Phytochemical profile using GC-FID and *invitro* biological activities of essential oil from *Boswellia serrata*

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Abstract

Boswellia serrata is a tree native to India, and is known as Indian frankincense (Guggul). It has been used for centuries in traditional Ayurvedic medicine to treat a wide range of health conditions, including arthritis, inflammation, and asthma. Apart from its importance, complete pharmacological and phytochemical studies are still in the early stages. In the current study, Guggul essential oil (GEO) was extracted from *Boswellia serrata* and evaluated for its GC-FID analysis, UV-VIS, FT-IR and Fluorescence analysis, phytochemicals, antimicrobial, anti-inflammatory, and anti-diabetic activities. Various antioxidant activities like DPPH (1,1-diphenyl-2-picrylhydrazyl), ABTS (2,2-azinobis-3-ethylbenzothiazoline-6-sulphonic acid), Hydroxyl radical, iron chelating, iron-reducing activity were performed. GC-FID revealed the presence of limonene, octanol, and caryophyllene, major components of *Boswellia serrata* essential oil and other bioactive compounds. High concentrations of phytochemicals like Tannin (1.309mg AA/ml), Flavonoids (0.056 mg RUE/ml), and Phenolics (0.653mg GAE/ml) were revealed through phytochemical analysis. The IC_{50} value of this oil for various antioxidant assays was: DPPH (6.061), ABTS (36.14), Iron chelating (30.82), Hydroxy radical scavenging activity (41.5), α-Amylase inhibition (24.86). *Boswellia serrata* essential oil shown anti-inflammatory and inhibits the alpha amylase in a competitive manner. Hence, from the results, it is confirmed that the GEO possesses a considerable number of bioactive compounds and can be used as an antibacterial and anti-diabetic agent in the pharmaceutical, food, perfume, and cosmetic industries.

Keywords: *Boswellia serrata*, biological activities, essential oil, phenols, Herbal Drug

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Introduction

According to Ayurvedic and Unani literature, medicinal plants initiated the development of medical sciences. (Sharma et al.,2022; Pattanayak et al., 2010). Traditional herbal medicine is currently occupying a special position among the treatments for a wide range of illnesses (Chauhan et al., 2021). Both the traditional and modern medical systems place a high value on herbal remedies (Suchita et al., 2021). Additionally, research on the most pharmaceutically significant plant metabolites that are useful for making medications on a large scale is aided by the use of herbal medicine (Rashan et al., 2019; Buyel et al., 2018). Plant essential oils are made up of biologically active compounds found in medicinal plants, especially those exhibited to have therapeutic potential. These molecules are typically produced as a defence mechanism in plant systems (Yildrim et al., 2001; Obafemi et al., 2017). Pure natural compounds are employed as pharmaceutical agents, while herbal treatments are practised using plants or specific parts of the plant. As a result, the bioactive compounds in the extracts have acted in synergy to demonstrate therapeutic activities (Gupta et al., 2022). Essential oils are volatile, natural, aromatic chemical compounds that have a highly strong odour. These are plants secondary metabolites (Sadhasivam et al., 2016). They can be synthesized by all plant organs (flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits and root) and therefore extracted from these parts, where they are stored in secretory cells, cavities, canals, epidermic cells or glandular trichomes (Bakkali et al., 2007). Essential oils are obtained by the steam distillation and hydro-distillation of plant parts such as flower bud, leaves, stem, roots, seed, fruit, gum resin, and barks. These are the conventional methods of oil extraction. There are many different kinds of compounds in essential oils, including terpenoids. The main volatile components are hydrocarbons (e.g., pinene, limonene, bisabolene), alcohols (e.g., linalool, santalol), acids (e.g., benzoic acid, geranic acid), aldehydes (e.g., citral), cyclic aldehydes (e.g., cuminal), ketones (e.g., camphor), lactones (e.g. bergaptene), phenols (e.g., eugenol), phenolic ethers (e.g., anethole), oxides (e.g., 1,8 cineole) and esters (e.g., geranyl acetate) (Opren et al., 1998). These oils are responsible for the distinctive aromas associated with individual plant species. The biologically active properties of essential oils include antibacterial, antifungal, anticancer, anti-inflammatory, and antioxidants (Syluestre et al., 2006). Essential oils are natural compounds, according to Ju et al. (2019), essential oils are also employed as natural food preservatives. Worldwide, Schippmann et al. reported that almost 50,000 plant species have been employed to produce various types of metabolites for medical purposes (Schippmann et al.,

2002). At present, approximately 3000 essential oils are known, 300 of which are commercially important especially, particularly for the medicinal, agronomic, food, sanitary, cosmetics, and perfume industries. Another biological characteristic of great interest is the antioxidant activity of essential oils, which may protect food from the harmful effects of oxidants. Additionally, the ability of essential oils to scavenge free radicals may be crucial in the prevention of certain diseases like brain dysfunction, cancer, heart disease, and immune system decline. More and more evidence points to the possibility that the cellular damage caused by free radicals might be the origin of these diseases (Miguel 2010). The pursuit of bioactive substances that inhibit harmful germs in healthcare or the food business has thus received increased attention in research. Additionally, the prolonged use of synthetic pesticides has raised more questions about how they affect the environment and people's health.

A medium-sized, branching tree, *Boswellia serrata* is part of the Burseraceae family. A gum resin is produced by Boswellia species (Burseraceae), which are trees that are native to Ethiopia, Somalia, India, and the Arabian Peninsula (Alam et al., 2012; Bogavac et al., 2022). The Ayurvedic and conventional medical systems have traditionally employed this gum resin. The existence of resin ducts in the barks, which create fragrant oleo-gum resins known as frankincense, olibanum in Arabic, Luban in Jibali, and lubari Arabic (Al-Saidia et al., 2012). Ayurveda's ancient and most holy herb is *Boswellia serrata*, also known as Salai Guggul. The Burseraceae family of plants, which is found worldwide all tropical climates, is distinguished by its 17 genera and 600 species. There are approximately 25 species in the genus Boswellia, and the majority of them are found in Arabia, along the north-eastern coast of Africa, and in India (Sultana et al., 2013). Commiphora (myrrh), Canarium (incense elemi), and Boswellia (frankincense), among others, are some of the genera found in Asia and Africa where studies and knowledge are concentrated despite the fact that Burseraceae species are found all over the world (Alraddadi et al., 2022). It has long been a vital component of the hawan samagri dhoop in Hindu ancient rituals since the medicated smoke of this resin is useful in curing several respiratory ailments like asthma, bronchitis, and laryngitis (Siddiqui 2011).

Plants use resin as protection; thus the essential oil also exhibits a number of advantageous properties. For flavouring and aroma purposes, frankincense (Boswellia) oils have traditionally been utilized whole. As a result of its outstanding antibacterial and antifungal properties, *Boswellia*

Serrata essential oil is appropriate for use in food preservation (Abdelsamadet al., 2020). According to certain research, frankincense oil (FO) can shield stored goods against fungi because it lowers the deadly aflatoxins that Aspergillus species excrete. Additionally, *Boswellia Serrata* essential oil has significant antibacterial action against a variety of food deterioration microorganisms (Mustafa et al., 2021).

The *Boswellia serrata* essential oil is typically light yellow in colour and has a pleasant aroma. It has been used to treat a variety of illnesses, including as cancer, arthritis, asthma, and psoriasis. In Ayurvedic and traditional Chinese medicine, this gum resin has been used for a very long time to treat a variety of health conditions, including inflammatory and arthritic disorders (Ni et al., 2012). For generations, its essential oils have been used to make perfumes, cosmetics, incense for aromatherapy, and for medicinal purposes (Lemenith et al., 2003). The anti-bacterial, anti-fungal, anti-tumor, and anti-inflammatory properties of *Boswellia spp* essential oil (also known as frankincense oil or Guggul essential oil, GEO) have been documented to have numerous benefits (supplementary table 1) (FadulAllA et al., 2021, Mustafa et al., 2021). Because of their potential anti-bacterial, anti-fungal, and antioxidant capabilities, it can be utilized as an efficient food preservative (FadulAllAet al., 2021). However, as far as we know, it is an accentuated plant that is not expansively grown due to the scarcity of proper research on its agroeconomic, medicinal, and pharmacological importance. It became interesting to analyze the essential oil of *Boswellia serrata* to evaluate the chemical composition, different antioxidant properties, anti-inflammatory and antimicrobial properties. Therefore, the objective of the present study was to extract the *Boswellia serrata* essential oil and GC-FID analysis, UV-VIS, FT-IR, Fluorescence analysis, its phytochemical, antioxidant content, antimicrobial activity, Anti-inflammatory activity, membrane integrity assay and anti-diabetic potential analysis of *Boswellia Serrata* essential oil.

Material and methods

FRANKINCENSE ESSENTIAL OIL EXTRACTION

The frankincense (guggul) essential oil was chosen for the current research. This oil was extracted by steam distillation from the resin of the *Boswellia serrata*. The essential oil investigated in the present study was kindly supplied by Amazon (Naturalis Essence of Nature, ISBT, Bengaluru, INDIA).

