. The percentage inhibition of protein denaturation was calculated (Chandra et al. 2012).

Results and discussion

Yield of essential oil

The essential oils from leaves and bark contained sesquiterpenes, saturated fatty acids, and hydrocarbons. The essential oil from leaves was yellow and green from bark with a characteristic smell (Fig. 1). The yield of essential oils from leaves was 0.35% (w/w) and 3.7% (w/w) from bark. The yield from leaves was lower than from bark, as also reported by Li et al. (2013). The higher oil yield from bark was due to the smaller particle size (0.8–3.4 mm) of the plant material, which not only increased the interfacial area but also released more oil from broken cells. The smaller particle sizes result in higher extraction efficiencies, increase in mass transfer surface and quantity of soluble fraction on the

surface. The maximum essential oil yield from *B. ovalifoliolata* was 0.35% for leaves and 3.7% for bark, which was higher than the previously reported yield of 0.17% for leaves and 0.2% for bark (Prasanna Anjaneya Reddy and Venkata Ratnam 2015; Kirti et al. 2013; Maksimovic et al. 2012).

The identification and chemical composition of essential oil by GC–MS analysis

The essential oils present in leaf and bark are shown in the GC–MS chromatogram (Fig. 2), and the chemical composition of the essential oils are listed in Table 1. Around 38 compounds were identified and quantified from leaves (96.1% of all essential oils) and 26 compounds from bark (96.8%). Essential oil of bark contained 13.4% β -Farnesene (sesquiterpene), 10.5% caryophyllene oxide (sesquiterpene), 6.6% spathulenol (sesquiterpene) 3.7% 2Z,6E-farnesol (sesquiterpene alcohol) 2.4% nerolidol (sesquiterpene) and 1% myrtenol (monoterpene alcohol). Leaves contained 11.1% spathulenol (sesquiterpene alcohol), 9.0% caryophyllene oxide (sesquiterpene) 6.3% decyl acetate (ester), 3.3% calacorene (sesquiterpene), 3.2% myrtenol (monoterpene alcohol), 3.1% nerolidol (sesquiterpene), 1.0% carveol (monoterpene alcohol), and 0.6% of manool (diterpene alcohol) as major compounds (Fig. 2).

Sesquiterpenoids were the major constituents in leaves and bark. Sesquiterpenoids are a class of terpene with a group of 15 carbon compounds consisting of three isoprenoid units. These are used as fixatives in the perfume industry, and in higher plants, they function as pheromones and juvenile hormones. The major constituents of essential oils were identified in leaves and bark from the plant. The identified compound β -farnesene is a clear, colorless liquid that is used as a flavoring additive in food and beverages. Caryophyllene oxide is also used to flavor food and beverages. Spathulenol is used in aromatizing compositions for food and as a flavoring agent in the food and cosmetics industries. Compounds such as caryophyllene oxide have antifungal properties, spathulenol is antibacterial, and farnesene has an insect repellent property. The variation in the results obtained by Prasanna Anjaneya Reddy and Venkata Ratnam (2015) may have been due to the presence of a high monoterpene content; however, in our case, sesquiterpene content was high because the essential oils were extracted

Table 2 Major compounds in essential oils of Burseraceae family (*Boswellia* genus) and their biological activities (Perez et al. 2011)

