



## *Trachyspermum ammi* (L.) Sprague: A Comprehensive Review of its Phytochemistry and Bioactivity

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### Abstract

*Trachyspermum ammi* L. Sprague, commonly known as ajwain, bishop's weed, joan, or carom seed, and referred to as nankha in Arabic-speaking regions. Ajwain is an annual herbaceous plant of notable significance in Ayurvedic medicine. It belongs to the family Apiaceae (Umbelliferae), which comprises approximately 300–455 genera and 3,000–3,750 species and many of which hold historical medicinal value. Like Umbelliferae fruits, ajwain is known for its biologically significant essential oil, having thymol as the major volatile constituent, therefore its fragrant odor is similar to that of thyme. *Trachyspermum ammi* contains a diverse array of bioactive metabolites, including flavonoids, phenolics, alkaloids, terpenoids, steroids, carbohydrates, vitamins, minerals, and essential oils which contribute to the herb's numerous biological activities such as anti-inflammatory, antioxidant, antibacterial, antifungal, insecticidal, carminative, antispasmodic, antihypertensive, antihyperlipidemic, and bronchodilatory effects. Furthermore, *T. ammi* has been traditionally recognized for its effectiveness in managing the stomach and respiratory disorders. This review presents a comprehensive overview of the chemical composition and associated bioactivities of *Trachyspermum ammi* (ajwain).

**Keywords:** *Trachyspermum ammi* L., essential oil, phenolics, biological activities

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## 1. Introduction

In recent years, spices and herbs have gained increasing importance since they not only impart taste, aroma, and color to culinary dishes, but also play a crucial role in preventing and treating a variety of illnesses, including gastrointestinal and respiratory disorders, diabetes, and other chronic disorders. Several spices such as coriander, cumin, turmeric, ginger, fenugreek, ajowan, dill, celery, nigella, cardamom, cinnamon, black pepper, clove, and anise are promising potential sources of a wide range of naturally occurring, biologically active phytochemicals with many beneficial effects including, anti-inflammatory, antioxidant, anticancer,

antidiabetic, antimicrobial, and hypolipidemic effects (Rathore, Saxena *et al.* 2013, Wani, Singh *et al.* 2022). *Trachyspermum ammi* (L.) Sprague is one of the oldest valued spices in the Apiaceae family, one of the world's largest and best-known families, with approximately 300–455 genera and 3,000–3,750 species (Thiviya, Gamage *et al.* 2021). *T. ammi* is an annual herbaceous plant, commonly known as bishop's weed, carom seed, Joan, and ajwain, and its widely grown in arid and semi-arid regions. This spice is a fundamental component in Indian cookery, providing essential flavor and odor. Additionally, it has been traditionally employed for treating many diseases, including gastrointestinal disorders, diarrhea, nausea, vomiting, respiratory problems,

headache, dysmenorrhea, and renal dysfunction (Ranjeetha, Raviteja *et al.* 2024). Although ajwain is native to Egypt, it is also widely distributed in various regions such as Iran, Pakistan, Afghanistan, Ethiopia, India, and Europe (Zarshenas, Moein *et al.* 2014). It is typically cultivated from October to November and harvested from May to June. Both *T. ammi* fruits, often misidentified as seeds, and leaves are the most often consumed parts (Wadikar and Premavalli 2012). Carom seeds contain carbohydrates (38.6%), fibers (11.9%), moisture (8.9%), proteins (15.4%), fats (18.1%), and minerals (7.1%). They also include bioactive compounds such as saponins, tannins, glycosides, flavonoids, terpenoids, coumarins, and vitamins, e.g., thiamine and riboflavin, in addition to their valuable essential oil (2% to 4%) with thymol as the major constituent, followed by  $\gamma$ -terpinene, *p*-cymene, and  $\beta$ -pinene (Nabi, Imanshu *et al.* 2023). Ajwain has been shown to possess many pharmacological activities such as carminative, antispasmodic (Bentley and Trimen 1880), diuretic, antimicrobial (Bonjar 2004), nematocidal, anti-inflammatory (Thangam and Dhananjayan 2003), antioxidant, hepatoprotective, antihypertensive, antihyperlipidemic (Anilakumar, Saritha *et al.* 2009), aflatoxin detoxification (Velazhahan, Vijayanandraj *et al.* 2010), abortifacient, galactagogue and broncho-dilating (Boskabady, Ramazani *et al.* 2003), antiplatelet, anthelmintic activities (Nabi, Imanshu *et al.* 2023). A detailed analysis of the phytochemical composition and major pharmacological activities associated with *Trachyspermum ammi* is presented, consolidating its therapeutic relevance in traditional and modern medicine.