GC-FID ANALYSIS

The Gas Chromatography (GC-FID) study of GEO was performed by using a method given in Amrita et al. (2023). Chemtron 2045 gas chromatograph coupled with flame ionization detectors. A stainless steel column (2 m long) filled with 10% OV-17 on 80–100% mesh Chromosorb W. (HP) was used. Nitrogen gas was used as carrier gas a with flow rate of 30 mL/min. The detector and injector temperature were kept at 200 and 250°C. 0.2 µl of the sample was injected. Ramping conditions for the oven were: 64°C maintained initially then ramped to 220°C at a rate of 3°C/min. Bioactive compounds were identified by comparing the relative retention time with known standards (eucalyptol, citral, eugenol and geraniol,Sigma-Aldrich (St. Louis, MO, USA)or with data published in the literature.

UV-FT-IR AND FLUORESCENCE ANALYSIS

A UV-VIS spectrophotometer (Labtronics) with a slit width of 2 nm, using a 10 mm cell at room temperature, was used to conduct the UV-VIS spectrum analysis of pure guggul essential oil (GEO) isolated from *Boswellia serrata*.The identification of numerous bioactive substances and their specific functional groups detected in GEO was conducted using FT-IR (Fourier Transfer Infrared) spectroscopy. The FT-IR spectrophotometer (Perkin Elmer, USA spectrophotometer) was used to capture the IR spectra. GEO was evaluated between 400 and 650 cm-1, and the spectra obtained were recorded and investigated. The Perkin Elmer spectrophotometer (FL6500) was used to record the fluorescence spectrum of GEO. With a slit width of 10 nm, emission starting at 410 nm and ending at 700 nm, and a scan speed of 240 nm/min at excitation wavelength 310 nm, the NN07 technique was employed. All experiments were performed at room temperature (25°C-30°C)

DETERMINATION OF ANTIOXIDANT PROPERTY

Antioxidant tests were performed following Sharma et al.(2021).Various antioxidant assays were performed to investigate the antioxidant capacity in the GEO.

Hydroxyl Radical Scavenging Activity

The reaction mixture for the generation of hydroxyl radicals consists of 1 ml of Phenanthroline (0.75 mM), 2 ml Sodium Phosphate Buffer (0.2M), 1 ml of FeSO4(0.75 mM), and 1 ml of H_2O_2

(0.01%). To this reaction mixture add GEO in increasing volume (10-50 µl). Then incubate the reaction mixture for 30 min at 37°C. After the completion of the incubation time, absorbance was recorded at 532 nm against deionized water as a blank solution, while ascorbic acid (1mg/ml) was used as standard/positive control. The experiment was conducted in a set of two. The **%age** Hydroxyl Radical Scavenging Activity was calculated by using the given formula: Scavenging Activity(%)= (Abs of sample- Abs of blank)/(Abs of control- Abs of blank)*100,whereas;Abs of control= Absorbance of water in the reaction mixture in place of H_2O_2 and sample (GEO).

DPPH Free radical scavenging activity

The free radical scavenging capacity of GEO was evaluated using the DPPH test. Three millilitres of DPPH solution are added to different volumes of GEO (10-50 µl). The reaction mixture was incubated for 1 hour at room temperature (25°C-30°C) in the dark. After incubation absorbance was measured at 517 nm and 82% methanol was taken as black. Three millilitres of DPPH in 82% methanol were taken as control. Ascorbic acid acts as a standard/positive control. The test was performed in duplicates. The free radical scavenging activity for GEO is demonstrated by the percentage of the scavenging activity using the following formula: Scavenging activity% = A_0-A_1 / A_0* 100, Where,A_0 = Absorbance of DPPH solution(control)A_1 = Absorbance of the sample to be tested.

ABTS Radical Scavenging Activity

To generate the cation of ABTS first mix the 50 ml of ABTS with 50 ml of potassium persulphates (2.45 mM) and kept in the dark for 15-16 hrs until reaction completion. Measure the absorbance of generated ABTS cations and compensate the absorbance to 0.7(±0.05) with ethanol. Three millilitres of ABTS-generated radicals were added to various amounts of the GEO (10-50 µl). Incubated for 6 min. The absorbance was measured at 734 nm and ethanol is taken as blank. Ascorbic acid served as a positive control standard. Control was pure ABTS radical. Examined in duplicates. The ABTS scavenging activity of GEO was counted in terms of percentage scavenging activity by applying the following given formula: Percentage scavenging activity = A_0-A_1/A_0*100Where,A_0 = absorbance of the controlA_1= absorbance of oil with various volumes.

Iron Reducing Assay.

Iron Reducing Assay of GEO was measured by following the procedure of Obafemi et al. (2017). Different volumes of the GEO (10-50 µl) were mixed with the 1.25 ml of Sodium Phosphate Buffer (200 mM, 6.6 pH). Add 1.25 ml of potassium ferricyanide (1%) in the above reaction mixture and incubate at 50°C for 20 minutes. After incubation, the reaction mixture was acidified with 1.25 ml TCA (10%) and centrifuged at 3000 rpm for 10 minutes. Add 1.5 ml H_2O to 1.5 ml of the upper phase of centrifuged reaction mixture. Finally, add 0.1 ml of ferric chloride to the upper phase of the reaction mixture. Leave the solution for incubation at room temperature for 10-15 minutes. The absorbance was measured at 700 nm in the spectrophotometer.

Iron Chelating Ability Assay

The iron chelating activity of GEO was examined by the following method by Obafemi et al. (2017).**900 µl of aqueous ferrous sulphate (500µM) to different volumes of GEO (10-50µl) was added** and incubated for 5 minutes at room temperature. 78 µl of 1,10-phenanthroline (0.25% w/v) was added. **The absorbance at 510 nm was measured** the with a spectrophotometer. The ***invitro*** iron chelating ability of the GEO was estimated by using the following formula: Chelating ability(%) = $(A_0 – A_S)/A_0$ * 100Where, A_0= Absorbance of the control ($FeSO_4$ alone)A_s= Absorbance of the reaction mixture in the presence of ascorbic acid/ GEO

Total Phenolic Content

The total phenolic content of GEO was measured by the Folin-Ciocalteu method in Sharma et al.(2021). Different volumes of GEO (10-50µl) were made up to 3 ml with distilled water. Added 0.5 ml of Folin-Ciocalteu Reagent to the above mixture. Incubated the reaction mixture for 3 minutes. Two millilitres of 20%(w/v) Sodium Carbonate were added to each sample. The final solution was incubated for 60 minutes in the dark protected from light. The absorbance of samples was counted at 650 nm in the spectrophotometer. Gallic acid is used as a standard or for calibration curve. The results articulated as mg of Gallic acid equivalent per ml of GEO standard curve equation y=0.0038x + 0.078.

Total Flavonoid Content

The total Flavonoid contents of GEO was determined by the Aluminum Chloride colorimetric method by Sharma et al.(2021). Different volumes of GEO (10-50 µl) were made up to one millilitres with methanol. 0.3 ml of $NaNO_3$ (5%) was added to each sample. The reaction mixture was incubated at room temperature for 6 minutes. To the above reaction mixture, 0.3 ml of $AlCl_3$ (10%) was added and incubated at room temperature for 6 minutes. Finally, two millilitres of NaOH (1M) solution were added and made up a final volume of 10 ml with distilled water. The reaction mixture was incubated for 15 minutes in the dark protected by light at room temperature. After incubation absorbance reading was noted down at 510 nm. Rutin served as the calibration curve. The results were expressed in the form of mg of the equivalent of Rutin/ml of GEO by implying standard curve y= 0.0031x + 0.0822.

Total Condensed Tannins

The Total condensed tannins content of GEO was determined by the method given by Sharma et al., (2021). A three millilitres solution of vanillin (4%) in methanol was added to the various amounts of the GEO (10-50µl). Mix 1.5 ml of conc. HCL to each. The mixture was incubated for 15 minutes at room temperature. The absorbance was taken at 500 nm against methanol used as blank. The results were stated as mg of ascorbic acid equivalent per ml of GEO using standard curve equation y=0.0002x + 0.0321.

Anti-Inflammatory Activity (Protein Denaturation)

The Protein denaturation assay was examined according to the protocol of Sharma et al., (2021). The reaction mixture comprises 0.4 ml of (1%) BSA, 4.78 ml of (1%, pH 6.4) PBS, and different volumes of GEO (100-300 µl). The reaction mixture was incubated at 37°C for 15 minutes in a water bath. The reaction mixtures were heated at 70°C for 5 minutes and instantly cooled down. 1 ml of each mixture was exposed to fluorescent spectroscopy after cooling for fluorescent analysis. NN05_2 method was used at an excitation wavelength of 280 nm and fluorescence emission spectra were observed at the range of 300-400 nm wavelength.

