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Phytochemical profiling via GC-MS analysis of *Blepharis intgrefolia* using methanol extract

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Abstract
The present study aimed to identify and characterize the phytochemical constituents of the methanolic extract of *Blepharis integrifolia* using Gas Chromatography-Mass Spectrometry (GC-MS) analysis. The chromatogram revealed 30 bioactive compounds with retention times ranging from 14.27 to 32.13 minutes. The major constituents identified were n-Hexadecanoic acid (16.56%), 9, 12, 15-Octadecatrienoic acid (12.46%), 8, 11, 14-Docosatrienoic acid, methyl ester (9.07%), 11, 14-Eicosadienoic acid (6.31%), and Stigmasterol (4.64%). Other notable compounds included Vitamin E, Squalene, Phytol, and Gamma-sitosterol, which are known for their antioxidant, anti-inflammatory, and antimicrobial properties. The presence of diverse fatty acids, sterols, and terpenoids suggests the pharmacological potential of *B. integrifolia* as a source of natural bioactive molecules.

Keywords: *Blepharis integrifolia*, GC-MS, Methanolic extract, Phytochemical profiling

Introduction
The World Health Organization (WHO) recognizes the potential medicinal effects of natural shrubs and their bioactive components [1, 2]. Herbal plants, their components, and their functional biomolecules have long been used in homeopathy, Chinese medicine, Unani medicine, and Ayurvedic medicine to prevent illnesses. India must learn about important medicinal herbs, and this can only be accomplished if the herbal product has undergone contemporary standardization processes [3]. Active secondary metabolites, or phytochemicals, are found in many therapeutic plants and are important in traditional medicine. However, only 2% of the roughly 350,659 known species of medicinal plants worldwide have received scientific analysis to evaluate their beneficial bioactivities and phytochemical components [4]. Applications for gas chromatography are quite diverse. However, its primary application is in the analysis and separation of multi-component mixtures, including solvents, hydrocarbons, and essential oils [5, 6, 7].
GC-MS has been shown to be a useful technique for the study of non-polar components and volatile essential oils, fatty acids, lipids, and alkaloids; it has been used more frequently for the investigation of medicinal plants [8-11].
The physiological impact of utilizing two distinct analytical techniques to detect the phytocomponents and chemicals found in the diluted methanolic extract of *Terminalia chebula*, *Aloe vera*, and *Chamaecostus cuspidatus* [12].
The pharmaceutical industry makes extensive use of GC-MS for analytical research and development, quality assurance, control, manufacturing, pilot plant departments for active pharmaceutical ingredients (API), bulk medications, and formulations [13].

Materials and Methods
The plant material was collected from Dhanbad district and identified using HH Haines (The Botany of Bihar and Orissa). Thermo GC Trace Ultra Ver-5.0, Thermo MS DSQ II system, and a gas chromatograph interfaced to a mass spectrometer (GC-MS) are used to do GC-MS analysis. This was done using the DB 35-MS capillary standard column (30 x 0.25 mm ID x 1 µM df) using the electron impact mode at 70 eV. With a constant flow rate of 1 mL/min and an injection volume of 1 µL, the carrier gas was helium (99.999%). The oven was supposed to be isothermal at 70 °C for two minutes, then rise to 260 °C at a rate of 6 °C per minute.
The GC took 37.53 minutes to run in total. A calibrated micro syringe was used to deliver 1 µL of the sample to the sample port, and the carrier gas (helium) subsequently transported it to the column.

The chemicals separate as the mobile phase carrying the sample moves through the stationary phase. After going through the mass spectrometer detector, the sample is then sorted based on the mass-to-charge ratio. The computer, which contains a sample library, then receives the data. To determine which components were eluted at a certain retention time, data is shown in the mass spectrum at that exact moment in the chromatogram. To determine the chemicals included in the plant sample, GC-MS spectra were compared to a computer on a NIST Ver.2.1 MS data repository. The National Institute of Standards and Technology (NIST) database, which contains over 62,000 patterns, was used to interpret mass spectrum GC-MS.

Result and Discussion

Gas Chromatography-Mass Spectrometry (GC-MS) analysis of the methanolic extract of *Blepharis integrifolia* revealed the presence of 30 phytochemical compounds representing diverse chemical classes, including fatty acids, esters, alcohols, sterols, terpenoids, and hydrocarbons. The chromatographic peaks were identified based on retention time (RT), molecular weight, and comparison with the NIST library spectra. The identified compounds, their molecular formulas, molecular weights, and percentage peak areas are presented in Table 1. The major constituents detected were n-Hexadecanoic acid (16.56%), 9,12,15-Octadecatrienoic acid (12.46%), 8,11,14-Docosatrienoic acid, methyl ester (9.07%), and 11,14-Eicosadienoic acid (6.31%), which together contributed significantly to the total composition. Other notable compounds included 9,12-Octadecadienoic acid (Z,Z)-, methyl ester (5.29%), Stigmasterol (4.64%), Gamma-sitosterol (4.00%), Phytol (3.57%), Vitamin E (3.49%), and Squalene (3.14%). Several minor constituents such as Diethyl phthalate, Neophytadiene, Loliolide, and Campesterol were also recorded in trace amounts (< 2%).

