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SHORT COMMUNICATION

Phytochemical Evaluation, GC-MS Analysis of Ethanol Extract of Endemic Plant of *Boswellia ovalifoliolata* Leaves

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Abstract

Background: In many nations, medical plants are an important part of the basic healthcare systems. Plant medicinal properties are found by the biological substances present in the plant components that are used to produce medicinal remedies. *Boswellia ovalifoliolata* is classified under the *Burseraceae* family. It is extensively spread out in the Eastern Ghats of India. Traditionally, leaves of this plant have been extensively used to aliment leprosy, cancer, as an emetic, and for headaches. **Aim:** The current study used gas chromatography-mass-spectrometry analysis and phytochemical assessment to determine the phytochemical components of *B. ovalifoliolata* leaves. **Methods:** The shade-dried leaves were ground and extracted with ethanol using the cold maceration method; we conducted a phytochemical examination to evaluate the characteristics of secondary metabolites and used Gas Chromatography-Mass- Spectrometry (GC-MS) to find the different phytocompounds in the ethanol leaf extract. Phytochemicals were determined using molecular weights (m/z) obtained from GC-MS chromatograms. Phytocompounds were identified using NIST library databases and spectral peak interpretation. **Results:** The phytochemical analysis found flavonoids, steroids, tannins, phenols, and glycosides. *B. ovalifoliolata* leaf extract included 28 phytochemical substances by GC-MS. The ethanol extract contained three major bioactive compounds: 1,2,3-Benzenetriol (36.16%), Quinic acid (21.84%), and Lupeol (11.75%), as well as eight minor compounds: Levoglucosan (3.53%), Hexadecanoicacid, Methylester (1.59%), n-Hexadecanoicacid (3.29%), Ethylester (1.64%), and Methyl-linolenate (1.30%). Bioactive substances are detected by retention duration, peak area, molecular formula, and likelihood. **Conclusion:** The findings suggest that *B. ovalifoliolata* may possess several beneficial properties, including as anti-cancer, antiulcer, antioxidant, anti-microbial, anti-diabetic, hepatoprotective, and hypocholesterolemic activities.

Major Findings: Identification of key bioactive compounds, including flavonoids, steroids, tannins, phenols, and glycosides, in the ethanol extract of *Boswellia ovalifoliolata* leaves. GC-MS analysis revealed volatile and semi-volatile compounds, such as essential oils and secondary metabolites, with anti-inflammatory, antimicrobial, and antioxidant properties.

Keywords: Phytochemical Evaluation, GC-MS Analysis, *Boswellia ovalifoliolata*, Antioxidant, Anti-cancer, Bioactive Compounds

1. Introduction

In traditional medicine, medicinal plants are considered an essential component of drugs for treatment. The kingdom of plants is an ideal spot for finding new potential medications, and the importance of medicinal plants has come to prominence in recent years. Medicinal plants generate a diversity of bioactive molecules and are significant providers of several medicinal compounds. Herbal plant extracts are immensely helpful as well as one of the main forms of medicine. These serve as vital to fostering production and preventing an array of illnesses. These are the lower-cost sources for effective therapies and treatments for multiple infections. Because they hold an extensive

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number of secondary metabolites, like flavonoids, alkaloids, phenolics, and tannins, which enhance innate immune response, growth, and resistance to disease toward pathogenic microorganisms in humans and other organisms, extracts from medicinal plants have recently gained attention as a viable substitute. Approximately 80% of people in affluent nations utilize a variety of medicinal plants as traditional medicines, such as antifungals, anticancer medications, and antibacterial pharmaceuticals, in a variety of ways. Secondary metabolites, which are incredibly varied chemically and taxonomically with unknown roles, are abundant in medicinal plants. Numerous phytochemicals are employed extensively in scientific study, veterinary medicine, agriculture, and human therapy[1-4].