## 2. Phytochemicals derived from *Trachyspermum ammi* essential oil and different extracts.

### 2.1. Carbohydrates

Numerous studies have reported that the *Trachyspermum ammi* contains a significant amount of carbohydrates, present in both free and glycosidic forms (Ishikawa, Sega *et al.* 2001, Chauhan, Kumar *et al.* 2011, Debnath and Sharma 2022). The isolated sugars and their structures were summarized in Table 1.

### 2.2. Fatty acids

Although detailed data on the fatty acid composition of *T. ammi* remains relatively limited, it was found that the petroselinic acid is a distinctive fatty acid for Apiaceae plants (Hajib, El Harkaoui *et al.* 2023). Morshed, Alam *et al.* (2012) reported that the *T. ammi* fatty acid content is made up of petroselinic acid

(89.35%), oleic acid (5.86%), and linoleic acid (4.79%), while Daga, Vaishnav *et al.* (2022) revealed that the *Carum copticum* contained oleic acid as the major fatty acid, followed by linoleic, palmitic,  $\alpha$ -linolenic, stearic, vaccenic, and myristic acids.

### 2.3. Steroids

Although the presence of steroids in *T. ammi* has been documented, a comprehensive profiling of its sterol constituents is still lacking, warranting further investigation.

### 2.4. Volatile organic compounds

The essential oil of *T. ammi* consists of a complex mixture of volatile aromatic compounds, including hydrocarbon terpenes and their oxygenated derivatives such as phenols, alcohols, oxides, and ketones. Previous studies have reported significant variation in both the chemical composition and yield of ajwain essential oil, likely due to a range of factors including geographical origin, extraction techniques (Khajeh, Yamini *et al.* 2004), climate changes, planting time, and developmental stage of the plant (Singh, Luthra *et al.* 1989, Chahal, Dhaliwal *et al.* 2017, Soltani Howyzeh, Sadat Noori *et al.* 2018).

Vitali, Beghelli *et al.* (2016) reported 19 volatile organic compounds in *T. ammi* fruits with thymol as the main compound (67.4%), followed by non-phenolic components, *p*-cymene (17.9%), and  $\gamma$ -terpinene (11.3%). Conversely, Eftekhari, Hoseinsalari *et al.* (2019) reported *p*-cymene as the most abundant compound, rather than thymol. Paliwal, Dwivedi *et al.* (2023) analyzed the essential oil extracted from ajwain seeds at different temperatures; 70 °C, 80 °C and 90 °C, observing that oil yield decreased with increasing temperature, while thymol concentration peaked at 90 °C. In contrast, Choudhury, Ahmed *et al.* (1998) detected no thymol in seeds cultivated in India's Barpeta district, instead identifying carvone as the dominant compound. Balbaa, Hilal *et al.* (1973) reported that *T. ammi* fruits grown in Egypt yielded higher amounts of essential oil (4.40%-8.90%) than the aerial herb (2.40%-2.88%) and both plant parts shared the same chemical components but in different concentrations. Furthermore, Syed, Al-Haq *et al.* (2015) proved that the end of October was the favorable time to plant the seeds as it provided a greater yield of essential oil with the highest contents of thymol (61.85%) and *p*-cymene (11.79%) compared to other months. Additionally, immature green seeds lacked thymol and contained  $\gamma$ -terpinene as the main terpene, followed by  $\alpha$ -phellandrene,  $\delta$ -carene, *p*-mentha-1,3,8 triene, *p*-cumin-7-ol,  $\beta$ -pinene,  $\beta$ -myrcene, and *cis*-myrtenol (Singh, Ali *et al.* 2008). The observed differences in the volatile composition across the

mentioned studies may result from geographical origin, extraction method, planting time, and developmental stages of different plant parts. Table 2 illustrates the diverse terpene composition found in *T. ammi*.

