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GC-MS analysis of root extracts of *Elephantopus scaber* L. using chloroform and petroleum ether

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ABSTRACT

The objective of this study was to identify bioactive compounds present in the chloroform and petroleum ether root extracts of *Elephantopus scaber* L. using Gas Chromatography-Mass Spectrometry (GC-MS). Air-dried leaves of *Elephantopus scaber* L. were powdered and initially extracted with methanol. The dried methanolic extract was subsequently partitioned using chloroform and petroleum ether. Both extracts were subjected to GC-MS analysis for compound identification. The most abundant compound identified was Lupeol (25.56%), detected at a retention time (RT) of 29.935 min. The second major constituent was Lup-20(29)-en-3-one (24.51%) at RT 29.588 min. Other significant triterpenoids included Lup-20(29)-en-3-ol acetate (3?-) (7.35%), β-Amyrone (7.06%), and β-Amyrin (2.04%). The collective abundance of these lupane-type compounds accounted for more than 65% of the total extract composition. GC-MS analysis of the petroleum ether root extract led to the identification of 44 compounds, representing 100% of the total chromatographic area. The most abundant peak corresponded to Phenol, 2,4-bis(1,1-dimethylethyl) phosphite (3:1) (56.17%), followed by Tris(2,4-di-tert-butylphenyl) phosphate (9.64%). These compounds are commonly reported as synthetic antioxidants or plastic-derived contaminants in GC-MS analyses and therefore should be interpreted cautiously as non-native phytochemical constituents. GC-MS analysis showed the chloroform root extract was rich in Lupane-type triterpenoids, dominated by Lupeol (25.56%) and Lup-20(29)-en-3-one (24.51%). In contrast, the petroleum ether extract was dominated by synthetic phosphite antioxidants, likely contaminants, indicating chloroform as the superior solvent for isolating bioactive triterpenoids. The chloroform root extract, exhibits strong anti-inflammatory, antioxidant, antimicrobial, hepatoprotective, and anticancer activities. The petroleum ether extracts shows antioxidant, chemo preventive, and mild antimicrobial properties, though major peaks suggest possible contaminant-derived activity.

Key Words - Bioactive compounds, Triterpenoids, Antioxidant

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INTRODUCTION

Medicinal plants have long served as a primary source of therapeutic agents in traditional systems of healthcare. Herbal medicine is widely regarded as an alternative to Western medical practice and is deeply interwoven with the spiritual beliefs and cultural traditions of indigenous communities (Nadkarni 2009). India, in particular, is richly endowed with vast botanical diversity and a wealth of medicinal flora that produce numerous bioactive secondary metabolites. Plants possess an extraordinary capacity to synthesize a wide array of secondary metabolites, which are distributed in

different plant parts such as leaves, fruits, buds, stems, flowers, bark, and roots (Yadav & Agarwal, 2011).

A large number of phytochemical constituents derived from medicinal plants have been employed by practitioners for centuries and continue to be administered under professional supervision to millions of individuals worldwide. Crude herbal extracts and plant-based formulations, including those developed by pharmaceutical industries, may result in extensive human exposure to naturally occurring compounds. The sustained reliance on herbal remedies is largely attributed to their therapeutic efficacy, accessibility, affordability, and comparatively lower incidence of adverse effect (Rios & Recio, 2005). Herbal preparations can exert pharmacological actions comparable to synthetic drugs and may stimulate the body's intrinsic healing mechanisms.

Since numerous pharmacologically active phytochemicals have been isolated from traditional Indian medicinal plants, systematic evaluation of their therapeutic potential is essential (Dzubak *et al.,* 2006; Patocka, 2003). In this context, Gas Chromatography–Mass Spectrometry (GC–MS) is recognized as a highly sensitive and reliable analytical technique for the separation and identification of structurally diverse and complex constituents present in plant extracts.

Traditional Indian medicine, particularly Ayurveda, incorporates thousands of classical and household formulations for managing a variety of ailments such as anxiety, depression, arthritis, hypertension, hormonal disturbances, insomnia, migraine, dermatological conditions, and several other disorders. The medicinal efficacy of a plant primarily depends on the presence of physiologically active biochemical compounds that contribute to its pharmacological properties (Yadav & Agarwal, 2011).