Boswellia ovalifoliolata Bal and Henry (*Burseraceae*) is a native, threatened arboreal plant found alone in the Tirumala highlands. It has therapeutic properties. It is commonly found growing on the foothill slopes of Tirumala forest. The species is distributed extensively through to an altitude of about 300m from Vinayaka temple northwards to north-east and extending west and south-west to the temple up to galigopuram I. Leaflets 2-6 pairs, ovate-oblong, glaucous beneath, flowers greenish white, in much branched panicles. Deciduous trees with bark greyish, smooth, exfoliating in thin flakes, branchlets red colored, glabrous, leaves imparipinnate, leaflets 5-13, coriaceous, ovate or elliptic, glabrous, glaucous beneath, entire, obtuse, secondary nerves 8-10 pairs, reddish, sessile, flowers greenish white, in much branched panicles, calyx 5 toothed, petals 6, imbricate, disc annular, stamens 10, ovary 3-locular, ovules-2 per locule, pendulous, style short, and stigma capitate drupes trigonous, pyrenes bony, seeds winged and compressed. Flowering and fruit season is March-June. Traditionally it is used as stem-stomach ulcers, diabetes, leaf- mouth and throat ulcers, gum- dysentery, inflammations, joint-pains, ulcers, arthritis, amoebic-dysentery, hydrocele, diabetes. Extracts of *B. ovalifoliolata* exhibit hypoglycemic and anti-inflammatory properties. The stem decoction is used as a treatment for whooping cough, the dried leaf powder is smoked to alleviate asthma symptoms, the leaf paste is applied to cleanse scars caused by pox wounds, the root bark is used to lower fever, fresh twigs serve as pesticides, and the dry leaf powder is utilised to preserve food grains. This research presents an investigation of bio-active compounds in the ethanol leaf-extract of *B. ovalifoliolata*[5-8].

2. Materials

2.1 Collection, Identification

Boswellia ovalifoliolata used for the phytochemical examination was collected from Tirupati, Andhra Pradesh, India. Dr. Sankararao Mudadla from the Botanical Survey of India in Hyderabad confirmed the authenticity of the plant. The voucher number is BSI/DRC/2021-22/Tech./Identification/522. The plant material of *B. ovalifoliolata* rinsed with tap water and dried in a shaded area.

2.2 Preparation of Extract

The botanical material was dried in the absence of direct sunlight and then pulverised into a rough powder using a mechanised crusher. The *B. ovalifoliolata* leaf powder was measured, placed into a reagent bottle, soaked in ethanol for a period of 72 hours, and then filtered using Whatman paper 41. The extracts were condensed using a rotary-evaporator at low pressure. The desiccated extracts were preserved at a temperature of 4°C in a hermetically sealed receptacle prior to conducting phytochemical and GC or MS analysis[9].



Figure 1. *Boswellia ovalifoliolata* plant.

2.3 Phytochemical Evaluation

The ethanol leaf extract of *B. ovalifoliolata* represented in Figure 1 was used to identify various secondary metabolites using standard methods[10].

2.4 GC-MS

The GC-MS analysis used Shimadzu TQ8040 NX GS-MS equipment with a TG-5-MS silica capillary-column (30m×0.25mm, film thickness 0.25µm). 70eV electron impact ionisation device with a scanning mass range of 29-400 (m/z) and helium carrier gas with a linear velocity of 41.4 cm/s was employed for GC-MS detection. The oven was heated to 60°C for 1 minute before phytochemical analysis. After this first phase, it set to grow to 300°C at 3°C/min and maintained isothermally for 15 min. The injector port, ion source, and detector temperatures were 280°C, 220°C, and 280°C. It took 20 minutes to run GC. The components names, molecular weights, and structures were obtained from the NIST Library database[11].

2.5 Statistical Analysis

Qualitative tests or analyses are done three times.

3. Results

3.1 Qualitative Phytochemical Screening

Qualitative analysis of the extracts from the leaves of *B. ovalifoliolata* showed the status of phytochemical constituents in ethanol extracts which is shown in Table 1.

3.2 Gas-Mass Spectroscopy (GC-MS)

GC-MS investigation of *B. ovalifoliolata* leaf ethanol extract active components found 28 chemicals. Table 2 lists active chemicals by Retention Time (RT), molecular formula, Molecular Weight (MW), and concentration (peak area %). Figure 2 shows the twenty-eight compound peaks in the GC-MS chromatogram. Presentation of mass spectroscopy-identified chemicals.

4. Discussion

It is evident from the Table 1 that the ethanol extract recorded the maximum number of chemical constituents including flavonoids, steroids, tannins, phenols, and glycosides. The presence of flavonoids, known to be effective free radical scavengers, indicates that this plant may have antioxidant qualities. Tannins and phenols are linked to anticancer, virucides, antioxidant, antimicrobial and anti-inflammatory, antdiabetic, wound healing, cardiovascular protection and antidiarrhoics. Steroids have been shown to be in reduction of inflammatory. Glycosides have been shown to be linked to the reduction of blood pressure. A total 28 active components found in *B. ovalifoliolata* ethanol extract investigated by using GC-MS. The ethanol extract contained three major components: 1,2,3-Benzenetriol (36.16%), Quinic acid (21.84%), and Lupeol (11.75%), as well as eight minor compounds: Levoglucosan (3.53%), Hexadecanoicacid, Methyl ester (1.59%), n-Hexadecanoicacid (3.29%), Ethylester (1.64%), and Methyl linolenate (1.30%).

