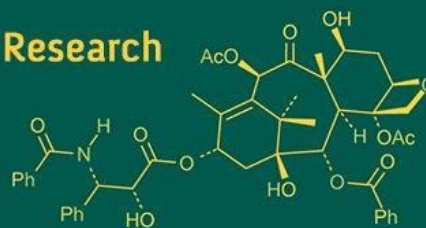


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K Lalawmpuii
Ph.D. Scholar, Guru Angad
Dev Veterinary and Animal
Sciences University, Ludhiana,
Punjab, India

Dr. Dhaval J Kamothi
Assistant Professor,
Department of Veterinary
Pharmacology and Toxicology,
College of Vet. Sci. & A.H.,
Kamdhenu University,
Junagadh, Gujarat, India

Lalrinkima
Assistant Professor, Institute
of Veterinary Sciences and
Animal Husbandry, SOA,
Odisha, India

Zarzoliani
Ph.D. Scholar, Guru Angad
Dev Veterinary and Animal
Sciences University, Ludhiana,
Punjab, India

Lakshmi Yadav
Ph.D. Scholar, ICAR-Indian
Veterinary Research Institute
(IVRI), Animal Reproduction
Division, ICAR-Indian
Veterinary Research Institute,
Izzatnagar, Uttar Pradesh,
India

Vanlalhmangaihsanga
Assistant Professor, Institute
of Veterinary Sciences and
Animal Husbandry, SOA,
Odisha, India

Muntasir Ashraf Wani
Assistant Professor, Institute
of Veterinary Sciences and
Animal Husbandry, SOA,
Odisha, India

Corresponding Author:
Dr. Dhaval J Kamothi
Assistant Professor,
Department of Veterinary
Pharmacology and Toxicology,
College of Vet. Sci. & A.H.,
Kamdhenu University,
Junagadh, Gujarat, India

GC-MS based phytochemical characterization of *Trachyspermum ammi* (Ajwain) and *Lansium parasiticum* (Langsat) for anthelmintic applications

K Lalawmpuii, Dhaval J Kamothi, Lalrinkima, Zarzoliani, Lakshmi Yadav, Vanlalhmangaihsanga and Muntasir Ashraf Wani

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Abstract

Helminth infections continue to pose significant challenges to both human and veterinary health, particularly in regions where access to modern anthelmintics is limited and resistance to synthetic drugs is increasing. Traditional medicinal plants offer a promising alternative owing to their diverse bioactive compounds. The present study focused on phytochemical screening of *Trachyspermum ammi* (Ajwain) seeds and *Lansium parasiticum* (Langsat) fruit extracts using ethanol as a solvent and gas chromatography-mass spectrometry (GC-MS) for compound identification. Extraction yields were found to be relatively low with ethanol, recording 1.9% for *T. ammi* and 2.26% for *L. parasiticum*. GC-MS profiling of *T. ammi* revealed the presence of key compounds including oleic acid (32.62%), linoleic acid (19.33%), thymol (12.98%), and palmitic acid (9.40%), along with minor constituents such as lupeol, squalene, and stigmasterol, all of which are known for their antimicrobial, antioxidant, and potential anthelmintic activities. Similarly, *L. parasiticum* extracts demonstrated a rich chemical profile, dominated by 1-heptadec-1-ynyl-cyclohexanol (36.55%), 7-tetradecenal (26.87%), and palmitic acid (14.19%), along with stearic acid, spiro derivatives, and sterols such as gamma-sitosterol. Many of these compounds have been reported to exhibit pharmacological effects including anti-inflammatory, antiparasitic, and antimicrobial properties. The findings highlight the chemical diversity of these two plants and support their traditional use in ethnomedicine as potential sources of novel anthelmintics. While further biological assays are needed to confirm their therapeutic efficacy, this study provides a phytochemical basis for considering *T. ammi* and *L. parasiticum* as promising candidates for developing plant-based, multi-targeted interventions against helminthic infections.