Anti-Diabetic Activity (Alpha-Amylase Inhibition Assay)

125µl of α-amylase solution (5mg/ml) was placed in test tubes and mixed with different amounts of GEO (50-250 µl). 0.5 ml Sodium phosphate buffer (0.02M, 6.9 pH) was added to the above mixture and pre-incubated for 10 minutes at 25°C. 500µl Starch solution (2%) prepared in 0.03M

sodium phosphate buffer (6.9 pH) was added and incubated at 25°C for 10 minutes. Then 500 µl of DNS Reagent was added to the solution to stop the reaction. For 10 minutes, the reaction mixture was kept at 100°C in the water bath. Consequently, 6 ml of water was added after cooling down. The optical density of samples was measured at 540 nm in a spectrophotometer. As a positive control, acarbose was used. Analyzed the α-amylase inhibition activity by the given formula: Percentage Inhibition= $A_{control}$-A_{sample}/$A_{control}$*100Where,$A_{control}$= Absorbance of the reaction mixture (without sample/positive control)A_{sample}=absorbance of the reaction mixture (with sample GEO /positive control Acarbose)

Mode of Alpha-Amylase Inhibition

Determination of the mode of alpha-amylase inhibition was performed according to Kazeem et al., (2013): The first set of reaction mixture containing 75µl of GEO was pre-incubated with 0.2 ml of alpha-amylase solution (prepared in 0.02M Sodium phosphate buffer) and 0.5 ml of 0.02M Sodium phosphate buffer at 25°C for 10 minutes. The second set of reaction mixture contains 0.5 ml of 0.02M Sodium phosphate buffer and was incubated with an alpha-amylase solution at 25°C for 10 minutes. To initiate the reaction, various volumes of the starch solution in increasing concentrations (50-250 mg) were added to both sets of test tubes. Incubation was given to the reaction mixture at 25°C for 10 minutes. To terminate the reaction 0.5 ml of DNS reagent was added followed by incubation at 100°C for 5 minutes. To determine the quantity of released reducing sugars and their conversion to reaction velocity maltose standard curve was used. A double reciprocal plot (1/V versus 1/S) between substrate concentration (S) and velocity (V) was plotted. The mode of inhibition of GEO on the alpha-amylase activity was recognized by analysis Lineweaver-Burk plot to use Michaelis-Menten kinetics.

***In Vitro* Determination of Anti-Bacterial Activity Of GEO**

Agar Disc Diffusion method was used to determine the antimicrobial activity of GEO against four test organisms, Gram-negative *Escherichia coli* (MTCC 40), *Pseudomonas aeruginosa* (MTCC 424), and Gram-positive *Staphylococcus aureus* (MTCC 3160), *Bacillus subtilis* (MTCC 121). Microorganisms were purchased from IMTECH (Institute of Microbial Technology, Chandigarh). The Fresh inoculum was prepared for the all-microbial strains from 12-hour-old cultures and the McFarland's standard was used as a reference to adjust the turbidity of the bacterial suspension culture. 0.5 MFU approximate cell density was $1.5x10^8$ cells/ml. 100 µl bacterial **cultures** were

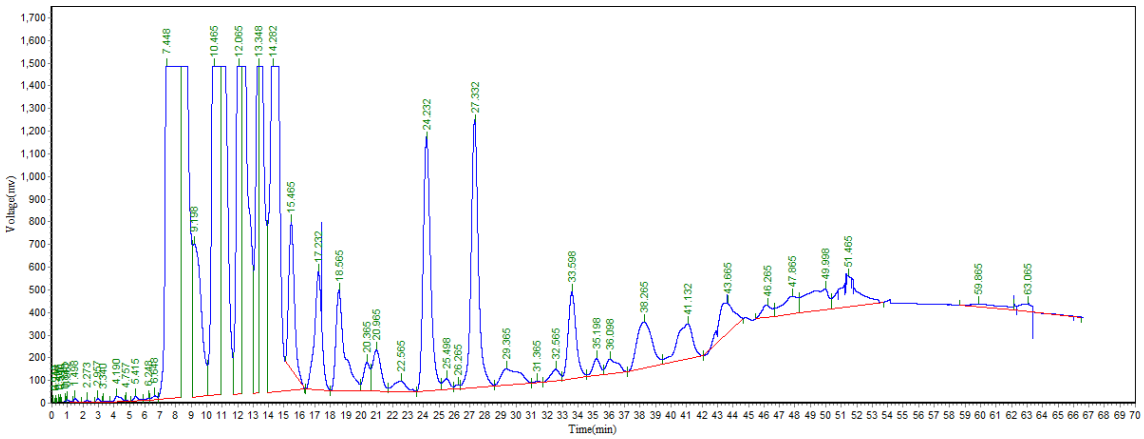
spread onto the LB-Agar plates and allowed to dry the 30 minutes. Paper discs which were flooded with 50 µl of GEO were placed in the centre of the Petri plates. The plates were left at room temperature for 20 minutes to allow oil diffusion, followed by incubation at 37°C for 24 hrs. After incubation, the antimicrobial activity was observed as a zone of inhibition around the discs.The positive control was streptomycin (10 mg/ml).

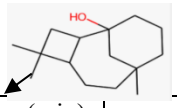
Results and Discussion

GC-FID Analysis of Bioactive Compounds in GEO

The chromatogram obtained by GC-FID was depicted in Figure.1.The observed peaks and their retention time is also given. The GC-FID analysis of guggul essential oil extracted from *Boswellia serrata* exposed 32 compounds. All identified compounds were: heptane, Methyl-2-butenal, Norbornene, Hexenol, Heptanone, *α*-Thujene, *α*-Pinene, Octanol,3-Carene, Limonene, Linalool, Campholene aldehyde, Caryophyllene alcohol, Phenyl ethyl acelate, Dihydro carveol, Hexyl 2-methyl butanoate; Thiazole d-acctyl-2,4-dimethyl, Chrysanthenyl acetate, Guaiacol acetate,Cinnalllyl alcohol, CycIoisolongifolene, Longipinocarvone, α-d-Mannofuranoside, farnesyl, Caryophyllene, Dodeccnal, Cadinene ɤ-Cycloisolongifol-5-o1, CedrolIsolongifolan-7-ol, Khusimol;Cedr-8-en-9-ol,acetate, Hexenyl anthranilate,Dihydroangelicin2',3',3'-trimethyl-2',3,Sandaracopimara-8(4),-diene, Phyllocladene, Octadecanol, Linoleic acid, Labd-I3E-8,15-diol, Totarolone. GC-FID chromatogram contained three major peaks along with many small peaks indicative of the presence of major compounds. The major compounds were Caryophyllene alcohol (19.6%),Octanol (15.9%), Limonene (10%),and α-d-Mannofuranoside, farnesyl (6.4%). The small peaks may be credited to the bioactive compounds present in the minor concentrations. Many compounds were identified in our work that was not seen in the essential oil prepared by Kubmarawa et al. (2006), who cited only 29 compounds. α -Pinene (45.7%) and α -terpinene (11.5%) were the predominant compounds found by Kubmarawa et al. (2006), but in our workCaryophyllene alcohol (19.6%), Octanol (15.9%), Limonene (10%) and α-d-Mannofuranoside, farnesyl (6.4%) were abundant.Kohoude et al., (2017) also show that 3-carene (27.72%),α -pinene (15.18%), p-cymene (9.54%), and β-phellandrene (8.48%) were dominant.Literature studies also reveal the presence of Linalool, Limonene, and Caryophyllene in Frankincenseessential oil extracted from*Boswellia spp*. (Kasali et al., 2002; Vuuren et al., 2010).

Different habitats, seasons, geographical areas, and harvesting periods can affect the quantitative composition and variation of essential oil in similar plant species from diverse regions. The difference in the quantity of the active ingredients implies the variation in the ecosystem diversity where plant species grow.





Peak no.	Retention Time (min)	Bioactive compound	Conc. **(%)**
1	2.273	heptane	0.0266
2	2.957	Methyl-2-butenal	0.0362
3	3.340	Norbornene	0.0139
4	4.190	Hexenol «2E)->	0.1181
5	4.757	Heptanone	0.0080
6	6.248	α-Thujene	0.0400
7	6.648	α-Pinene	0.0822
8	7.44	Octanol<3->	15.9369
9	9.19	Δ -3-Carene	4.3438
10	10.465	Limonene	10.0807

11	12.065	Linalool	5.7792
12	13.348	A--Campholene aldehyde	3.4698
13	14.282	Caryophyllene alcohol	19.6205
14	15.465	Phenyl ethyl acelate < 1->;Dihydro carveol	2.8019
15	17.232	Hexyl 2-methyl butanoate; Thiazole d-acctyl-2,4-dimethyl->	2.9397
16	18.565	Chrysanthenyl acetate<cis->;Guaiacol acetate<o->	2.9468
17	20.365	Cinnalllyl alcohol «E)->	0.6033
18	20.965	CycIoisolongifolene	1.1547
19	22.565	Longipinocarvone	0.4340
20	24.232	α-d-Mannofuranoside, farnesyl	6.2239
21	27.332	Caryophyllene<9-epi-(E); Dodeccnal «2Ej->	6.4664
22	29.365	Cadinene <-γ>;Cycloisolongifol-5-o1	0.9884
23	33.598	Cedrol <-epi->;Isolongifolan-7u-ol	2.3200
24	38.265	Khusimol;Cedr-8(l5)-en-9-!l-ol,acetate	2.3575
25	41.132	Hexenyl anthranilate	1.8934
26	43.665	Dihydroangelicin<2',3',3'-trimethyl-2',3'->	1.5691
27	46.265	Sandaracopimara-8(4),IS-diene	0.4545
28	47.865	Phyllocladene	0.9914
29	49.998	Octadecanol	1.7384
30	51.465	Linoleic acid	2.0010
31	59.865	Labd-I3E-8,15-diol	0.3679
32	63.065	Totarolone	0.3193