Table 1. Qualitative phytochemical analysis of *B. ovalifoliolata* leaf extract

S. No.	Phytochemicals	Tests or Reagents	Results
1	Alkaloids	Mayer's, Wagner's, Hager's, Dragendorff's, and Tannic acid test	-
2	Flavonoids	Lead acetate, Shinoda, Alkaline, and Zinc HCl test	+
3	Tannins and Phenols	$FeCl_3$ test, Lead acetate test	+
4	Steroids	Libermann-Buchard test, Salkowski test	+
5	Anthocyanins	Anthocyanins test	-
6	Glycosides	Glycoside test	+
7	Saponins	Honeycomb test, Foam test	-
8	Anthraquinone	Bontrager's test	-

Note: +ve-presence of constituents and -ve- absence of constituents.

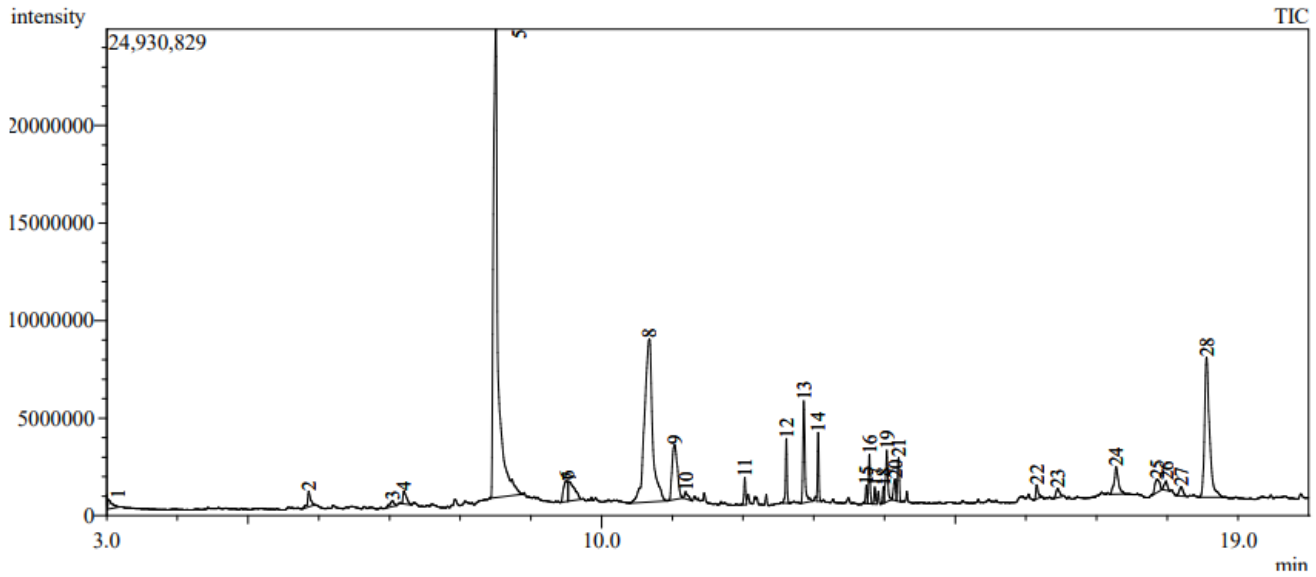


Figure 2. Gas-mass spectral analysis of *B. ovalifoliolata* leaf ethanol extract.

Table 2. Bioactive compounds identified in *B. ovalifoliolata* ethanol extract

S. No.	Bioactive Compound Name	Retention Time (min)	Peak Area %	Molecular Formula	Similarity	Biological Activity Reported	References
1	Furfural	3.025	0.64	$C_5H_4O_2$	81	Antibacterial, anticancer	[12]
2	Levoglucosenone	9.079	0.83	$C_6H_6O_3$	95	Antimicrobial, cytotoxic	[13]
3	1,4:3,6-Dianhydro-.alpha.-d-glucopyranose	7.041	0.43	$C_6H_8O_4$	82	Antibacterial, antioxidant	[14]
4	5-Hydroxymethylfurfural	7.206	0.74	$C_6H_6O_3$	91	Antioxidant, antiproliferative	[15]
5	1,2,3-Benzenetriol	8.503	36.16	$C_6H_6O_3$	96	Antimicrobial, antioxidant	[16]
6	Levoglucosan	9.505	1.50	$C_6H_{10}O_5$	93	Antibacterial	[17]
7	Levoglucosan	9.535	2.03	$C_6H_{10}O_5$	87	Antibacterial	[18]
8	Quinic acid	10.675	21.84	$C_7H_{12}O_6$	83	Antidiabetic, antioxidant, antimicrobial, anticancer activity, aging, analgesic effects, antiviral, anti-nociceptive protective	[19]
9	Quinic acid	11.033	4.83	$C_7H_{12}O_6$	81	antiviral, antibacterial antioxidant, antidiabetic, anti-nociceptive protective, antimicrobial, anticancer activity, aging, analgesic effects	[20]
10	Methyl-3,4-di-O-methylxyloside	11.188	0.39	$C_8H_{16}O_5$	76	Antioxidants, hypo-cholesterolemic, nematicide, and pesticide	[21]
11	Neophytadiene	12.024	0.74	$C_{20}H_{38}$	93	anti-inflammatory, anti-microbial	[22]