Elephantopus scaber L. is widely distributed in the tropical deciduous forests surrounding Ranchi, the capital of Jharkhand. These forests, characterized by rich biodiversity and a moderate climatic condition, offer a favorable habitat for the growth of this herb. In addition to forest ecosystems, the plant commonly occurs in disturbed habitats such as roadsides, open grasslands, fallow fields, and the margins of agricultural lands. It flourishes particularly well in areas receiving sufficient sunlight and growing in moderately disturbed, well-drained soils. Seasonal monsoon rainfall further supports its vigorous growth, and the plant is most abundantly observed between August and December, which corresponds to its flowering and fruiting phase.

In Jharkhand, *Elephantopus scaber* L. holds significant importance within the traditional knowledge systems of various tribal communities (Nadkarni, 2009; Rao & Rao, 1995). Indigenous healers locally referred to as "vaidyas," utilize this plant to treat a wide range of ailments, including common conditions such as fever, digestive disorders, and respiratory problems, as well as certain chronic illnesses. Decoctions prepared from the leaves and roots are frequently administered for the management of fever and gastrointestinal disturbances, as they are believed to possess antipyretic and antimicrobial properties. The roots are particularly valued for treating dysentery, while the leaves are often applied externally to promote wound healing. Hence, the present study was aimed to find out the bioactive compounds found in roots of *Elephantopus scaber* L. in chloroform and petroleum ether extracts.

MATERIALS & METHODS

Collection and Authentication of Plant Materials:

Fresh, healthy, young leaves of *Elephantopus scaber* L. used for the investigation were collected from forests of Nagri block of Ranchi district of Jharkhand, India. The plant was authenticated by Central National Herbarium, Botanical Survey of India, Kolkata.

Preparation of Plant Extracts:

The roots of plants were dried in the shade at room temperature 30°C for 30 days. Roots were grounded to fine powder with the assistance of mortar and pestle. 100 g of sample powder was saturated each in 1000mL Ethanol, chloroform, Petroleum ether,

Acetone and Distilled water (Sultana *et al.,* 2009). After 10 days, the extracts were filtered by Whatman filter paper. This process was applied triple time and the extracts were combined and then it was concentrated utilizing a rotary type of evaporator. The obtained extracts were then packaged and stored at in the refrigerator for maintaining temperature.

Gas Chromatography-Mass Spectroscopy (GCMS) Analysis:

The ethanolic extract of the sample was used for the determination of compounds through GC-MS. Shimadzu QP-2010 Plus with thermal desorption System TD 20 was used for GC-MS analysis and work done in Advanced Instrumental Research Facility (AIRF), Jawaharlal Nehru University (JNU), New Delhi, India. For GC-MS, use the following steps i.e. first: Sample preparation, second: injection, third: vaporization, fourth: separation, fifth:mass spectrometry, and finally sixth: identification. To evaluate the bioactivity of the identified phyto-constituents, a comprehensive literature survey was conducted using popular search engines such as Google Scholar and PubMed. The structure of the identified compound was retrieved from PubChem, providing valuable insights into the potential biological activities and medicinal properties associated with these specific phytochemicals. This approach ensures a thorough investigation into the bioactive properties of the compound by scientific literature available online.

RESULTS

Gas Chromatography–Mass Spectrometry (GC–MS) analysis of the chloroform root extract revealed the presence of 67 phytochemical constituents as shown in Table-1, representing 100% of the total chromatographic area. The chromatogram showed in Fig. 1 a predominance of pentacyclic triterpenoids, particularly Lupane-type derivatives, indicating a triterpenoid-rich profile (Mahato & Kundu, 1994). The most abundant compound identified was Lupeol (25.56%), detected at a retention time (RT) of 29.935 min. The second major constituent was Lup-20(29)-en-3-one (24.51%) at RT 29.588 min. Other significant triterpenoids included Lup-20(29)-en-3-ol acetate (3β-) (7.35%), β-Amyrone (7.06%), and β-Amyrin (2.04%). The collective abundance of these lupane-type compounds accounted for more than 65% of the total extract composition. Moderate quantities of Tris(2,4-di-tert-butylphenyl) phosphate (5.30%) and Methacrylic acid, 2,3-dimethylphenyl ester (7.04%) were also detected. Additionally, minor bioactive constituents such as Squalene (0.49%), Vitamin E (0.40%), Stigmasterone (0.93%), and Neryl nitrile (0.48%) were identified.