Plant name	Sample	Compounds identified	Activity
<i>Boswellia ovalifoliolata</i> (Present work)	Dried leaf powder	Spathulenol, caryophyllene oxide decyl acetate carveol, myrtenol, manool (38 compounds)	Anti-inflammatory
	Dried bark powder	β -Farnesene, caryophyllene oxide, spathulenol, farnesol (2Z,6E)-, myrtenol (26 compounds)	Anti-inflammatory
<i>Boswellia ovalifoliolata</i> (Prasanna Anjaneya Reddy and Venkata Ratnam 2015)	Oleo gum resin	Caryophyllene, α -pinene, β -myrcene, ledol, α -amarphene (25 compounds)	Antioxidant, antimicrobial
	Bark	β -Myrcene, caryophyllene, α -amarphene, cyclofenchene, cedr-8-ene (25 compounds)	Antioxidant, antimicrobial
	Leaf	α -Pinene, β -pinene, α -terpineol, caryophyllene (30 compounds)	Antioxidant, antimicrobial
<i>Boswellia rivae</i>	Resin	Limonene	
<i>Boswellia ameero</i>	Resin	(E)-2,3-Epoxy-carene, 1,5-isopropyl-2-methylbicyclo [3.1.0]hex-3-en-2-ol, acymene (3E,5E)-2,6-dimethyl-1,3,5,7-octatetraene, 1-(2,4-dimethylphenyl)ethanol, 3,4-dimethylstyrene, α -campholenal, α -terpineol	Antioxidant anticholinesterase
<i>Boswellia carterii</i>	Oleo-gum resin	Isoincensole, isoincensole acetate	Antifungal
<i>Boswellia dalzielii</i>	Dried leaf	α -Pinene, α -terpinene	–
<i>Boswellia dioscorides</i>	Bark	α -Thujene, α -pinene	Antimicrobial, antioxidant
<i>Boswellia elongate</i>	Resin	Diterpene verticiol, caryophyllene, methyl cyclo undecane carboxylate	Antioxidant, anticholinesterase
	Bark	Incensol	Antimicrobial, antioxidant
<i>Boswellia frereana</i>	Resin	<i>p</i> -Cymene	–
<i>Boswellia neglecta</i>	Resin	α -Thujene, α -pinene, terpinen-4-ol	–
<i>Boswellia papyrifera</i>	Oleo-gum resin	Isoincensole, isoincensole acetate, <i>n</i> -octanol, <i>n</i> -octyl acetate	Antifungal
	Resin	–	Inhibited <i>Staphylococcus epidermidis</i> and <i>Staphylococcus aureus</i> biofilms
	Branch	–	Antimicrobial
<i>Boswellia pirotta</i>	Resin	<i>Trans</i> -verbenol, terpinen-4-ol	–
<i>Boswellia rivae</i> Engler	Oleo-gum resin	Hydrocarbon, oxygenated monoterpenes	<i>Candida albicans</i>
	Resin	–	Active against <i>Candida albicans</i> biofilms
<i>Boswellia sacra</i>	Resin	<i>E</i> - β -Ocimene, limonene, <i>E</i> -caryophyllene	–
	Resin	Boswellic acids	Anticancer
	Branch	–	Antimicrobial
	Resin	α -Pinene	Antibacterial
<i>Boswellia serrata</i> Roxb.	Oleo-gum resin	Isoincensole, isoincensole acetate	–
	Resin	α -Thujene	–
	Bark	α -Pinene	–
	Resin	α -Thujene	–
<i>Boswellia socotrana</i> Balf. f.	Resin	(E)-2,3-Epoxy-carene, 1,5-isopropyl-2-methylbicyclo [3.1.0]hex-3-en-2-ol, and acymene, (3E,5E)-2,6-dimethyl-1,3,5,7-octatetraene, 1-(2,4-dimethylphenyl)ethanol, 3,4-dimethylstyrene, α -campholenal, α -terpineol	Antioxidant, anticholinesterase
	Bark	<i>p</i> -Cymene, 2-hydroxy-5-methoxyacetophenone, camphor	Antimicrobial, antioxidant

from fresh plant material and dry, powdered material. Monoterpene content extracted from fresh plant material is higher than from dry powder because some oil is lost when the material is dried (Ichi 2016). On the other hand, the total

essential oil content on a mass basis is higher in the dry powder material than in the fresh plant material because it has less water. Similarly, less dry plant material is needed than fresh material to achieve equivalent percentage yields.

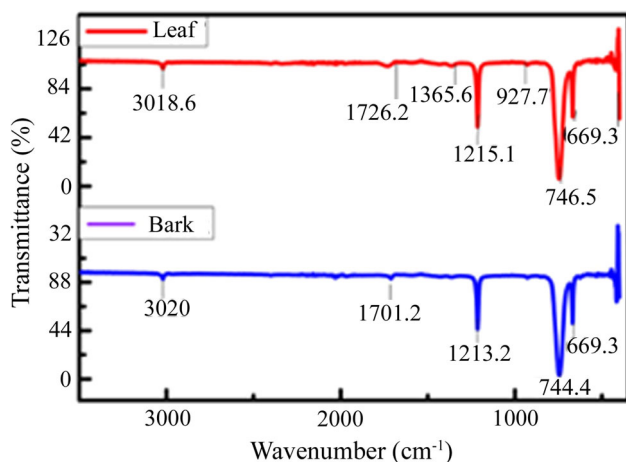


Fig. 3 FT-IR spectra of essential oils of *Boswellia ovalifoliolata*

There is also a small variation in the essential oil concentration, which is influenced by the sample size (Charles and Simon 1990).