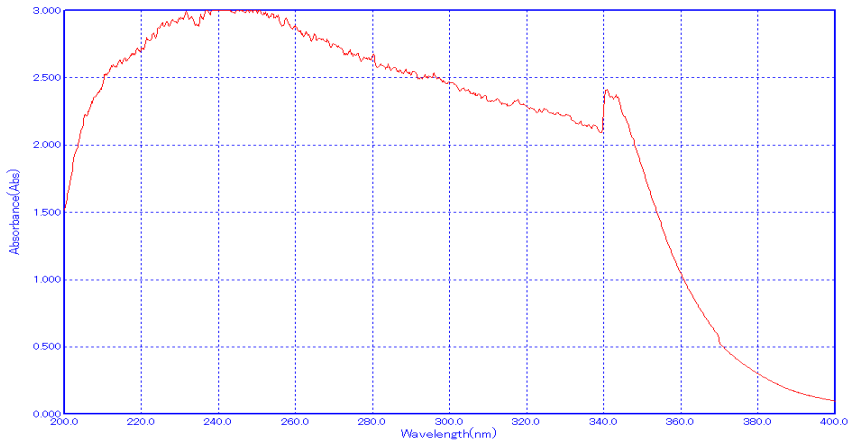
Figure 1: GC-FID analysis of GEO. Arrow indicates the major bioactive compound in GEO

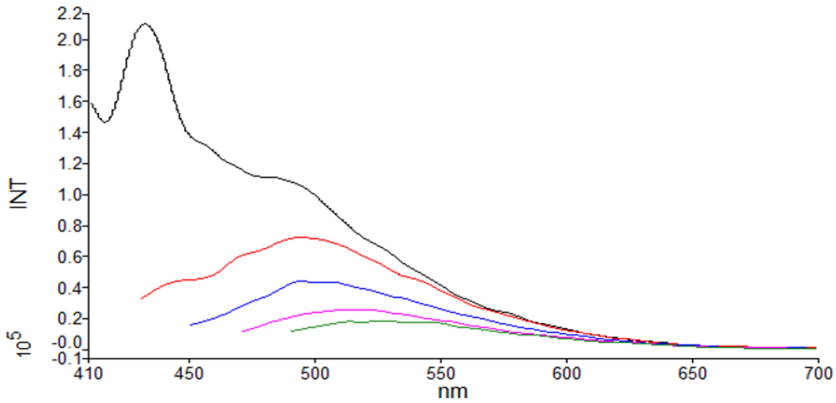
Phytochemical analysis

Plant cells produce both primary and secondary metabolites (carbohydrates, lipids, and proteins) (alkaloids, phenols, essential oils, terpenes, sterols, flavonoids, tannins, etc.). Natural compounds play an important role in the treatment of a number of illnesses, according to a review of the literature (Kaur et al., 2016). Essential oils are one of many secondary metabolites found in aromatic plants that are known to be biologically active and to have strong pharmacological effects. Due to safety concerns, natural and essential oils—which are obtained by extracting plants—are preferred to synthetic ones (Gao et al., 2012). Total phenolic, flavonoids, and tannin concentrations were estimated in the current study to be 0.653mg GAE/ml and 0.056 mg RUE/ml, and 1.30 mg AA/ml respectively. The Tannin content discovered was greater as compared to Phenolic and flavonoids. Based on the results, the presence of secondary metabolites in *Boswellia spp.*are phenolics, flavonoids, saponins, steroids,and tannins in relatively large quantities than others (Sitorus et al., 2022; Singh et al., 2012). The aforementioned phytocompounds are plant secondary metabolites which have antioxidant activity *in vitro* and *in vivo* conditions. Earlier studies have documented that plants pose medicinal and redox properties due to having these chemical structures (Khan et al., 2012). The data of the present study revealed that GEO contained a notable number of polyphenols compounds endowed with high antioxidant. We, therefore opine that polyphenols may contribute to the medicinal value of *Boswellia*. These findings provided good pharmacological logic for *Boswellia* use in folk and traditional medicine. The presence of the high content of polyphenols indicated the effectiveness of GEO as a potent source of bioactive compounds from *Boswellia*. This study data provided clues that due to its richness in bioactive components, essential oils can reduce the risk of many diseases by scavenging free radicals in biological pathways (Agnish et al., 2022). It has been reported in previous investigations that bioactive compounds such as phenolics from medicinal plants are immensely used to mitigate many diseases and scavenging activities (Khan et al., 2012). GEO has a variety of uses because of these phytochemicals, including being an effective antifungal and antibacterial agent and being used in perfumery, scenting soaps, hair oils, and cosmetics.

Fingerprint analysis

UV-VIS spectroscopy measures the scattering and absorption of light passing through the sample. UV-VIS, FT-IR, and fluorescent spectroscopy methods provide a very broad conception of the quantitative and qualitative formulation of bioactive compounds of plant origin (Amrita et al., 2023). The UV profile of GEO revealed a sharp peak at 240 nm with an absorbance of 3.0 Figure-2(a), signifying the presence of secondary metabolites (Agnish et al., 2022; Sharma et al., 2021). Various small peaks were observed in the spectra in the range of 250–340 nm, indicating the presence of flavonoids and phenolic compounds in the oil (Kumar et al.,2018). A further method fluorescent spectroscopy is more reliable and sensible for the detection of plant bioactive. The fluorescent spectrum of GEO is given in Figure-2(b). On the basis of excitation wavelength, all spectra are grouped together in one.





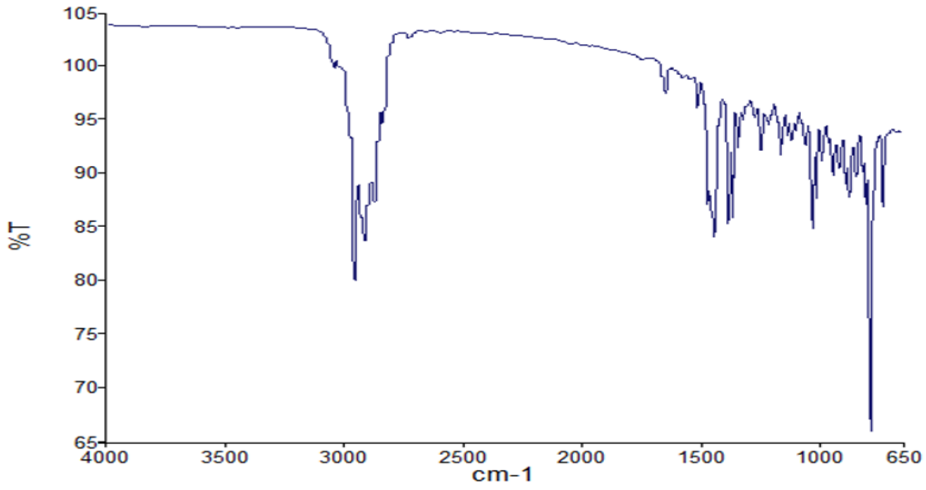
(a)

(b)

GEO

λexc	PEAK INTENSITY (INT)x10^{-5} (cm^{-1})				FLUORESCENCE WAVELENGTH (nm)			
	Ma		Mi		Ma		Mi	
390	2.2		1.2		431.60		500	
410	-		0.7		-		500	
430			0.4		-		500	
450			0.3		-		500	
470			0.2		-		500	

(c)



Peak No.	X (cm^{-1})	Y(%T)	Vibrational mode	Functional group
1	2958.42	80.00	C-H stretching	alkane
2	2914.92	83.68	C-H, C-H_2 stretching asymmetric	alkane
3	2875.65	87.34	C-H,-CH_2 stretching symmetric	alkane
4	1643.73	97.43	C=O stretching	Alkanes
5	1512.63	96.18	N-H bending	
6	1445.64	84.29	C-H stretching	Alkanes
7	1381.64	85.21	C-O stretching	amide
8	1363.84	85.87	O-H stretching	Phenols
9	1344.32	92.44	C-O stretching	
10	1242.69	92.24	C-N stretching	amine

11	1164.96	91.80	C-O stretching	Tertiary alcohol
12	1114.47	93.15	C-O stretching	ester
13	1025.22	84.85	C-O stretching	
14	1009.22	87.59	C-H bending	
15	986.93	91.19	C=C bending	alkene
16	938.75	89.87	C-H bending	Alkane
17	913.59	90.55	C-H bending	Alkane
18	873.26	87.77	C-H bending	Alkane
19	841.89	89.66	C=C bending	alkene
20	802.91	86.99	C-H bending	Alkane
21	778.71	65.84	C=C bending	Alkane
22	731.24	86.84	C-C bending	alkane

Figure 2: UV-VIS Spectrum of GEO (a), Fluorescence spectra of GEO at different excitation wavelengths (b), FT-IR spectra of GEO (c).

One major peak in the Green Fluorescent region (GFR) at 431 nm was detected. It was suggested that the bioactive near 500 nm (GFR) are Polyphenolics such as Flavonoids, flavins, and terpenoids (Mylle et al., 2013). As the fluorescence wavelength increased from 390 nm to 470 nm, the peak intensity decreased accopmaneid by one major peak disappeared. The FT-IR spectrum of GEO is shown in figure-2(c). FT-IR peek at 2958.42 cm^{-2}, 2914.92 cm^{-2}, 2875.65 cm^{-2}, 1643.73 cm^{-2}, 1512.63 cm^{-2}, 1445.64 cm^{-2} indicates the C-H stretching due to alkanes, C-H, CH_2 stretching asymmetric due to alkanes, C-H,-CH_2 stretching symmetric due to alkanes, C=O stretching, N-H bending due to aromatic compounds, C-H stretching due to alkanes respectively. It reveals the presence of terpenoids, aromatic compounds (Taylan et al., 2021). 1381.64 cm^{-2}, 1363.84 cm^{-2}, 1344.32cm^{-2}, 1242.69cm^{-2}, 1164.96cm^{-2}, 114.47cm^{-2}, 1025.22cm^{-2}, 1009.22cm^{-2}, 986.93cm^{-2}, 938cm^{-2}, 913cm^{-2}, 873cm^{-2}, 841cm^{-2}, 802.91cm^{-2}, 778.71.71cm, 731.24cm^{-2}indicates the C-H stretching, C-O stretching due to amide, O-H stretching due to phenols, C-O stretching, C-N stretching due to amine, C-O stretching due to tertiary alcohol, C-O stretching due to esters, C-H bending, C=C bending, C-H bending, C-H bending, C=C bending, C-C bending due to alkanes respectively (Berechet et al., 2015; Benoudjit et al., 2020).All these compounds belong to the secondary plant metabolites (Paulraj et al., 2011). All these observations imply that all GEO is

rich in secondary metabolites. The presence of above said secondary metabolites may be the reason for the medicinal properties of GEO.