Keywords: *Trachyspermum ammi* (Ajwain), *Lansium parasiticum* (Langsat), phytochemical screening, GC-MS analysis, bioactive compounds

Introduction

Herbal medications have garnered interest as viable alternatives to synthetic anthelmintics, particularly in regions where traditional practices are prevalent. Numerous reports indicate the anthelmintic activity of various seed oils, such as grape oil, carvacrol, pomegranate oil, eugenol, and cumin oil (Pandey and Madhuri, 2010) [17]. Herbal remedies offer multiple advantages, including a reduced risk of resistance due to their diverse mechanisms of action, environmental sustainability, and additional health benefits from their antioxidant properties (Jabbar *et al.*, 2006) [10]. Several herbal medicines derived from plants such as chamomile, nettle, shatavari, neem, karela, ashwagandha, fenugreek, pippali, wormwood, and ajwain have also demonstrated anthelmintic potential. These formulations contain various secondary metabolites, including polyphenolic compounds like alkaloids, flavonoids, and tannins, which have been shown to possess anti-helminthic activity (Mushtaq *et al.*, 2018) [16]. Some plant extracts have been investigated for their inhibitory effects on various stages of parasitic development, such as embryonation, egg hatching, exsheathing of infective larvae, and larval settling.

Despite the prevalence of helminthic infections, the scarcity of medications and extensive records of traditional remedies highlight that only a small number of natural resources have been investigated for their potential in modern medicine. Furthermore, the efficacy of a single plant extract can vary against different parasites and exhibit distinct modes of action.

This research gap emphasizes the importance of comparing the efficacy of synthetic anthelmintics with herbal extracts against abomasal nematodes. Identifying and evaluating the most effective herbal treatments can contribute to a more diverse and sustainable approach to parasite management.

Phytochemicals are natural components of plants, including alkaloids, flavonoids, tannins, saponins, steroids, terpenoids, and phenolic compounds. Phytochemical screening is essential for identifying new plant-derived medications, as many traditional remedies have paved the way for the development of modern pharmaceuticals. Screening methods can be categorized into qualitative and quantitative approaches. Qualitative tests identify the presence of broad groups of substances, such as alkaloids and flavonoids, while quantitative methods, including spectrophotometry, measure their concentrations. Advanced techniques like chromatography, high-performance liquid chromatography (HPLC), high-resolution mass spectrometry (HRMS), nuclear magnetic resonance (NMR), and gas chromatography-mass spectrometry (GC-MS/LC-MS) provide detailed analysis of phytochemical components.

To effectively harness the potential of herbal remedies, it is crucial to identify the bioactive compounds responsible for their anthelmintic activity. Plant composition can vary based on environmental and geographical factors, affecting result consistency, while improper dosages may lead to toxicity (Ademola *et al.*, 2008) [2]. Phytochemical screening not only elucidates the active constituents but also provides a scientific foundation for the therapeutic applications of these plants. Understanding the phytochemistry of potential anthelmintics can aid in developing more targeted therapies, improving efficacy and reducing the likelihood of resistance. A comprehensive analysis may be required to identify the specific compounds contributing to their anthelmintic effects. This research will not only enrich the scientific knowledge base but could also pave the way for formulating new treatments derived from natural sources.

Materials and Methods

Collection and preparation of herbal extract

For the experiment, an unrefined whole seed of *Trachyspermum ammi* (Ajwain) was purchased from a local market in Ludhiana. Additionally, *Lansium parasiticum* (Langsat) fruit was procured from the local market in Cooch Behar, West Bengal. The fruits were washed several times, the cover was peeled off, then the pulps and seeds were separated from each other and left to air dry in an oven for 7-8 days at 60 °C. The two plants were then ground into

powder form using a grinder separately. Subsequently, extracts were dissolved in ethanolic extracts using maceration method (Abubakar and Haque, 2020) [1]. 100 g of plant powder was added to 200 ml of ethanol in a conical flask with the mouth of the flask plugged and shaken in a rotatory shaker for 24 hours. Then, the contents were filtered using a muslin cloth followed by sterile Whatman filter paper no.1. Then, the filtrate was dried in a hot air oven for 24-72 hours. Then the dried extracts were collected and stored at 4 °C for further use.