The essential oils in the Burseraceae family have many biological properties such as antifungal, anticancer, antioxidant, anticholinesterase, antibacterial, antimalarial and larvicidal. *Cedrelopsis grevei* belongs to the Burseraceae family, and its essential oils possess anti-inflammatory, anticancer, antimalarial, and antioxidant properties. There are no reports yet on an anti-inflammatory property of the essential oil from the *Boswellia* genus (Murthy et al. 2016); however, compounds and their biological activities have been identified for other species from the Burseraceae family (Table 2).

The GC-MS for the essential oils from the bark and leaves showed that caryophyllene oxide and spathulenol are the two major compounds common to both plant parts. In addition, the chemical composition, appearance, and percentage yield of the essential oils differed between the leaf and bark.

FT-IR analysis of essential oils

In the FT-IR analysis of the functional groups, absorption bands at 927, 744 and 3020 cm^{-1} corresponded to C=C stretching, and carbonyl C=O stretching was confirmed by a peak between 1726 and 1701 cm^{-1} (Fig. 3). Significant stretching for C-O was observed at 1213 cm^{-1} , and the absorption band at 1365 and 669 cm^{-1} indicated C-H bending and halide stretching of the compounds.

Photophysical properties of essential oils

In the photophysical study using UV-visible (UV-Vis) and fluorescence spectroscopy method, the leaf and bark oils yielded three maximum absorption peaks, and the

absorption band of the leaf at 250 nm and the bark at 245 nm corresponded to a $\pi-\pi^*$ conjugation system and electron transfer in the benzene ring (Fig. 4a). The peak at 325 and 402 nm indicates an $n-\pi^*$ transition (Fig. 4b) and the presence of a keto group in the essential oil. The absorption bands at 285 and 290 confirm an $n-\pi^*$ transition. The UV-Vis spectra confirmed the presence of a conjugation system in the essential oil. The fluorescence excitation wavelength of the essential oils from the leaf and bark showed significant shifts in the emission maxima for each.

The fluorescent compounds in the essential oil had an emission range from blue to red shifts. Among the three excitation wavelengths, the excitation wavelength at 402 nm of bark and 325 nm of leaf provided a larger emission peak at 456 and 414 nm, respectively. The maximum emission wavelength for bark oil is due to the presence of conjugated double bonds in bark oil. The luminescence is due to the response of the plant to stress and shock. Similarly, fluorescence may also be correlated with senescence and stress (Boschi et al. 2011). The difference in the emission may be associated with chemical compounds, the main component of essential oil is sesquiterpenoids with a different degree of conjugation.

Cyclic voltammetry analysis

Cyclic voltammetry was used to characterize the interaction between electroactive species in the essential oils of the leaf and bark and the platinum electrode surface and to measure the peak reductive potential using an anodic current. Oxidation and reduction peak potentials were observed between -1.0 and 1.0 eV. The onset oxidation potential of the leaf and bark was observed at 0.75 and 0.74 eV; subsequently, onset reduction potentials were observed at -0.44 for the leaf oil and -0.56 eV for the bark oil. Figure 4c shows the cyclic voltammogram recorded during the cathodic potential sweep of the leaf and bark essential oils of *Boswellia ovalifoliolata*. The presence of the leaf and bark essential oil reduced the current value, which indicates the inhibition of the charge transfer process as a result of the oxygen electroreduction. Thus, the cyclic voltammetry allows the determination of the antioxidant molecules present in the essential oil of *B. ovalifoliolata* (Goncalves et al. 2009; Masek et al. 2013). The presence of a chemical compound in essential oil by photophysical and electrochemical techniques is reported here for the first time (Prasanna Anjaneya Reddy and Venkata Ratnam 2015).

In vitro anti-inflammatory activity of essential oil

Anti-inflammatory activity of the essential oils was investigated using the protein denaturation method.