Antioxidant analysis

The assessment of the antioxidant activity of medicinal plants has turned into a critical part of the cycles utilized in unwinding the therapeutic potential of such plants (Prior et al., 2005). Earlier reports reported that molecules such as phenolics, flavonoids, and tannins, etc., were accountable for the therapeutic value of the plants in which they were found due to their antioxidant activity (Abdelsamad et al., 2020). Authors stated that the aromatic plants based on natural antioxidants from are fetching progressively importance, not only in the nutritional area (food preservation and stability) but also in preventive and complementary medicine. In the present study antioxidant activity of *Boswellia serrata* was examined. The number of essays like ABTS, DPPH, Hydroxyl radical scavenging, iron-reducing, iron chelation, and anti-diabetic assay were estimated. The IC_{50} value of this oil for various antioxidant assays was observed in the following order: DPPH (6.061), ABTS (36.14), Iron chelating (30.82), and Hydroxy radical scavenging activity (41.5) (Table-1).

TABLE 1. IC50 values for different antioxidant assays of GEO

ACTIVITY	IC50 **[µl/ml]**
DPPH radical scavenging activity	6.0611
ABTS radical scavenging activity	36.14
Iron chelating activity	30.8269
Hydroxyl radical scavenging activity	41.5

The linear correlation analysis was also followed to check the correlation between various antioxidant activities and total flavonoid content, total tannin content, and total phenolic content, results are given in Table-2. A strong correlation between polyphenols content and different antioxidant activities were observed. For instance, the R^2 value for phenolic content and DPPH activity is 0.8148, and the R^2 value for flavonoid content and ABTS is 0.9721.

This observation was in consonance with earlier studies reporting tight correlation with polyphenols contents and antioxidant activities (Khan et al.,2012). A positive correlation between the number of phenolic compounds and the antioxidant activity of plants has been reported in

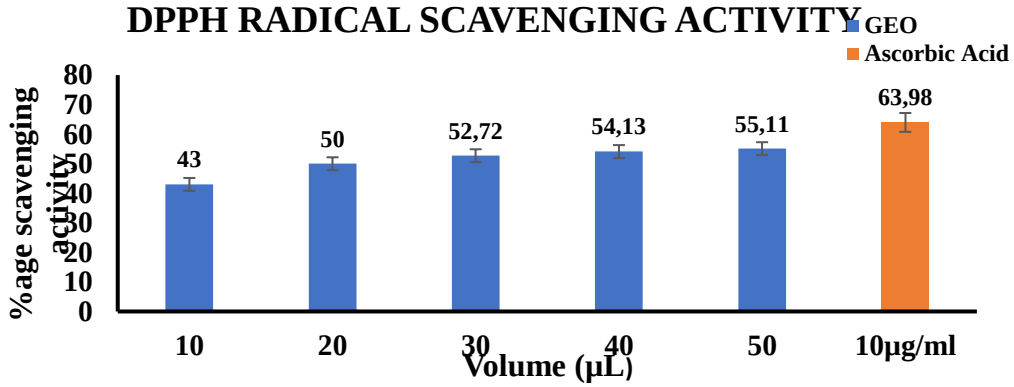
numerous studies (Brasileiro et al. 2015). Due to this antioxidant and antimicrobial activities, GEO from various plant species is used as preservative in the food industry (Nieto et al., 2018). Herbal products may contain a variety of substances with various antioxidant capabilities. The antioxidant values of herbal derivatives are connected to one another (Khan et al., 2012). We therefore believe that the presence of flavonoids and other phenolic compounds found in this study may be the cause of the essential oil's notable antioxidant effects.

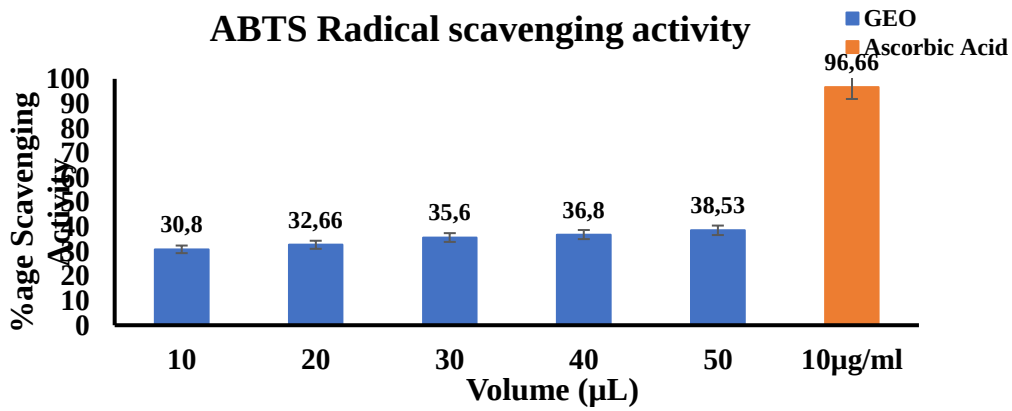
It is a rapid, simple, inexpensive, and widely used method to measure the ability of compounds to act as free radical scavengers or hydrogen donors, and to evaluate the antioxidant activity of natural compounds or essential oils (Kedare et al.,2011). DPPH radical consists of an odd electron that is in charge of the absorbance at 517 nm and for noticeable deep purple colour. By gaining an electron from an antioxidant, it decolorizes and can be measured in a decrease in absorbance (Hasan et al., 2009). In the present study, GEO at different concentrations was used to measure DPPH radical scavenging activity, as shown in Figure-3. The results show that DPPH radical scavenging activity was 55.11% activity at its higher concentration (50µl), whereas 63.98% activity was shown by ascorbic acid at a concentration of 10µg/ml. The scavenging activity of DPPH radical was found to be dose-dependent, with the %age of scavenging activity of DPPH increasing as the volume increases as depicted in the previous study by Obafemi et al., (2017).

The principle of this method is to monitor the decay of the radical cation (ABTS•+) resulting from the oxidation of ABTS. Since ABTS•+ is soluble in both aqueous and organic solvents and is not affected by ionic strength, this method was widely used to indicate the antioxidant activity of a wide range of samples (Saeio et al., 2011). ABTS+• is a blue chromophore produced by the reaction between ABTS and potassium persulphate and in the presence of the GEO. Performed cation radical gets reduced and the remaining radical cation concentration after reaction with an antioxidant compound was then quantified (Obafemi et al., 2017). In the present study as presented in Figure-3, GEO showed a dose-dependent increase in the scavenging activity at 50µl which was the highest concentration of GEO tested showed 38.53% scavenging activity. Ascorbic acid which was used as standard showed 96.66% scavenging activity at a concentration of 10µg/ml as shown in Amrita et al. (2023) the *Cymbopogan martini*exhibiteddose-dependent ABTS activity.

Hydroxyl radical (OH–) is one of the vastly reactive and damaging species in free radicals, able to damage all molecules formed in a cellular system. It induces severe damage to biomolecules,

but also causes breakage of DNA strands and Denaturation of DNA which leads to carcinogenesis, cytokinesis, and mutagenesis (Thirunavukkarasu et al., 2011). The current study was designed to reveal the OH–radical scavenging activity of GEO. The results are shown in Figure-3. GEO showed dose-dependent manner Hydroxyl Radical Scavenging Activity, with 13.26% activity at its highest concentration of 50µl and Ascorbic acid which was used as standard showed 13.18% scavenging activity at a concentration of 10µg/ml. The reducing potential is mainly coupled with the existence of reductones and their antioxidant potential arises due to the breaking of the free radical chain by accepting a hydrogen atom from the antioxidant content present in the sample (Gordon and Hudson 1990).





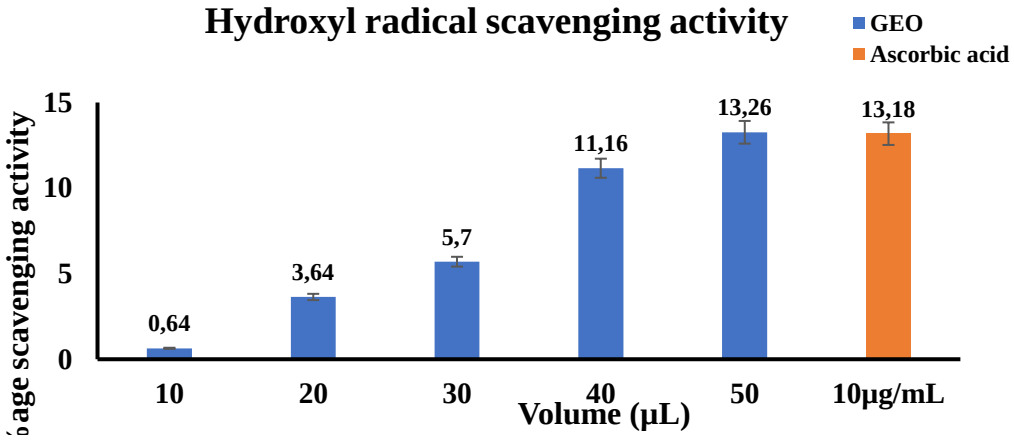


Figure 3: DPPH scavenging, ABTS scavenging, Hydroxyl radical scavenging activity of GEO.