### 2.5. Phenolic compounds

Phenolics represent the most abundant group of secondary metabolites in nature, recognized for their strong antioxidant properties and diverse

pharmacological activities. This class includes thousands of structurally diverse compounds, such as simple phenols, phenolic acids, flavonoids, tannins, lignans, and stilbenes (Kumar, Khan *et al.* 2022). Like other members of the Apiaceae family, *T. ammi* is notable for its valuable phenolic profile, particularly phenolic acids, flavones, and flavonols. Phenolic compounds previously identified in this plant are listed in Table 3, 4, and 5.

**Table 1: Examples of isolated and identified glucides from *Trachyspermum ammi* (fruits/ seeds).**

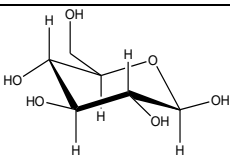
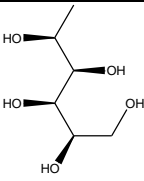
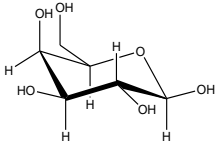
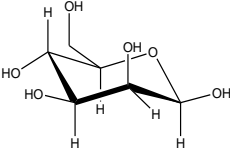
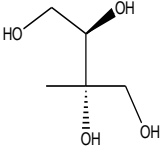
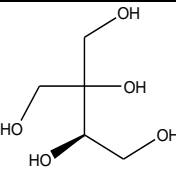
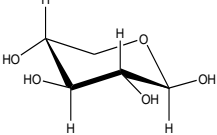
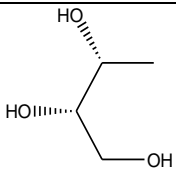
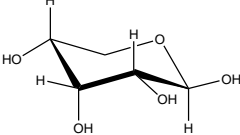
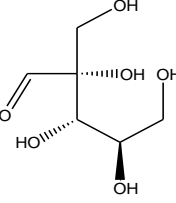
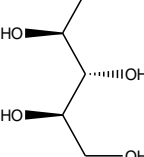
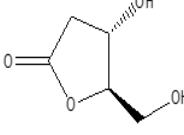
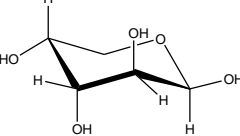
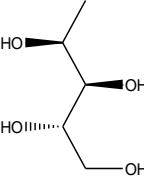
Name	Structure	Organ	Extract	Technique	Reference
D-Glucose		Seeds	Aqueous	Sephadex G-25 column (GPC) and GC-MS	(Basu, Ghosh <i>et al.</i> 2017)
1-Deoxy-D-glucitol		Fruits	Aqueous fraction of methanolic extract	Column chromatography and NMR	(Ishikawa, Sega <i>et al.</i> 2001)
D-Galactose		Seeds	Aqueous	Sephadex G-25 column (GPC) and GC-MS	(Basu, Ghosh <i>et al.</i> 2017)
D-Maltose		Seeds	Aqueous	Sephadex G-25 column (GPC) and GC-MS	(Basu, Ghosh <i>et al.</i> 2017)
(2S,3R)-2-methylbutane-1,2,3,4-tetrol		Fruits	Aqueous fraction of methanolic extract	Column chromatography and NMR	(Ishikawa, Sega <i>et al.</i> 2001)
(3R)-2-hydroxymethyl butane-1,2,3,4-tetrol		Fruits	Aqueous fraction of methanolic extract	Column chromatography and NMR	(Ishikawa, Sega <i>et al.</i> 2001)
D-xylose		Seeds	Aqueous	Sephadex G-25 column (GPC) and GC-MS	(Basu, Ghosh <i>et al.</i> 2017)

Table 1: Cont.