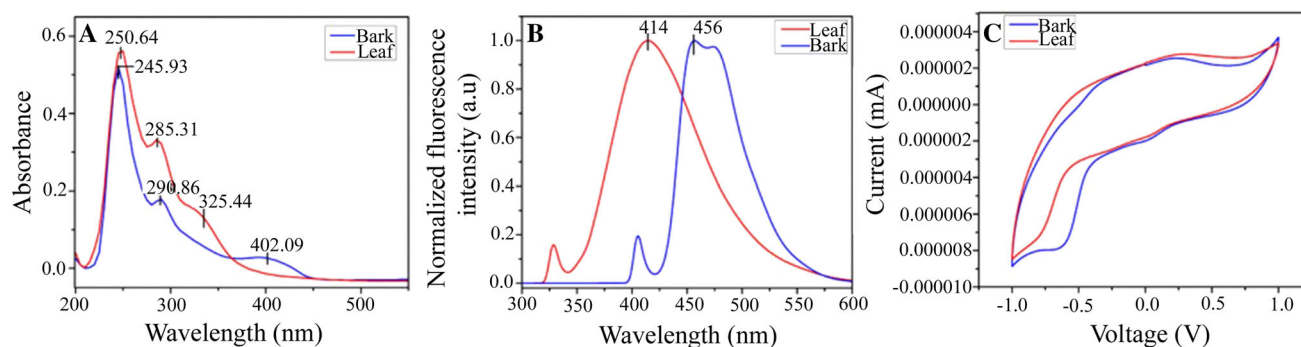


Fig. 4 UV-visible, **a** and fluorescence, **b** spectra and cyclic voltammogram, **c** for leaf and bark essential oils of *Boswellia ovalifoliolata*

Table 3 In vitro anti-inflammatory activity leaf and bark oil

Concentration ($\mu\text{g mL}^{-1}$)	Inhibition (%)		
	Bark oil	Leaf oil	Diclofenac sodium
200	9 \pm 0.8	46.2 \pm 2.45	–
400	124.4 \pm 1.17	195.6 \pm 1.55	–
600	176.8 \pm 2.05	255.2 \pm 1.5	37.4 \pm 1.05
800	209.2 \pm 2.55	303.3 \pm 1.66	70.5 \pm 0.3
1000	254.8 \pm 1.95	360.6 \pm 1.96	102.9 \pm 0.2
IC ₅₀ value	271.3	202.1	670.36

Denaturation of a protein is a process whereby the proteins lose their secondary and tertiary structures by external stress or compounds such as an acid or base, an inorganic salt, an organic solvent, or heat. Denaturation occurs when biological proteins lose their biological function. The denaturation of a protein is a well-known method in the cause of inflammatory and arthritic diseases. The anti-inflammatory activity of the leaf and bark oils was compared with that of diclofenac sodium as the reference drug. The IC₅₀ value of leaf was 202.1 and 271.3 $\mu\text{g/mL}$ oil for bark; thus, the anti-inflammatory activity of the oils was higher than that of diclofenac sodium (Table 3). The essential oil may inhibit the release of the lysosomal content of neutrophils at the inflammation site (Govindappa and Poojashri 2011). The neutrophil lysosomal constituents include proteinases and bactericidal enzymes, which lead to tissue inflammation and damage upon their extracellular release. *Canarium schweinfurthii* and *Aucoumea klaineana*, (Burseraceae) an essential oil using the lipoxygenase method, only the *C. schweinfurthii* essential oil was active with *p*-cymene, limonene and α -terpineol as the major compounds (Leelaprakash et al. 2011).

Conclusion

In summary, the use of GC–MS, FT-IR, UV–Vis, fluorescence spectroscopy and cyclic voltammetry provided the necessary information on chemical composition and the

variability of the leaf and bark of *Boswellia ovalifoliolata* essential oils. This study is the first report of the presence of a chemical compound determined using a spectrophotometric and electrochemical technique. Spectrophotometric techniques are faster and more cost-effective than chromatographic methods, which are often time-consuming and may require large amounts of toxic solvents. The fluorescence of leaf and bark essential oils was high at 414 and 456 nm, and the cyclic voltammetry showed good onset reduction potential at -0.44 and -0.56 eV. Caryophyllene oxide and spathulenol were the major constituents of the essential oils and anti-inflammatory property of essential oil was reported, and the fluorescence property of the essential oils needs to be studied for various biological applications.

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