The final extracts obtained from the solvent was weighed in order to obtain the percentage extraction yield (Y%). The Y% was calculated by the following formula:

$$Y\% = \frac{\text{Weight of residue obtained}}{\text{Weight of plant powder}} \times 100$$

Phytochemical screening of active constituents

Understanding the phytochemical composition of herbal extracts is crucial for evaluating their medicinal potential. Phytochemicals not only contribute to the therapeutic effects of these plants but also serve as indicators of their bioactive properties.

The qualitative screening for phytochemical constituents of ethanol extracts of both *T. ammi* and *L. parasiticum* was carried out by Gas-chromatography mass spectroscopy (GC-MS).

Phytochemical Analysis Using GC-MS

The ethanol extracts of both *T. ammi* and *L. parasiticum* were dissolved in ethanol solvent at a concentration of 2 mg/ml and filtered using a sterile 0.22-micron syringe filter. The phytochemical constituents of the extracts were analyzed at the Central Instrumentation Laboratory (CIL) at the Central University of Punjab, Bathinda, using gas chromatography-mass spectrometry (GC-MS).

Results

Extraction yields of *T. ammi* and *L. parasiticum* dissolved in various solvents

The extraction of the remarkable herbal plants was conducted using a range of solvents: aqueous, hydro-ethanol, and ethanol, employing the maceration method. The final extracts obtained from *T. ammi* and *L. parasiticum* were meticulously weighed to determine the percentage extraction yield (Y%).

Table 1: Extraction Yield of Various Extracts of *T. ammi* and *L. parasiticum*

Solvents	Initial Weight (g)	Weight of <i>T. ammi</i> Residue (g)	Y%	Weight of <i>L. parasiticum</i> Residue (g)	Y%
Ethanol	100	1.9±0.1	1.9%	2.26±0.7	2.26%

The results reveal that the ethanol extracts yield a mere 1.9% for *T. ammi* and 2.26% for *L. parasiticum*. This relatively low yield can be attributed to the non-polar nature of ethanol, which limits its ability to extract a wide array of compounds. In contrast, the aqueous and hydro-ethanol extracts showcase significantly higher yields, with the aqueous solvent excelling in extracting polar compounds. Water's superior solvation capability allows for the effective

extraction of beneficial compounds such as flavonoids, phenolic compounds, and glycosides.

Phytochemical Screening of Ethanol Extracts Using GC-MS

Ethanol extracts of both *T. ammi* and *L. parasiticum* were subjected to GC-MS analysis to identify additional phytochemicals.