Name	Structure	Organ	Extract	Technique	Reference
1-Deoxy-L-erythritol		Fruits	Aqueous fraction of methanolic extract	Column chromatography and NMR	(Ishikawa, Sega <i>et al.</i> 2001)
D-Ribose		Seeds	Aqueous	Sephadex G-25 column (GPC) and GC-MS	(Basu, Ghosh <i>et al.</i> 2017)
D-Hamamelose = 2-C-Hydroxymethyl-D-ribose		Fruits	Aqueous fraction of methanolic extract	Column chromatography and NMR	(Ishikawa, Sega <i>et al.</i> 2001)
1-Deoxy-D-ribitol		Fruits	Aqueous fraction of methanolic extract	Column chromatography and NMR	(Ishikawa, Sega <i>et al.</i> 2001)
2-deoxy-D-ribino-1,4-lactone		Fruits	Aqueous fraction of methanolic extract	Column chromatography and NMR	(Ishikawa, Sega <i>et al.</i> 2001)
D-Arabinose		Seeds	Aqueous	Sephadex G-25 column (GPC) and GC-MS	(Basu, Ghosh <i>et al.</i> 2017)
1-deoxy-L-arabinitol		Fruits	Aqueous fraction of methanolic extract	Column chromatography and NMR	(Ishikawa, Sega <i>et al.</i> 2001)

**Table 2: Terpenoids (monoterpenes, phenolic terpenes and their glycosides) in different organs of *Trachyspermum ammi* L.**

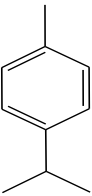
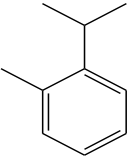
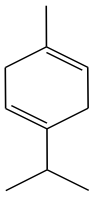
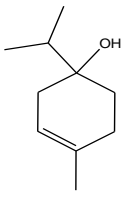
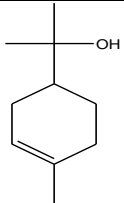
Name	Structure	Organ	Extract/ method	Technique	Reference
<i>p</i> -Cymene		Herb, fruits, and seeds	Essential oil Hydrodistillation	GC-MS	(Balbaa, Hilal <i>et al.</i> 1973). (Eftekhari, Hoseinsalari <i>et al.</i> 2019). (Syed, Al-Haq <i>et al.</i> 2015).
<i>o</i> -Cymene		Seeds	Essential oil Hydrodistillation	GC-MS	(Paliwal, Dwivedi <i>et al.</i> 2023).
$\gamma$ -Terpinene		Herb, fruits, and seeds	Essential oil Hydrodistillation	GC-MS	(Balbaa, Hilal <i>et al.</i> 1973). (Vitali, Beghelli <i>et al.</i> 2016). (Syed, Al-Haq <i>et al.</i> 2015).
Terpinen-4-ol		Fruits and seeds	Essential oil Hydrodistillation	GC-MS	(Vitali, Beghelli <i>et al.</i> 2016). (Paliwal, Dwivedi <i>et al.</i> 2023).
$\alpha$ -Terpineol		Fruits and seeds	Essential oil Hydrodistillation	GC-MS	(Vitali, Beghelli <i>et al.</i> 2016). (Paliwal, Dwivedi <i>et al.</i> 2023).

Table 2: Cont.