12	Hexadecanoic acid, methyl ester	12.613	1.59	$C_{17}H_{34}O_2$	97	Anti-hyper-cholesterolemic, Antioxidant, Antimicrobial, Antiandrogenic 5-Alphareductase inhibitor activity	23-24
13	n-Hexadecanoic acid	12.860	3.29	$C_{16}H_{32}O_2$	93	Hypo-cholesterolemic, 5-Alpha reductase inhibitor activity, Anti-inflammatory property. Antioxidant	25-26
14	Hexadecanoic acid, ethyl ester	13.062	1.64	$C_{18}H_{36}O_2$	94	hypocholesterolemic, antioxidants, nematicide	27
15	Methyl linoleate	13.741	0.42	$C_{19}H_{34}O_2$	93	Antioxidant	28
16	Methyl linolenate	13.788	1.30	$C_{19}H_{32}O_2$	95	Antioxidant	29
17	Phytol	13.863	0.46	$C_{20}H_{40}O_2$	98	Neuroprotective, Anticonvulsant activity, Antimicrobial, Anticancer, Antiinflammatory Antioxidant, Antidepressant, Anti-diabetic, Anti-diuretic properties	30-31
18	10(E),12(Z)-Conjugated linoleic acid	13.985	0.41	$C_{18}H_{32}O_2$	92	Anticancer	32
19	(9, 12, 15-Octadecatrienoic acid(Z,Z,Z)-	14.034	1.85	$C_{18}H_{30}O_2$	96	Antimicrobial	33
20	trans, trans-9,12-Octadecadienoic acid, propyl ester	14.148	0.84	$C_{21}H_{38}O_2$	90	antimicrobial activity. anti-inflammatory, antioxidant	34
21	Ethyl. alpha. -linolenate	14.199	1.16	$C_{20}H_{34}O_2$	94	Antimicrobial	35
22	2-Hexadecanoyl glycerol	16.153	0.52	$C_{19}H_{38}O_4$	93	Antimicrobial	36
23	Stigmasterol	16.451	0.55	$C_{29}H_{48}O$	85	antioxidant, anti-osteoarthritis anticancer immunomodulatory, antidiabetic, antibacterial, antiinflammatory, neuroprotective, anti-parasitic, anti-fungal	37
24	gamma. -Sitosterol	17.274	1.98	$C_{29}H_{50}O$	80	Antibacterial, antifungal	38
25	beta. -Amyrin	17.855	1.06	$C_{29}H_{50}O$	95	Antifungal	39
26	Germanicol	17.980	0.49	$C_{30}H_{50}O$	90	Antibacterial	40
27	(1R,2S,8R,8Ar)-8-acetoxy-1-(2hydroxyethyl)-1, 2, 5, 5tetramethyl-trans-decalin	18.197	0.58	$C_{16}H_{30}O_2$	71	Antibacterial	41
28	Lupeol	18.555	11.75	$C_{30}H_{50}$	94	Anti-inflammatory, antiprotozoal, anticancer	42

5. Conclusion

Using phytochemical screening, the study found a number of phytochemicals, including flavonoids, steroids, tannins, phenols, and glycosides. Gas chromatography-mass spectrometry was used to analyze the ethanol extract of *B. ovalifoliolata* leaves and discover twenty-eight chemical components. Because plant leaves contain a number of bioactive substances, traditional healers employ them to cure a variety of illnesses. The results of this investigation suggest that the identified phytocomponents have demonstrated biological activity in the form of anti-inflammatory, anti-microbial, antioxidant, hepatoprotective, and anti-cancer properties[37]. *B. ovalifoliolata* is therefore recommended as a potential source of phytopharmaceutical advantages.

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7. Author Contribution

Conceptualization and Study Design: Garla Venkateswarlu, Shashank K. Singh and Ramesh Bodla; Sample Collection and Preparation: Garla Venkateswarlu; Writing and Drafting: Garla Venkaetswarlu, Shashank K. Singh and Dr. Ramesh Bodla. All authors have read and agreed to the published version of the manuscript.

8. References

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