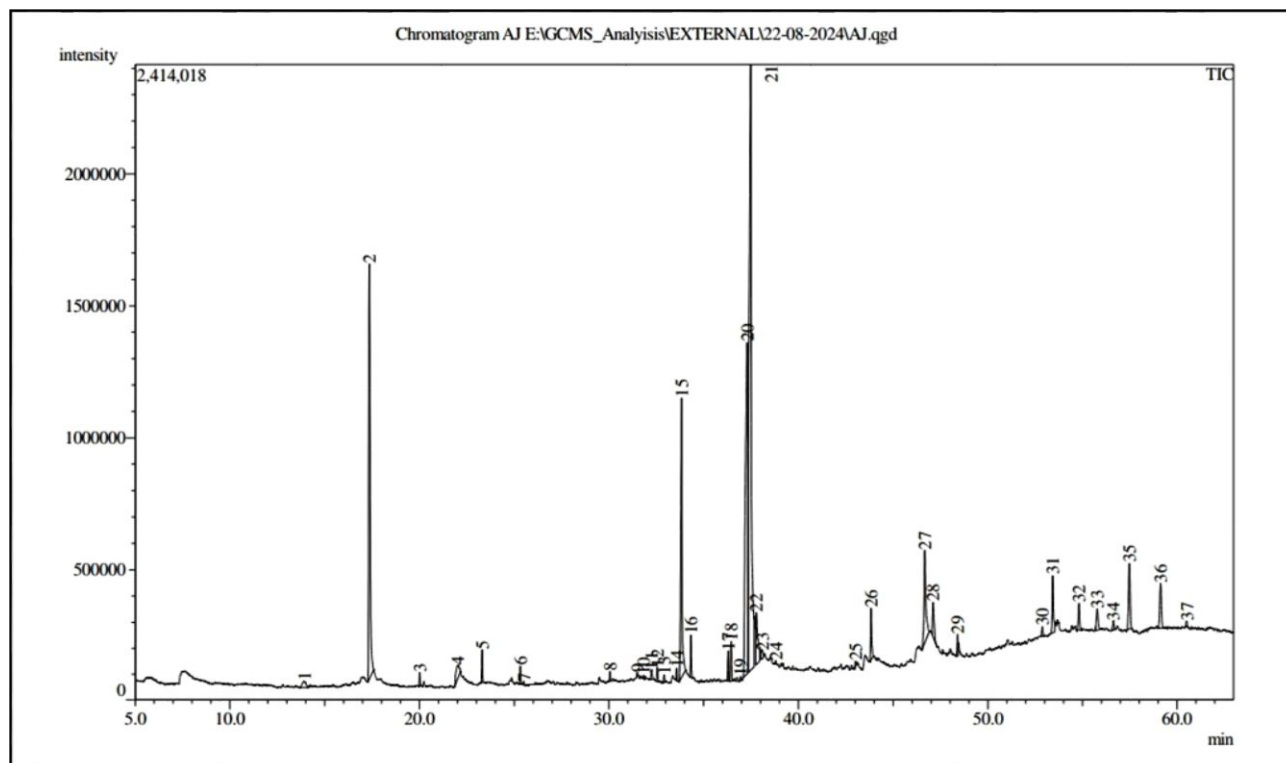


Fig 1: Graph showing the peaks obtained from GC-MS of *T. ammi* ethanol extract.

Table 2: Phytochemical constituents of *T. ammi* ethanol extract obtained from GC-MS.

Retention time	Area%	Compound
13.914	0.57	Azulene
17.355	12.98	Phenol, 2-methyl-5-(1-methylethyl)-
20.011	0.29	1-Tetradecene
22.017	1.09	Guanosine
23.307	0.66	2, 4-Di-tert-butylphenol
25.320	0.40	1-Pentadecene
25.517	0.09	Hexadecane
30.053	0.17	1-Heptadecene
31.500	0.08	Dewar benzene, hexamethyl
31.826	0.05	8-Octadecanone
32.245	0.27	3-Benzylsulfonyl-2, 6, 6-trimethylbicyclo(3.1.1)heptane
32.551	0.31	7, 9-Di-tert-butyl-1-oxaspiro(4, 5)deca-6
32.912	0.16	Hexadecanoic acid, methyl ester
33.577	0.27	Dibutyl phthalate
33.838	9.40	n-Hexadecanoic acid
34.324	0.97	Benzenepropanoic acid, 3, 5-bis(1, 1-dimethylethyl
36.296	0.61	8, 11-Octadecadienoic acid, methyl ester
36.44	0.90	6-Octadecenoic acid, methyl ester
36.933	0.04	Heptacosanoic acid, methyl ester
37.296	19.33	10E, 12Z-Octadecadienoic acid
37.482	32.62	6-Octadecenoic acid
37.781	2.42	Octadecanoic acid
38.117	0.34	Nerolidolisoobutyrate
38.810	0.05	Octanoic acid, cyclobutyl ester
43.011	0.13	1-(3-Isopropenyl-2, 2-dimethylcyclopropyl
43.834	1.37	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester
46.665	4.21	8-Dodecen-1-ol, acetate, (Z)-
47.105	1.30	4(5H)-Indanone, 6-ethyl-6, 7-dihydro-1, 5-dihydroxy-2, 2, 5, 7-tetramethyl
48.403	0.52	Squalene
53.420	1.75	-2, 7, 8-Trimethyl-2-((3E, 7E)-4, 8, 12
54.807	0.83	Stigmastero
55.769	0.89	Chondrillasterol
56.613	0.29	alpha.-Amyrin
57.470	2.48	Lupeol
59.125	1.77	Lup-20(29)-en-3-ol, acetate, (3.beta.)
60.517	0.18	Tris(2, 4-di-tert-butylphenyl) phosphate