The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. At 50µl the GEO showed 0.471 and the standard showed 0.343 absorbance values of ascorbic acid at 10µg/ml figure-4. This implies that the GEO shows a high reducing capacity in comparison to ascorbic acid. The result shows that GEO consists of hydrophilic polyphenolic compounds that cause greater reducing power (Sharma et al., 2012). Reducing capacity is generally associated with the presence of reductones and the antioxidant action of reductones is based on the breaking of the free radical chain by donating a hydrogen atom (Gordon and Hudson, 1990).

Chelating agents form σ-bonds with metal are effective as secondary antioxidants because they reduce the redox potential, thereby stabilizing the oxidized form of the metal ion (Kumaran and Karunakaran, 2006). Figure-4 shows the percentage iron-chelating activity of GEO. The results show that at 50µl the GEO showed 73.11% higher iron chelating activity as compared to standard EDTA shows 62.64% iron-chelating activity at 10µg/ml. The study shows that GEO has great iron chelating capacity, suggesting the presence of polyphenols (Sharma et al., 2012).

According to Khan et al. (2012), antioxidant activities against DPPS and ABTS were related to concentration, chemical structures and polymerization degree of antioxidants. It was also reported that high molecular weight phenolics like tannins have more ability to mitigate free radicals. Earlier study by Borotová et al. (2023) via DPPH and ABTS assays evaluated the antioxidant potential of *Boswelliacarterii* essential oil and showed that 10 µL of FEO can neutralize 73.88 ±

0.35% of DPPH radical and 97.09 ± 0.48% of ABTS radical cation. The findings are consistent with a prior study that found Boswellia serrate oleo gum resin essential oil to have high free radical scavenging capability (Gupta et al., 2016). Remarkable DPPH radical scavenging activity (51.68%) was also reported from *B. carterii* essential oil (Prakash et al., 2014). In another study the antioxidant activity of B. sacra essential oil, based on the DPPH- and ABTS-radical scavenging activity, were found to be 57.50% and 48.66%, respectively (Rahmati-Joneidabad and Alizadehbehbahani, 2021). It was also reported that the Linalool, Limonene, and Caryophyllene compounds present in our GEO were found to be endowed with a strong antiradical and ferric reducing power activity (Kasali et al., 2002). According to Khan et al. (2012), antioxidant activities against DPPS and ABTS were related to concentration, chemical structures and polymerization degree of antioxidants. It was also reported that high molecular weight phenolics like tannins have more ability to mitigate free radicals. Authors noticed that antioxidant activity is variable and related to chemical composition and can be affected by specific substances. Previously, Viuda-Martos et al. (2010) also documented that oxygenated monoterpene, probably monoterpenoid ketones may have the greatest contribution to the antioxidant capacity of essential oils.

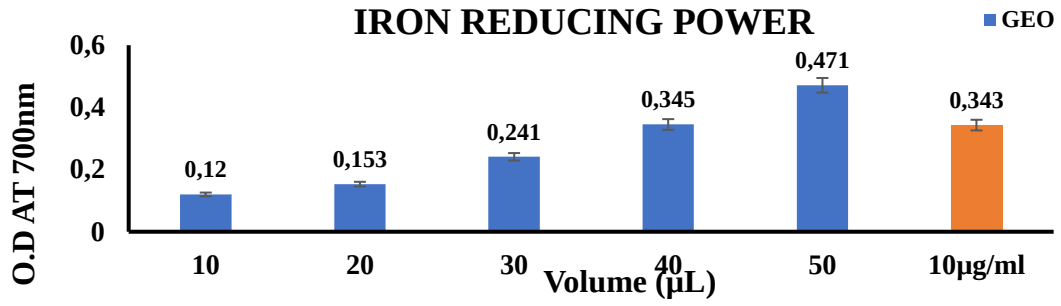
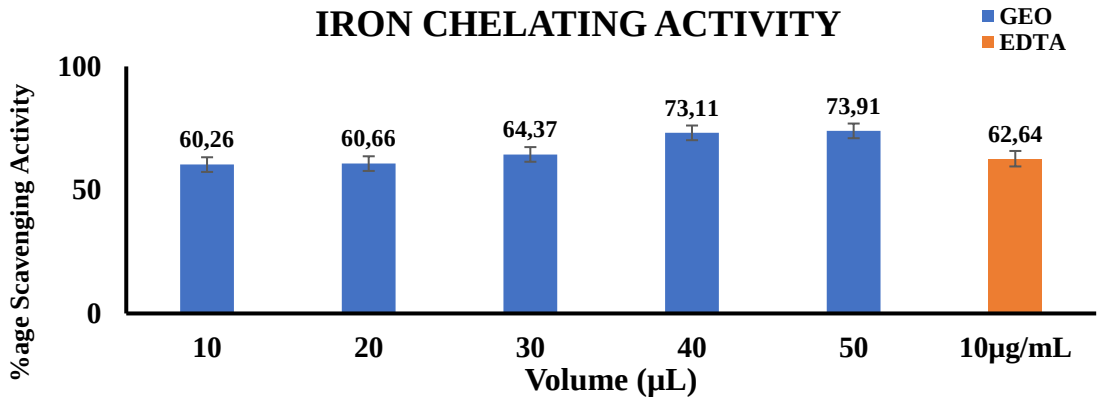


Figure 4:Iron-reducing power and Iron Chelating activity of GEO.



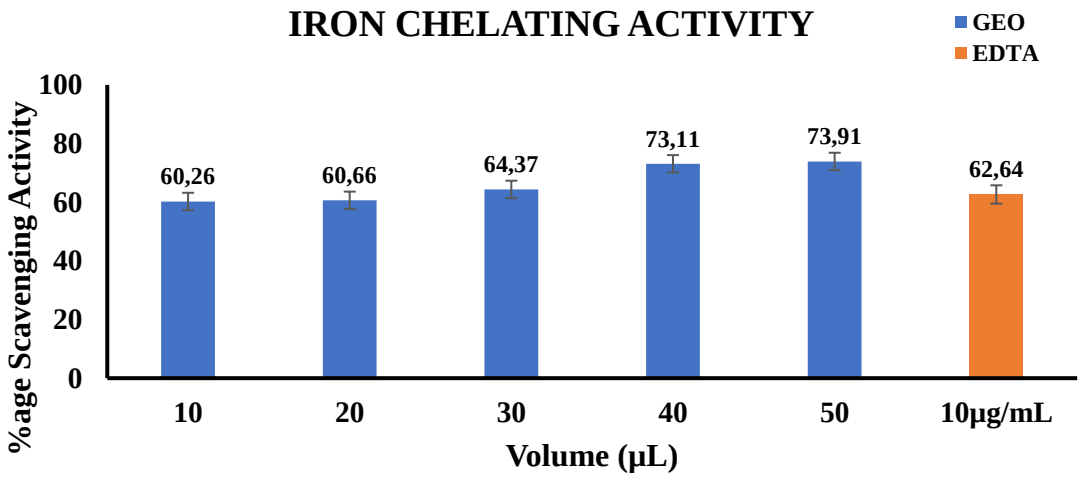


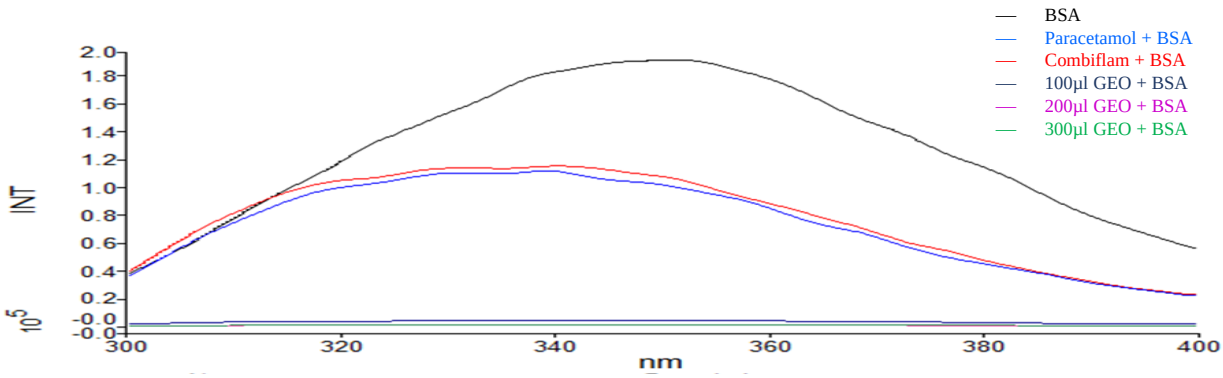
TABLE 2: R2 values for different activities of GEO