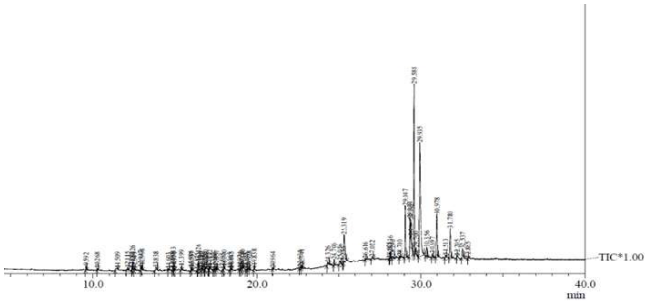

Fig. 1: Chromatogram of Chloroform root extract of *Elephantopus scaber* L.

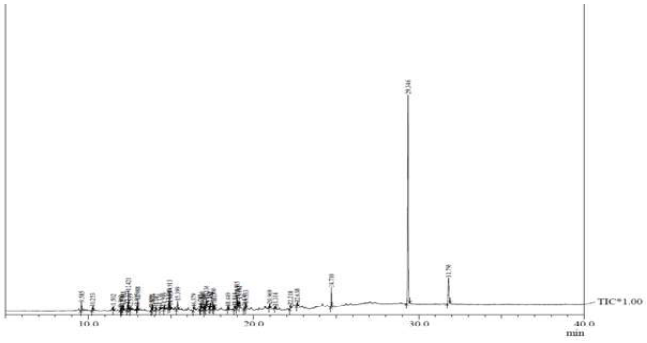

Fig. 2: Chromatogram of Petroleum ether extract of *Elephantopus scaber* L.

Gas Chromatography–Mass Spectrometry (GC–MS) analysis of the petroleum ether root extract led to the identification of 44 compounds, representing 100% of the total chromatographic area. The extract was predominantly composed of long-chain hydrocarbons, fatty acid derivatives, and minor triterpenoid constituents. The most abundant peak corresponded to Phenol, 2,4-bis(1,1-dimethylethyl), phosphite (3:1) (56.17%), followed by Tris(2,4-di-tert-butylphenyl) phosphate (9.64%). These compounds are commonly reported as synthetic antioxidants or plastic-derived contaminants in

GC–MS analyses and therefore should be interpreted cautiously as non-native phytochemical constituents.Among the confirmed plant-derived metabolites, Squalene (3.08%) was identified as the major authentic bioactive compound. Squalene is a triterpene hydrocarbon widely recognized for its antioxidant and chemopreventive properties. Its presence indicates the extraction of non-polar bioactive constituents by petroleum ether. Fatty acid methyl esters (FAMEs) were also detected in appreciable amounts, including 9,12-octadeca dienoic acid (Z, Z)-, methyl ester (1.93%) and 9-octadecenoic acid, methyl ester (E)- (0.83%). These compounds are associated with anti-inflammatory and antimicrobial activities. Additionally, saturated hydrocarbons such as eicosane (2.49%, 2.00%, and 1.67%), tetracosane, dotriacontane (1.22%), and tetrapentacontane (1.45% and 0.74%) were identified, indicating a hydrocarbon-rich profile. The GC–MS profile of the petroleum ether root extract demonstrates a predominance of long-chain alkanes and fatty acid derivatives, with squalene representing the principal bioactive phyto constituent. The results suggest that petroleum ether preferentially extracts non-polar lipidic and hydrocarbon components from the root matrix.

Table 1. Major phytochemical constituents identified in chloroform root extract of *Elephantopus scaber* L. by GC–MS