GC-MS analysis of the ethanol extract of *T. ammi* revealed the presence of several phytochemical constituents, identified by their retention times and quantified by their relative area percentages. The most abundant compound identified was 6-octadecenoic acid (oleic acid), comprising 32.62% of the extract. The second major component was 10E, 12Z-octadecadienoic acid (linoleic acid), detected at a retention time of 37.296 minutes and constituting 19.33% of the extract. Phenol, 2-methyl-5-(1-methylethyl) (thymol) was also a significant component, accounting for 12.98%, followed by n-hexadecanoic acid (palmitic acid) at 9.4%. An ester compound, 8-dodecen-1-ol acetate (Z), was also identified and contributes to the extract's chemical profile. Among the minor compounds, lupeol was present at 2.48%, squalene at 1.75%, and stigmasterol at 0.83%. These findings highlight the chemical diversity of the ethanol extract of *T. ammi*, with a range of fatty acids, phenolic compounds, esters, and sterols identified.

GC-MS analysis of the ethanol extract of *L. parasiticum* revealed the presence of various chemical constituents, identified based on their retention times and quantified by their relative area percentages. The most abundant compound identified was 1-heptadec-1-ynyl-cyclohexanol, comprising 36.55% of the extract. The second major compound was 7-tetradecenal, detected at a retention time of 37.415 minutes, accounting for 26.87%. N-hexadecanoic acid (palmitic acid) constituted 14% of the total extract. Other identified compounds included octadecanoic acid (stearic acid) and spiro(4.5)decane-1, 6-dione, comprising 2.86% and 2.31% of the extract respectively. 2-isobutoxymethyl oxirane, an epoxide compound, was also detected at 2.51%. Minor components detected in the extract included stigmast-4-en-3-one (1.11%), gamma-sitosterol (0.41%), and squalene (0.89%). The diversity and composition of these compounds indicate a complex phytochemical profile in the ethanol extract of *L. parasiticum*.

Discussion

From the GC-MS data analysis, we identified various compounds in the ethanol extract of *T. ammi*, determined by their retention times and quantified by their relative area percentages. Among the major components, 6-octadecenoic acid (area: 32.62%), commonly known as oleic acid, is present in the highest abundance. This monounsaturated fatty acid is widely found in plant oils and offers several health benefits, including anti-inflammatory properties (Rodriguez *et al.*, 2016) [22]. At a retention time of 37.296 minutes, we identified 10E, 12Z-octadecadienoic acid, or linoleic acid, which accounts for 19.33%. This polyunsaturated fatty acid is known to support cardiovascular health (Ghosh *et al.*, 2014; Wu & Zhou, 2014) [7, 27].

Additionally, we identified phenol, 2-methyl-5-(1-methylethyl), known as thymol, which covers 12.98% of the extract. Thymol has documented anthelmintic effects against various parasites, potentially disrupting their cell membranes and causing paralysis, which ultimately leads to death (Bairwa *et al.*, 2012) [4]. It is also recognized for its antimicrobial and antioxidant properties, contributing to the medicinal and preservative qualities of the extract (Marchese *et al.*, 2016; Bassole and Juliani, 2012) [15, 5].