ACTIVITY	DPPH RADICAL SCAVENGING ACTIVITY	ABTS RADICAL SCAVENGING ACTIVITY	α- AMYLASE INHIBITION ACTIVITY	IRON REDUCING ACTIVITY	IRON CHELATING ACTIVITY	HYDROXYL REDICAL SCAVENGNG ACTIVITY
TOTAL PHENOLICS	0.8148	0.9721	0.9141	0.977	0.8846	0.985
TOTAL FLAVONOIDS	0.6869	0.913	0.8072	0.994	0.8135	0.9318
TOTAL TANNIN	0.6552	0.8713	0.7499	0.982	0.7873	0.9194

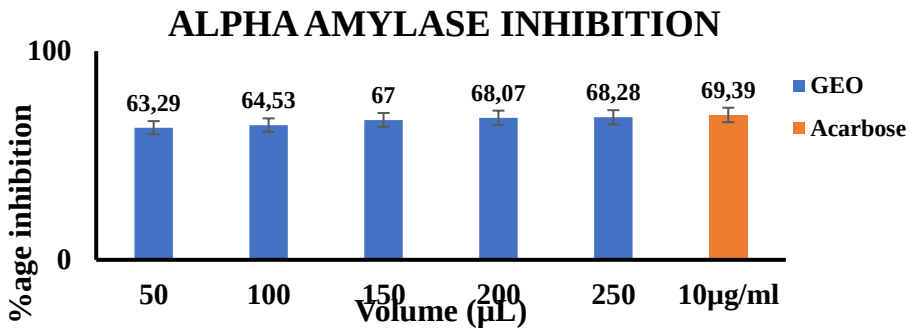
Anti-Inflammatory (Protein Denaturation) Activity

Inflammation is a natural defensive response triggered by tissue damage or disease, and it has the power to fight off invaders (microorganisms and non-self-cells) besides being able to eliminate dead or damaged host cells. Increased blood leukocyte aggregation into the interstitial space, an oxidative burst, and the appearance of cytokines (interleukins and cancer rot factor-(TNF-)) are all characteristics of the inflammatory response. Additionally, a few catalysts (oxygenases, nitric oxide synthases, and peroxidases) as well as the breakdown of arachidonic corrosive is induced simultaneously. Furthermore, cell grip atoms such intercellular bond particles (ICAM) and vascular cell attachment particles (VCAM) are expelled throughout the provocative cycle (Miguel et al., 2010). The primary cause of tissue inflammation, which leads to loss of function, swelling, redness, and heat release around the affected areas, is protein denaturation. Bioactive with the ability to prevent the generation of heat-induced protein denaturation have been proposed as potential therapeutic anti-inflammatory agents(Amrita et al., 2023). The protein denaturation inhibitory action of GEO was determined in the present study by the fluorescent technique. Characteristic fluorescent spectra of denatured BSA (Bovine Serum Albumin) and BSA + GEO at different concentrations is shown in Figure-5(a). A notable increase in the inhibition of protein Denaturation was shown by GEO, as there is a decrease in fluorescent intensity to a large extent afterward the addition of the GEO to BSA. These results grant strong verification for the potential of the GEO as an anti-inflammatory agent. The high protein denaturation inhibition ability of GEO might be credited to complex compounds having synergistic associations of numerous compounds or involvement of minor components. The essential oil activity is due to the combined anti-inflammatory activity of compounds such as b-caryophyllene (19.62%), a-pinene (0.082%), and linalool (5.7%). Linalool was previously demonstrated to exhibit anti-inflammatory properties by Peana et al., (Sibanda et al., 2004). The main compounds found in the essential oil of *Boswellia serrata* were caryophyllene and linalool. This corroborates the results of Kohoude et al. (2017), who concluded that these bioactive chemicals are responsible for the anti-inflammatory effect. Previously reported by Chao et al., (2005) in *Cinnamomum osmophloeum* and -caryophyllene, and observed in the essential oils of *Syzygium cumini* and *Psidium guajava*, having promising anti-inflammatory activity, as stated by Siani et al., (2013). The anti-inflammatory significance depicted by the plant essential oils, primarily caryophyllene oxide. The differences between the data of our findings and the previously published literature are caused by the variation among

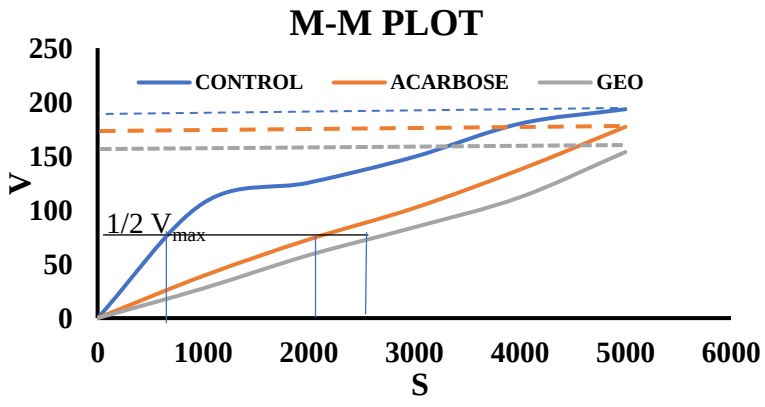
plant species as well as the fact that the various plant species possess a variety of active constituents that are influenced by a variety of factors, including habitat, climatic and edaphic factors, and water availability. Accordingly, the anti-inflammatory activity of essential oil from *Boswellia serrata* is worthy of investigation. According to earlier research, essential oils' bioactive components are a key component of anti-inflammatory lotions, ointments, and sprays (Aginish et al., 2022)



(b)



(c)



(d)

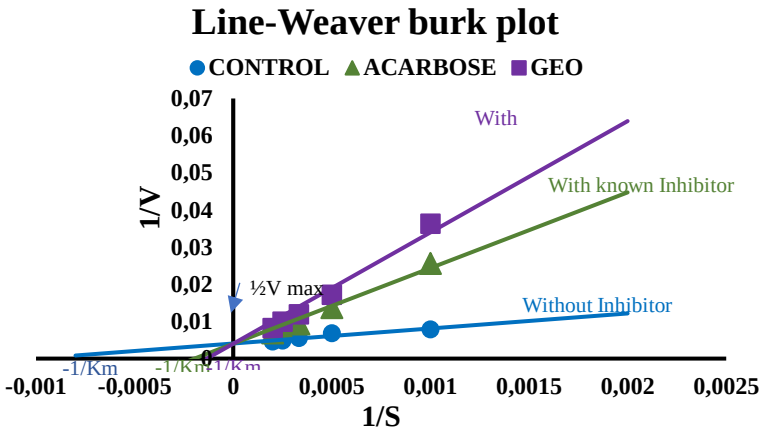


Figure 5: Anti-inflammatory (Protein denaturation) activity (a) of GEO, α-amylase inhibition assay (b), Michaelis-Menten Plot (c), and Lineweaver-burk plot (d) showing competitive inhibition of α-Amylase activity by GEO.

Anti-Diabetic (α-Amylase Inhibition) Activity

Alpha amylase is a prominent enzyme found in the pancreatic juice and saliva which breaks down large insoluble starch molecules into absorbable molecules (Afifi et al., 2008).Therefore, by blocking this enzyme, carbohydrate digestion can be delayed, which in turn slows down the rate at which glucose is absorbed (Kwon et al., 2007). One method used to treat diabetes mellitus is the inhibition of carbohydrate-digesting enzymes like -amylase. This lowers the body's blood glucose levels by reducing gastrointestinal glucose absorption. This is an alternative to looking for elective medications from therapeutic plants with expanded power and lesser antagonistic impacts than existing medications.α-Amylase Inhibition potential of GEO was determined as shown in Figure-5(b). It is clear from the figure that GEO showed inhibition in a dose-dependent manner and showed 68.28% inhibition of α-amylase at its higher concentration of 250µl as compared with Acarbose (synthetic drug known inhibitors), which showed 69.39% inhibition at 10µg/mL concentration. The Michaelis-Menten plot shows that enzyme activity increases as substrate concentration increases. The mode of inhibition of GEO was determined by the Lineweaver-Burk plot and Michaelis-Menten likewise showed Figures-5(c)(d), which displays GEO inhibits α-amylase ina near competitive manner. This proposes that the GEO's active components compete

with the enzyme's substrate for binding to the active site, preventing the breakdown of oligosaccharides into disaccharides(Matsuda et al., 2002; Shai et al., 2010). This anti-diabetic activity may be due to the presence of flavonoids. As in a previous study, Ahmed et al. (2020) described anti-diabetic activity might be due to the presence of flavonoids in *A. cepa* oil. Flavonoids have been reported to have alpha-amylase inhibitory potential. According to earlier research by Najibullah et al., (2021), Basak et al., (2013), and Jelassi et al., (2017), essential oil contains these active components, including linalool, limonene, and caryophyllene oxide, which provides the investigated sample a promising ability. Bigham et al., (2010) and Riyaphan et al., (2021) have shown in the plant species *Teucrium polium* and *Musca domestica*, essential oils are rich sources of monomers that have the potential capacity to serve as antidiabetics. In our research linalool, limonene and caryophyllene are bioactive compounds of GEO. It can be used as an anti-diabetic agent.