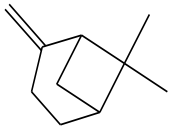
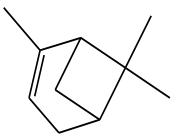
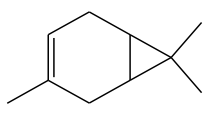
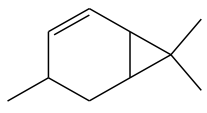
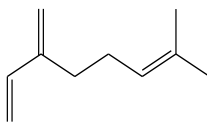
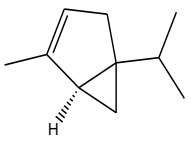
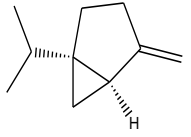
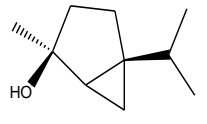
Name	Structure	Organ	Extract/ method	Technique	Reference
$\beta$ -Pinene		Herb, fruits, and seeds	Essential oil Hydrodistillation	GC-MS	(Balbaa, Hilal <i>et al.</i> 1973). (Syed, Al-Haq <i>et al.</i> 2015). (Eftekhari, Hoseinsalari <i>et al.</i> 2019).
$\alpha$ -Pinene		Herb, fruits, and seeds	Essential oil Hydrodistillation	GC-MS	(Balbaa, Hilal <i>et al.</i> 1973). (Vitali, Beghelli <i>et al.</i> 2016). (Paliwal, Dwivedi <i>et al.</i> 2023).
3-Carene		Herb, fruits and seeds	Essential oil Hydrodistillation	GC-MS	(Balbaa, Hilal <i>et al.</i> 1973). (Vitali, Beghelli <i>et al.</i> 2016). (Paliwal, Dwivedi <i>et al.</i> 2023).
4-Carene		Seeds	Essential oil Hydrodistillation	GC-MS	(Paliwal, Dwivedi <i>et al.</i> 2023).
Myrcene		Herb, fruits, and seeds	Essential oil Hydrodistillation	GC-MS	(Balbaa, Hilal <i>et al.</i> 1973). (Vitali, Beghelli <i>et al.</i> 2016). (Choudhury, Ahmed <i>et al.</i> 1998).
$\alpha$ -Thujene		fruits and seeds	Essential oil Hydrodistillation	GC-MS	(Vitali, Beghelli <i>et al.</i> 2016). (Choudhury, Ahmed <i>et al.</i> 1998).
Sabinene		Fruits and seeds	Essential oil Hydrodistillation	GC-MS	(Vitali, Beghelli <i>et al.</i> 2016). (Khan, Jamila <i>et al.</i> 2020).
Trans-Sabinene hydrate		Fruits and seeds	Essential oil Hydro-distillation	GC-MS	(Vitali, Beghelli <i>et al.</i> 2016). (Khan, Jamila <i>et al.</i> 2020).

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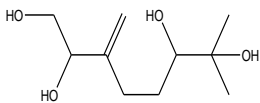
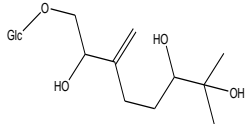
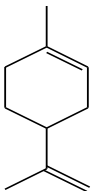
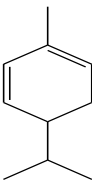
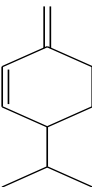
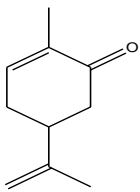
Name	Structure	Organ	Extract	Technique	Reference
3,7-dimethyloct-3(10)-ene-1,2,6,7-tetrol		Fruits	Aqueous fraction of methanolic extract	Column chromatography and NMR	(Ishikawa, Sega <i>et al.</i> 2001).
(2S,6z)-3,7-dimethyloct 3(10)-ene-1,2,6,7-tetrol 1-O-β-D-glucopyranoside		Fruits	Aqueous fraction of methanolic extract	Column chromatography and NMR	(Ishikawa, Sega <i>et al.</i> 2001).
Limonene		Herb, fruits, and seeds	Essential oil Hydro-distillation	GC-MS	(Balbaa, Hilal <i>et al.</i> 1973). (Vitali, Beghelli <i>et al.</i> 2016). (Choudhury, Ahmed <i>et al.</i> 1998).
α- Phellandrene		Fruits and seeds	Essential oil Hydro-distillation	GC-MS	(Vitali, Beghelli <i>et al.</i> 2016). (Choudhury, Ahmed <i>et al.</i> 1998).
β-phellandrene		Fruits	Essential oil Hydro-distillation	GC-MS	(Vitali, Beghelli <i>et al.</i> 2016).
Carvone		Seeds	Essential oil Hydro-distillation	GC-MS	(Choudhury, Ahmed <i>et al.</i> 1998).

Table 2: Cont.