The detected phytoconstituents are known to possess a wide range of biological activities. Fatty acids and their methyl esters (e.g., palmitic, stearic, and linolenic acids) are recognized for anti-inflammatory, antimicrobial, and antioxidant properties. Sterols such as Stigmasterol, Gamma-sitosterol, and Campesterol have been associated with cholesterol-lowering and cytoprotective effects, while Squalene and Vitamin E are potent natural antioxidants. The presence of these bioactive molecules supports the ethnomedicinal relevance of *B. integrifolia* and indicates its potential as a source of pharmacologically important compounds.

The components of volatile oil from plant extract and polyherbal formulation were examined using GC-MS analysis and compared with mass spectra of recognized chemicals from the NIST library database. A useful method for evaluating the consistency and quality of herbal treatments is the combination of hyphenated approaches. By combining chromatographic and mass spectrum data of the sample with reference standards, HPTLC and GC-MS help identify particular chemical elements within herbal formulations, ensuring authenticity and confirming the identity of important chemicals work done by [14].

Chamaecostus cuspidatus included 25 chemicals, according to the GCMS data. The highest reported area peak was 47.92%, while the lowest peak was 0.3%. The plant extract of *Aloe vera* included 20 compounds with a maximum area peak of 11.22% and a minimum area peak of 0.8%, whereas *Terminalia chebula* had 30 compounds with a maximum area peak of 12.51% and a minimum area peak of 0.21% work done by [15].

Tricosane, docosane, transcaryophyllene, nonacosane, beta-Cubebene, bis (2-ethylhexyl) phthalate, the most common chemicals discovered in extracts were trichlorfon, naphthalene, 1,2,3,4,4a, 5,6,8a-octahydro-4a, 8-dimethyl -2-(1-methylet henyl) -, [2R, 3-Aminophenol] work by [16].

The extract's GC-MS analysis identified three primary compounds: ethyl alpha-dglucopyranoside (21.05%), 4-vinylphenol (14.5%), and linoleic acid (10.8%) by [17].

Blepharis ciliaris, found in the Hail Mountains of Saudi Arabia, has strong antioxidant and antibacterial properties. GC-MS analysis found 17 bioactive chemicals, while DPPH experiments demonstrated significant antioxidant activity [18].

Table 1: GC-MS analysis of methanol extract of *Blepharis integrifolia* leaves

Sl. No	RT	Compound name	Molecular Formula	Molecular weight	Peak area in %
1	14.276	Diethyl Phthalate	C12H14O4	222	4.11
2	16.433	Loliolide	C11H16O3	196	0.41
3	16.941	Neophytadiene	C20H38	278	0.67
4	17.714	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	C17H24O3	276	0.46
5	17.854	Hexadecanoic acid, methyl ester	C17H34O2	270	1.52
6	18.292	n-Hexadecanoic acid	C16H32O2	256	16.56
7	19.461	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	C19H34O2	294	5.29
8	19.519	8,11,14-Docosatrienoic acid, methyl ester	C23H40O2	348	9.07
9	19.622	Phytol	C20H40O	296	3.57
10	19.761	Methyl stearate	C19H38O2	298	0.88
11	19.891	11,14-Eicosadienoic acid	C20H36O2	308	6.31
12	19.945	9,12,15-Octadecatrienoic acid	C18H30O2	278	12.46
13	20.135	Octadecanoic acid	C18H36O2	284	3.42
14	22.879	Hexatriacontane	C36H74	506	0.47
15	23.035	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	C19H38O4	330	3.50
16	24.380	Hexatriacontane	C36H74	506	0.46
17	24.586	Octadecanoic acid, 2,3-dihydroxypropyl ester	C21H42O4	358	1.13
18	25.016	13-Docosenamide	C22H43NO	337	2.09
19	25.200	Squalene	C30H50	410	3.14
20	25.425	Cyclononasiloxane, octadecamethyl-	C18H54O9Si9	666	0.50
21	25.777	Tetracontane	C40H82	562	0.49
22	27.335	Vitamin E	C29H50O2	430	3.49
23	27.403	Alpha-Tocopherolquinone	C29H50O3	446	1.40
24	28.043	Campesterol	C28H48O	400	2.07
25	28.207	Stigmasterol	C29H48O	412	4.64
26	28.623	Gamma, Sitosterol	C29H50O	414	4.00
27	28.717	Phytonadione	C31H46O2	450	0.80
28	30.155	Glutinol	C30H50O	426	2.94
29	30.232	Phytyl stearate	C38H74O2	562	2.57
30	32.132	Phytyl linoleate	C38H70O2	558	1.60