Sl. No.	Compound Name	Retention Time (min)	Peak Area (%)	Molecular Formula	Reported Biological Activity
1	Lupeol	29.935	25.56	$C_{30}H_{50}O$	Anti-inflammatory, anticancer, antioxidant, hepatoprotective, antimicrobial
2	Lup-20(29)-en-3-one	29.588	24.51	$C_{30}H_{48}O$	Anti-inflammatory, cytotoxic, antimicrobial
3	Lup-20(29)-en-3-ol acetate (3β-)	30.978	7.35	$C_{32}H_{52}O_2$	Anti-inflammatory, wound healing, antimicrobial
4	β-Amyrone	29.047	7.06	$C_{30}H_{48}O$	Anti-inflammatory, antioxidant, analgesic
5	β-Amyrin	29.392	2.04	$C_{30}H_{50}O$	Anti-inflammatory, antimicrobial, hepatoprotective
6	Methacrylic acid, 2,3-dimethylphenyl ester	25.319	7.04	$C_{12}H_{14}O_2$	Reported antioxidant and antimicrobial potential (requires confirmation)
7	Tris (2,4-di-tert-butylphenyl) phosphate	31.78	5.3	$C_{42}H_{63}O_4P$	Antioxidant stabilizer (commonly reported as GC–MS contaminant)
8	Squalene	24.706	0.49	$C_{30}H_{50}$	Antioxidant, chemopreventive, immunomodulatory
9	Vitamin E	27.022	0.4	$C_{29}H_{50}O_2$	Potent antioxidant, membrane stabilizing, anti-aging
10	Stigmasterone	29.7	0.93	$C_{29}H_{46}O$	Anti-inflammatory, cholesterol-lowering
11	Neryl nitrile	24.326	0.48	$C_{10}H_{15}N$	Antimicrobial, fragrance-related bioactivity
12	2-Pentadecanone, 6,10,14-trimethyl	16.58	0.37	$C_{18}H_{36}O$	Antimicrobial, antioxidant
13	1-Hexadecanol	18	0.19	$C_{16}H_{34}O$	Antimicrobial, emollient activity
14	1-Eicosanol	19.838	0.26	$C_{20}H_{42}O$	Antimicrobial, surface-protective activity
15	Ursa-9(11),12-dien-3-one	28.136	0.69	$C_{30}H_{46}O$	Anti-inflammatory, cytotoxic potential

Table 2. Major phytochemical constituents identified in petroleum ether root extract of *Elephantopus scaber* L. by GC–MS

Sl. No.	Compound Name	Retention Time (min)	Peak Area (%)	Molecular Formula	Reported Biological Activity
1	Phenol, 2,4-bis(1,1-dimethylethyl)-, phosphite (3:1)	29.346	56.17	$C_{42}H_{63}O_3P$	Synthetic antioxidant stabilizer (commonly reported GC–MS contaminant)
2	Tris(2,4-di-tert-butylphenyl)phosphate	31.795	9.64	$C_{42}H_{63}O_4P$	Antioxidant stabilizer; frequently reported as laboratory contaminant
3	Squalene	24.71	3.08	$C_{30}H_{50}$	Antioxidant, chemopreventive, immunomodulatory
4	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	18.985	1.93	$C_{19}H_{34}O_2$	Anti-inflammatory, antimicrobial, hypocholesterolemic
5	9-Octadecenoic acid, methyl ester (E)-	19.043	0.83	$C_{19}H_{36}O_2$	Antioxidant, cardioprotective, antimicrobial
6	Eicosane	12.421	2.49	$C_{20}H_{42}$	Antimicrobial, anti-inflammatory (reported in plant waxes)
7	Eicosane	15.399	2	$C_{20}H_{42}$	Antimicrobial activity
8	Eicosane	14.913	1.67	$C_{20}H_{42}$	Mild bioactive hydrocarbon
9	Dotriacontane	17.136	1.22	$C_{32}H_{66}$	Antimicrobial, protective surface activity
10	Tetrapentacontane	19.142	1.45	$C_{54}H_{110}$	Reported antimicrobial activity
11	Tetracosane	17.56	0.94	$C_{24}H_{50}$	Antimicrobial, anti-inflammatory
12	Hexadecane, 2,6,10,14-tetramethyl-	18.844	0.88	$C_{20}H_{42}$	Antioxidant, antimicrobial
13	Heptadecane	11.961	0.26	$C_{17}H_{36}$	Reported antimicrobial activity
14	Heneicosane	12.03	0.41	$C_{21}H_{44}$	Antimicrobial, insecticidal
15	Hexadecanoic acid, methyl ester	17.392	0.39	$C_{17}H_{34}O_2$	Anti-inflammatory, antioxidant

DISCUSSION

The GC–MS analysis of the chloroform root extract of *Elephantopus scaber L.* revealed a chemically rich profile dominated by pentacyclic triterpenoids, particularly lupane-type derivatives. The predominance of Lupeol (25.56%) and Lup-20(29)-en-3-one (24.51%) indicates that the chloroform solvent efficiently extracted non-polar to moderately polar triterpenoid constituents from the root matrix. Together with Lup-20(29)-en-3-ol acetate, β-Amyrone, and β-Amyrin, these compounds accounted for more than 65% of the total extract composition, establishing a strong triterpenoid signature.