Antibacterial activity

GEO showed temperate antibacterial activity against *E. coli* (MTCC 40 Gram –ve) while equable inhibition against *P. aeruginosa* (MTCC 424 Gram -ve), and moderate activity against *B. subtilis* (MTCC 121 Gram +ve) and *S. aureus* (MTCC 3160 Gram +ve). The zone of inhibition of different bacterial strains was shown in the Figure-6 and Table-3. Maximum antibacterial activity of GEO was observed against pathogen MTCC 3160 with zones of inhibition 0.6 cm. The difference in antibacterial activity of GEO against bacterial strains could be due to the incidence of multiple targets or single target for their activity. Scholars suggest that the antimicrobial properties of essential oils result from synergistic interactions of their major and minor compounds(Asbahani et al., 2015).Essential oils are known to exert their antibacterial effect either by inhibiting the synthesis of functional and structural molecules (Micheal et al., 2014). Furthermore, the multi-component nature of essential oils enables them to have multiple target sites for their antimicrobial action, hence, they may be able to deal with otherwise resistant microorganisms. According to Nazzaro et al. (2013), the antibacterial action of essential oils and their respective constituents is linked to their lipophilic nature which enables them to accumulate in membranes, thus making cell membranes the main target site. However, the antibacterial action of essential oils has also been attributed to the bacterial cell wall degradation, reduction in proton motive force and reduction of

intracellular adenosine triphosphate (ATP) levels. Moreover, they can also increase the membrane permeability of the bacteria because of toxic effects on membrane structure and function (Davies et al., 2013) or by damaging the proton pump which induces an interruption of the energy production into the bacterial cell. The chemical structure of essential oils affects their antibacterial activity. Limonene, linalool and 3-carene are bioactive components of GEO. Previous studies report that limonene and linalool inhibit the growth of several Gram-positive bacterial strains (Camarda et al., 2007).3-carene, which is a monoterpene hydrocarbon and, its antibacterial activity could be attributed to the methylene group (Maczka et al., 2020).Current findings revealed that the GEO is promising exploratory new and effective antimicrobials.

CONCLUSION

GC-FID analysis confirmed that the *Boswellia Serrata* essential oil possesses a considerable amount of bioactive compounds linalool, caryophyllene alcohol, limonene and can be used in the pharmaceutical due to its capability of antioxidant and anti-inflammatory. High concentrations of phytochemicals like Tannin (1.309mg AA/ml), Flavonoids (0.056 mg RUE/ml), and Phenolics (0.653mg GAE/ml) were detected in GEO. The IC_{50} value of this oil for various antioxidant assays was: DPPH (6.061), ABTS (36.14), Iron chelating (30.82), Hydroxy radical scavenging activity (41.5), α-Amylase inhibition (24.86). *Boswellia serrata* essential oil showed anti-inflammatory and inhibits the alpha amylase in a competitive manner. Maximum antibacterial activity was observed of *Staphylococcus aureus* with ZOI 0.6 cm. Due to the aroma fragrance of *Boswellia serrata* oil, it can be used in the cosmetics, perfumes and room fresheners. It has anti-diabetic activity so it can use as a natural antidiabetic agent. Due to its antibacterial activity, it can be used as an antimicrobial agent. It can be used as food additives and as food preservatives because it has antibacterial activity.

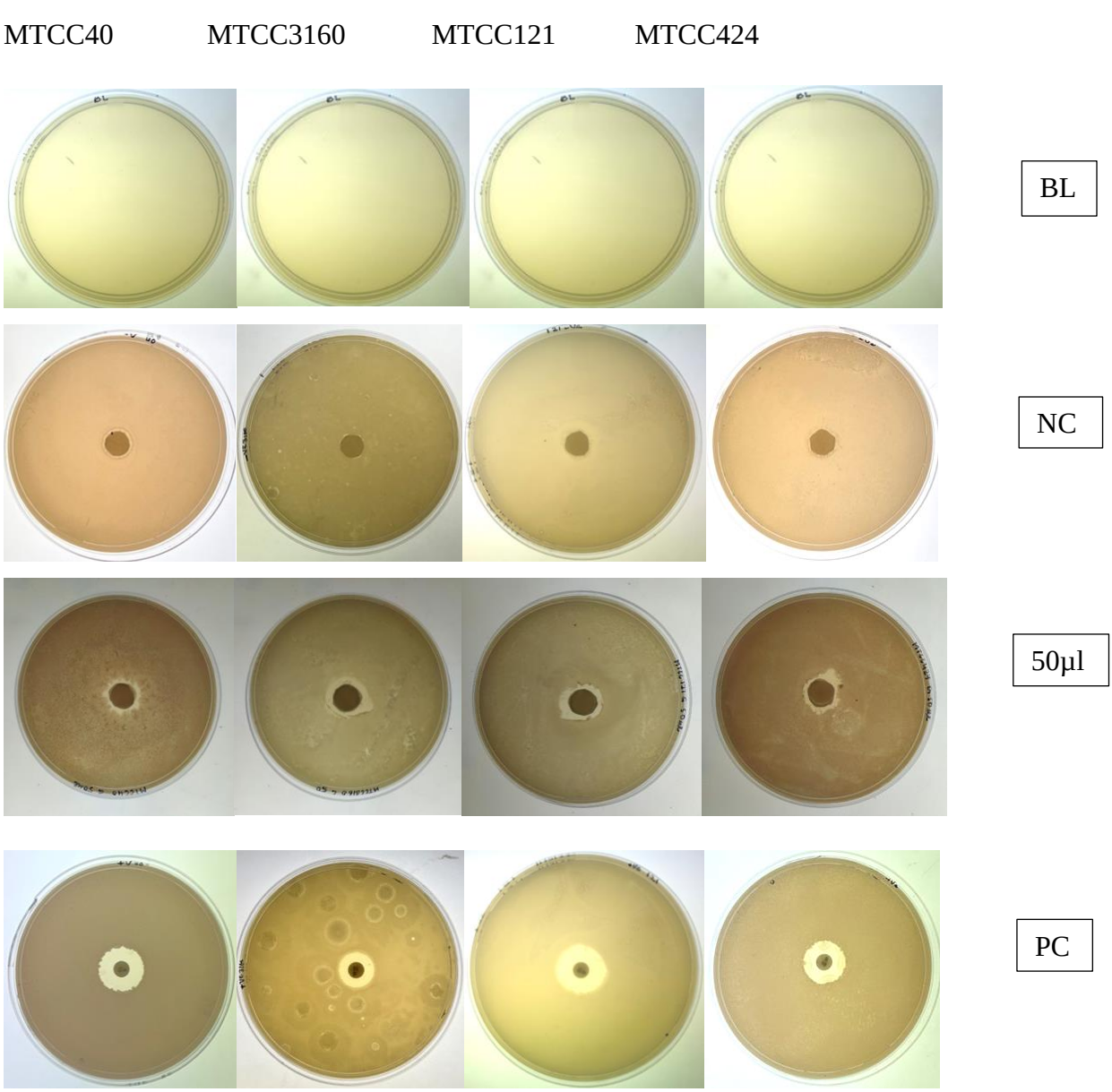


Figure 6:Anti-microbial Activity of GEO against MTCC40, MTCC3160, MTCC424, MTCC121.Note:BL: Blank (Only Media),NC: Negative Control (Empty Disc), PC: Positive Control (Streptomycin 10 mg/disc).

TABLE 3: Antibacterial activity of GEO

S.NO.	MICROBIAL CULTURE	SAMPLE NAME	GEO/CONTROL VOLUME(µl)	ZONE OF INHIBITION (cm)
1	MTCC 40 *Escherichia coli*	NC	0	NI
2		PC	Streptomycin(10 mg/ml)	0.3
3		GEO	50	0.2
4	MTCC 121 *Bacillus subtilis*	NC	0	NI
5		PC	Streptomycin(10 mg/ml)	0.8
6		GEO	50	0.4
7	MTCC 424 *Pseudomonas aeruginosa*	NC	0	NI
8		PC	Streptomycin(10 mg/ml)	0.3
9		GEO	50	0.2
10	MTCC 3160 *Staphylococcus aureus*	NC	0	NI
11		PC	Streptomycin(10 mg/ml)	1.1
12		GEO	50	0.6

Note: NC: negative control, PC: positive control

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Supplementary Table 1: Biological activities of essential oil from *Boswellia* spp.

Species Name	Bioactive component	Activities	Reference
Boswellia sacra	duva-3,9,13-trien-1,5α-diol-1-acetate	immunomodulatory activity, antifungal, antibacterial, anti-inflammatory, anti-leukotriene, antiacetylcholinesterase, anticancer,	Al-Harrasi et al., 2008 Mothana et al. 2011
	octyl acetate		
	2-β-pinene		
	α-thujene		
	E-β-ocimene		
	sabinene		
	myrcene		
	α-pinene		
	Limonene		
Boswellia. Papyrifera,	incensole acetate	anti-inflammatory, stimulating immune defenses, antimicrobial, anti-infectious	Fatouma et al., 2020
	soincensole		
	incensole		
	neryl acetate		
Boswellia. socotrana	2-Hydroxy-5-methoxy-acetophenone	Anti-bacterial, anti-fungal, antioxidant, anticancer, anti-inflammatory, immunomodulatory, antimicrobial and antiviral	Mothana et al. 2011
	camphor		
	terpinen-4-ol		
	p-cymene		
	limonene		
Boswellia. dioscorides	a-thujene	Anti-bacterial, anti-fungal, antioxidant, anticancer, anti-inflammatory, immunomodulatory, antimicrobial and antiviral	Mothana et al. 2011
	a-pinene		
Boswellia. elongata	verticilla 4(20),7,11-triene	Anti-bacterial, anti-fungal, antioxidant, anticancer, anti-inflammatory, immunomodulatory, antimicrobial and antiviral	Mothana et al. 2011
	incensole		
	incensole acetate		