Another significant compound is n-hexadecanoic acid, or palmitic acid, which constitutes 9.4% of the extract. This saturated fatty acid is commonly found in both plants and animals and is used in the manufacture of soaps and cosmetics, as well as being a precursor for biodiesel (Krittawong *et al.*, 2020) [13]. While less documented, palmitic acid is believed to possess anthelmintic properties, disrupting the cell membranes of parasites and impacting their viability (Kaur and Michael, 2014) [11]. We also identified an ester, 8-dodecen-1-ol acetate (Z), which is widely used for flavors and fragrances and may contribute to the scent profile of the extract (Schreiner and Korn, 2004) [23].

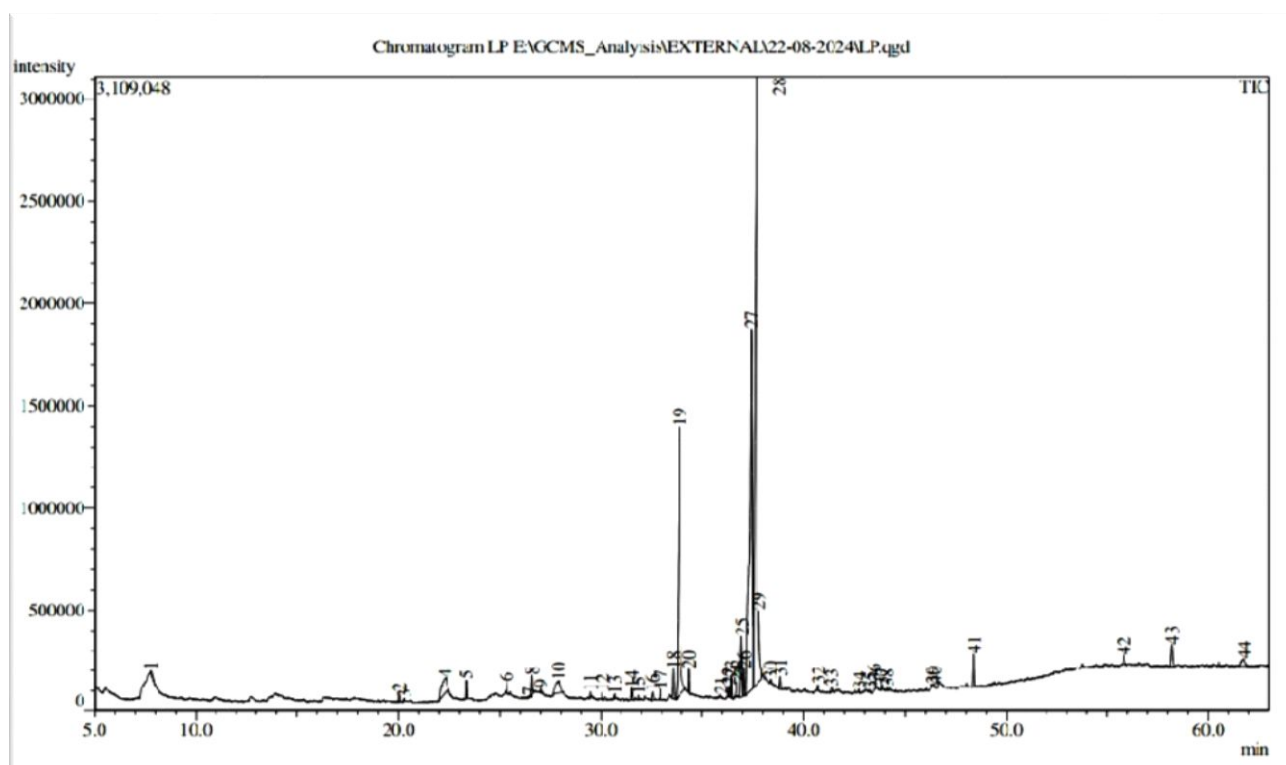


Fig 2: Graph showing the peaks obtained from GC-MS of *L. parasiticum* ethanol extract.