Lupane-type triterpenoids are widely documented for anti-inflammatory, antioxidant, antimicrobial, hepatoprotective, and anticancer activities (Dzubak *et al.,* 2006; Sohretoglu *et al.,* 2017; Kim *et al.,* 2011). The high abundance of lupeol, in particular, provides a biochemical basis for the traditional medicinal applications of *E. scaber* (Gallo & Sarachine, 2009; Siddique & Saleem, 2011), especially in inflammatory disorders and wound healing (Bani *et al.,* 2006; Saleem. 2009). Minor constituents such as Squalene and Vitamin E further enhance the antioxidant potential of the extract through membrane stabilization and free radical scavenging mechanisms (Spanova & Daum, 2011).

The presence of stigmasterone and neryl nitrile also contributes to the overall pharmacological relevance of the chloroform fraction. Moderate levels of Tris(2,4-di-tert-butylphenyl) phosphate and methacrylic acid derivatives were detected; however, these compounds are frequently reported as synthetic stabilizers or laboratory contaminants in GC–MS analyses. Their occurrence should therefore be interpreted cautiously and confirmed through repeated extraction or alternative analytical techniques. In contrast, the petroleum ether root extract exhibited a markedly different phytochemical profile. Although 44 compounds were identified, the chromatogram was dominated by phenolic phosphite derivatives, which are commonly associated with plasticware or instrumental contamination rather than genuine plant metabolites. Excluding these likely contaminants, the petroleum ether extract was primarily characterized by long-chain hydrocarbons and fatty acid methyl esters. Among the confirmed plant-derived metabolites, Squalene (3.08%) emerged as the principal authentic bioactive compound. Squalene is well recognized for its antioxidant, chemopreventive, and immuno modulatory properties (Spanova & Daum, 2011), indicating that petroleum ether effectively extracted highly non-polar triterpene hydrocarbons. The detection of fatty acid methyl esters, including linoleic and oleic acid derivatives, suggests additional anti-inflammatory and antimicrobial potential. Saturated hydrocarbons such as eicosane, tetracosane, dotriacontane, and tetrapentacontane further confirm the lipid-rich and hydrocarbon-dominant nature of this fraction. Comparatively, the chloroform extract demonstrated a substantially higher concentration of pharmacologically significant triterpenoids than the petroleum ether extract (Sultana *et al.,* 2009). This highlights the influence of solvent polarity on phytochemical recovery, with chloroform proving more efficient for isolating bioactive lupane-type compounds, whereas petroleum ether preferentially extracted lipidic and hydrocarbon constituents. This study finding indicates that the chloroform root extract of *E. scaber* possesses greater therapeutic potential due to its triterpenoid-rich composition, with lupeol serving as the principal chemotaxonomic and pharmacologically active marker compound.

Comparative Interpretation

The present GC–MS investigation demonstrated that the root extracts of *Elephantopus scaber L.* possess distinct phytochemical profiles depending on the extraction solvent used. The chloroform root extract was predominantly enriched with pentacyclic triterpenoids, particularly Lupeol and Lup-20(29)-en-3-one, which together constituted more than 50% of the total extract. The collective abundance of lupane-type derivatives (>65%) confirms a triterpenoid-rich composition and supports the plant's reported anti-inflammatory, antioxidant, antimicrobial, and anticancer potential (Hiradeve *et al.,* 2010; Rajkapoor *et al.,* 2007).

In contrast, the petroleum ether root extract was mainly characterized by long-chain hydrocarbons and fatty acid methyl esters, with Squalene identified as the principal authentic bioactive constituent. The predominance of synthetic phosphite derivatives in this fraction suggests possible analytical contamination and highlights the importance of careful interpretation of GC–MS data.

Overall, chloroform proved to be the more effective solvent for extracting pharmacologically significant triterpenoids from the root matrix. The findings provide a scientific basis for the medicinal relevance of *E. scaber* roots and establish lupeol as a key chemotaxonomic and bioactive marker compound.

CONCLUSION

The present GC–MS study revealed significant variations in the phytochemical composition of chloroform and petroleum ether root extracts of *Elephantopus scaber L.*. The chloroform extract was predominantly rich in pentacyclic triterpenoids, particularly Lupeol and Lup-20(29)-en-3-one, which together accounted for a major proportion of the total composition. The high abundance of lupane-type derivatives confirm the triterpenoid-rich nature of the root and support its reported anti-inflammatory, antioxidant, antimicrobial, and

anticancer properties. In contrast, the petroleum ether extract was mainly composed of long-chain hydrocarbons and fatty acid methyl esters, with Squalene as the principal authentic bioactive compound. This study proved that chloroform as a solvent is more efficient in extracting pharmaco logically significant constituents, establishing lupeol as a key bioactive and chemotaxonomic marker of the root.

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