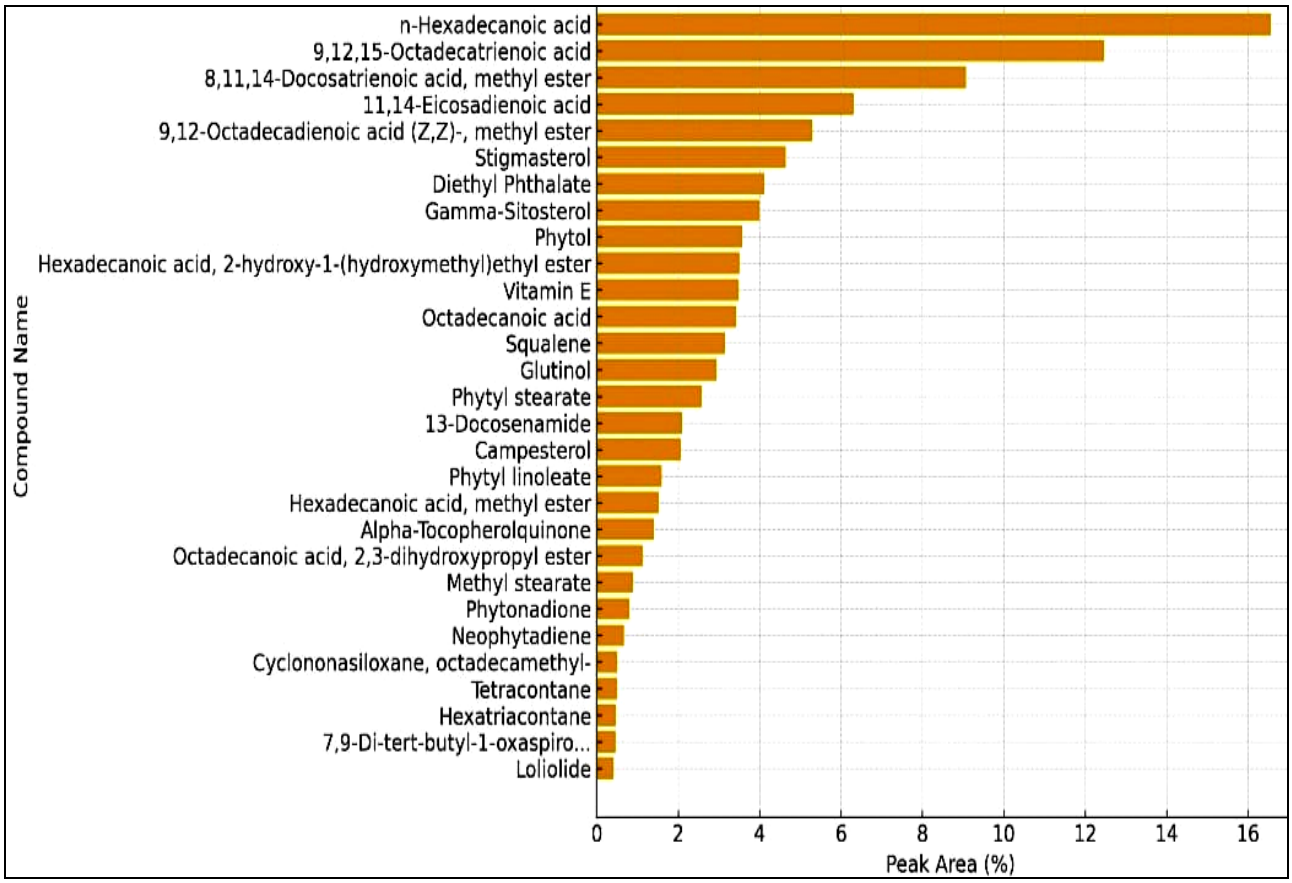

Fig 1: Relative abundance of phytochemical compounds identified in methanolic extract of *Blepharis integrifolia*

Conclusion

The present study provides a comprehensive GC-MS profile of the methanolic extract of *Blepharis integrifolia*, revealing the presence of 30 bioactive phytochemical compounds belonging to diverse chemical classes such as fatty acids, esters, sterols, terpenoids, and hydrocarbons. Among these, *n*-Hexadecanoic acid, 9, 12, 15-Octadecatrienoic acid, 8, 11, 14-Docosatrienoic acid methyl ester, and 11, 14-Eicosadienoic acid were identified as the major constituents. These compounds are known for their potent antioxidant, anti-inflammatory, antimicrobial, and cytoprotective properties.

The findings support the traditional and ethnomedicinal uses of *B. integrifolia* and emphasize its potential as a rich source of pharmacologically active natural products. The presence of key bioactive molecules such as sterols, fatty acids, and antioxidants further indicates its possible role in drug development and nutraceutical applications. Future studies focusing on bioassay-guided isolation, structural characterization, and pharmacological validation of the major compounds are recommended to establish their therapeutic significance and mechanisms of action.

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