Table 3: Phytochemical constituents of *L. parasiticum* ethanol extract obtained from GC-MS.

Retention time	Area%	Compound
7.776	0.30	Glycerin
20.031	0.26	3-Hexadecene, (Z)
20.253	0.07	Hexadecane
22.310	2.51	2-(Isobutoxymethyl)oxirane
23.326	0.50	2, 4-Di-tert-butylphenol
25.330	0.25	Cetene
26.408	0.06	Pregn-4-ene-3, 20-dione, 17, 21-dihydroxy-, bis (O-methyloxime)
26.592	0.61	2-Pentanone, 4-cyclohexylidene-3, 3-diethyl-
27.010	0.10	2H-Bisoxireno[2, 3:8, 8a]azuleno[4, 5-b]furan-7(3aH)-one, octahydro-3a, 8c-dimethyl-6-methylene
27.879	1.69	3-O-Methyl-d-glucose
29.482	0.15	Tetradecanoic acid
30.053	0.18	1-Nonadecene
30.664	0.12	98 1-Oxaspiro[2.5]octane-2-carboxylic acid, ethyl ester
31.509	0.44	1, 2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester
31.829	0.10	1, 8-dimethyl-8, 9-epoxy-4-isopropyl
32.549	0.33	7, 9-Di-tert-butyl-1-oxaspiro(4, 5) deca-6, 9-diene-2, 8-dione
32.914	0.27	Hexadecanoic acid, methyl ester
33.561	0.94	Dibutyl phthalate
33.868	14.19	n-Hexadecanoic acid
34.327	0.66	Benzenepropanoic acid, 3, 5-bis (1, 1-dimethylethyl
35.900	0.06	Testosterone, 10-demethyl-10-hydroxy
36.295	0.31	9, 12-Octadecadienoic acid, methyl ester
36.424	0.56	8, 11, 14-Docosatrienoic acid, methyl ester
36.675	0.48	Cyclohexane, 1, 1'-ethylidenebis
36.928	2.31	Spiro[4.5]decane-1, 6-dione
37.065	0.79	Coronarlin E
37.415	26.87	7-Tetradecenal
37.673	36.55	1-Heptadec-1-ynyl-cyclohexanol
37.781	3.24	Octadecanoic acid
38.354	0.12	4-Isopropyl-5-methylhexa-2, 4-dien-1-ol
38.851	0.45	(2, 2, 6-Trimethyl-bicyclo[4.1.0]hept-1-yl
40.716	0.16	Spiro[4.5]decan-7-one, 1, 8-dimethyl-8, 9-epoxy-4-isopropyl-
41.362	0.18	1-Heptadec-1-ynyl-cyclohexanol
42.731	0.10	Androstane, (5.alpha.)
43.242	0.02	1-Octadecanol
43.479	0.59	2-Ethylbutyric acid, eicosyl ester
43.837	0.14	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester
44.094	0.08	Bis(2-ethylhexyl) phthalate
46.333	0.09	Trichloroacetic acid, tetradecyl ester
46.442	0.06	1-Tetracosene
48.405	0.89	Squalene
55.810	0.41	gamma.-Sitosterol
58.182	1.11	Stigmast-4-en-3-one
61.773	0.74	Stigmastane-3, 6-dione, (5.alpha.)

Minor components include lupeol (2.48%), squalene (1.75%), and stigmastanol (0.83%). Lupeol, a triterpene, is known for its potential anti-cancer, anti-inflammatory, and antimicrobial properties (Siddique and Saleem, 2011) [25]. Squalene, a precursor in cholesterol synthesis, also exhibits antioxidant properties (Spanova and Daum, 2011; Zarrouk *et al.*, 2012) [26, 28]. Additionally, squalene has shown activity against protozoans and other parasites, possibly disrupting their cell membranes or exerting antioxidant effects, which may contribute to its anthelmintic properties (Hamid and Aiyelaagbe, 2012; Spanova and Daum, 2011) [8, 26]. Stigmastanol, a plant sterol, is associated with anti-inflammatory effects and has been shown to lower cholesterol levels (Cabral and Klein, 2017; Khalid *et al.*, 2018) [6, 12]. It has also demonstrated anthelmintic effects against certain nematodes by interfering with lipid metabolism in parasites (Ramalhete *et al.*, 2010) [20]. From the GC-MS data analysis, we identified various compounds in the ethanol extract of *L. parasiticum*, based

on their retention times and quantified by their relative area percentages.

Among the major compounds identified, 1-heptadec-1-ynyl-cyclohexanol accounts for 36.55% of the extract, making it the most abundant component. Derivatives of cyclohexanol have been screened for anti-cancer and antimicrobial properties, suggesting the potential bioactive effects of this compound (Shin *et al.*, 2020) [24]. At a retention time of 37.415 minutes, we found 7-tetradecenal, which covers 26.87% of the extract. This aliphatic aldehyde contributes to aroma characteristics and is commonly used in fragrances and flavoring agents (Paravisini *et al.*, 2012) [18].

We also identified n-hexadecanoic acid (palmitic acid), which comprises 14% of the extract. This fatty acid is widely used in the manufacturing of cosmetics and soaps, and serves as a precursor for biodiesel (Mancini *et al.*, 2015) [14]. Although less documented, palmitic acid is believed to possess anthelmintic properties, potentially disrupting the

cell membranes of parasites and impacting their viability (Kaur and Michael, 2014)^[11].

Additionally, octadecanoic acid (stearic acid) was detected, commonly used in cosmetics, soaps, and pharmaceuticals (Al-Salem, 2019)^[3]. It is noted for its antiparasitic properties, likely due to its hydrophobic nature, which facilitates the disruption of parasite cell membranes (Ribeiro *et al.*, 2023)^[21]. A spiro compound, spiro(4.5)decane-1, 6-dione, was also found, accounting for 2.31% of the extract; this compound has been screened for therapeutic effects, including antioxidant and anti-inflammatory properties (Pecio *et al.*, 2023)^[19]. An epoxide compound, 2-isobutoxymethyl oxirane, was identified, covering 2.51% of the extract; it is well-known for its reactive nature and is used in polymer synthesis (Herzberger *et al.*, 2016)^[9].

Minor compounds such as stigmast-4-en-3-one (1.11%), gamma-sitosterol (0.41%), and squalene (0.89%) further contribute to the diversity of this extract. Squalene has demonstrated activity against protozoans and other parasites, disrupting cell membranes and exhibiting antioxidant mechanisms, which may indirectly contribute to its anthelmintic effects (Hamid and Aiyelaagbe, 2012; Spanova and Daum, 2011)^[8, 26]. Similarly, gamma-sitosterol and stigmast-4-en-3-one have shown anthelmintic effects in certain nematodes by interfering with lipid metabolism (Ramalhete *et al.*, 2010)^[20]. Beyond its cholesterol-lowering properties (Cabral and Klein, 2017; Khalid *et al.*, 2018)^[6, 12].

These bioactive compounds highlight the therapeutic potential of *L. parasiticum* in industrial applications and its potential anthelmintic effects, providing a foundation for further studies into the functional roles of these compounds.

Conclusion

Phytochemical screening is an important approach for determining the medicinal potential of plant-based drugs. To further understand the capabilities of ethanol extracts of *T. ammi* and *L. parasiticum*, we examined the phytochemical constituents of these extracts using established chemical methods and GC-MS analysis. This comprehensive analysis underscores the diverse phytochemical profile of *T. ammi* and *L. parasiticum* unveiling a rich arsenal of bioactive compounds, positioning botanical extracts as potent, multi-targeted therapeutic agents with substantial promise in combating complex nematode infestations. These findings pave the way for further exploration into its therapeutic applications.

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