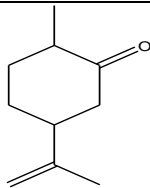
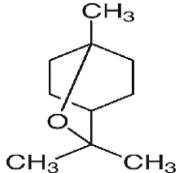
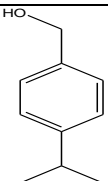
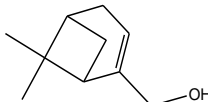
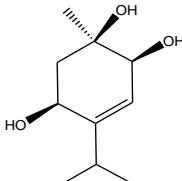
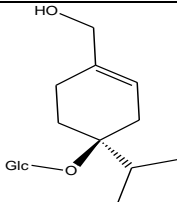
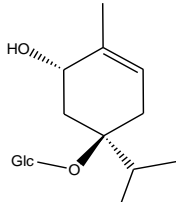
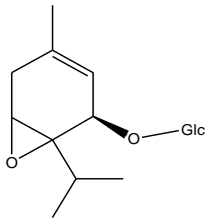
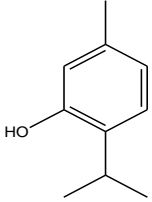
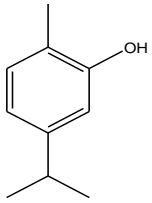
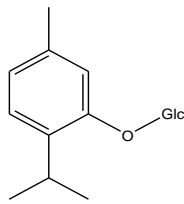
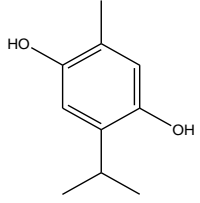
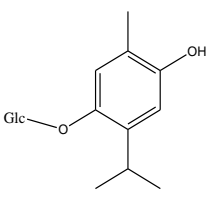
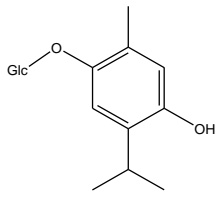
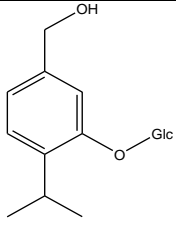
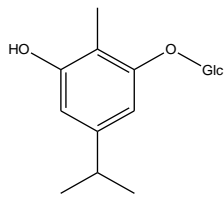
Name	Structure	Organ	Extract	Technique	Reference
Dihydrocarvone		Seeds	Essential oil Hydro-distillation	GC-MS	(Choudhury, Ahmed <i>et al.</i> 1998).
1,8-Cineole		Fruits and seeds	Essential oil Hydro-distillation	GC-MS	(Vitali, Beghelli <i>et al.</i> 2016). (Khan, Jamila <i>et al.</i> 2020).
<i>p</i> -cumin-7-ol		Fruits	Essential oil Hydro-distillation	GC-MS	Singh, Ali <i>et al.</i> (2008).
Cis-Myrtenol		Fruits	Essential oil Hydro-distillation	GC-MS	Singh, Ali <i>et al.</i> (2008).
<i>p</i> -Menth-3-ene-1 $\beta$ ,2 $\beta$ ,5 $\beta$ -triol.		Fruits	Aqueous fraction of methanolic extract	Column chromatography and NMR	(Ishikawa, Sega <i>et al.</i> 2001).
(4S)- <i>p</i> -menth-1-ene-4,7-diol 4- <i>O</i> - $\beta$ -D-glucopyranoside		Fruits	Aqueous fraction of methanolic extract	Column chromatography and NMR	(Ishikawa, Sega <i>et al.</i> 2001).
(4R,6S)- <i>p</i> -menth-1-ene-4,6-diol 4- <i>O</i> - $\beta$ -D-glucopyranoside		Fruits	Aqueous fraction of methanolic extract	Column chromatography and NMR	(Ishikawa, Sega <i>et al.</i> 2001).

Table 2: Cont.

Name	Structure	Organ	Extract	Technique	Reference
3 $\beta$ -hydroxy- <i>p</i> -menth-1-en-4 $\beta$ ,5 $\beta$ -oxide 3- <i>O</i> - $\beta$ -D- glucopyranoside		Fruits	Aqueous fraction of methanolic extract	Column chromatography and NMR	(Ishikawa, Sega <i>et al.</i> 2001).
Thymol		Herb and fruits  Seeds	Essential oil (Hydro-distillation). Methanolic extract	GC-MS  UPLC-MS	(Balbaa, Hilal <i>et al.</i> 1973). (Vitali, Beghelli <i>et al.</i> 2016). (Dutta, Kundu <i>et al.</i> 2021).
Carvacrol		Herb, fruits and Seeds	Essential oil Hydro-distillation	GC-MS	(Balbaa, Hilal <i>et al.</i> 1973). (Vitali, Beghelli <i>et al.</i> 2016). (Paliwal, Dwivedi <i>et al.</i> 2023).
Thymol - $\beta$ -D-glucoside		Seeds	Methanolic extract	UPLC-MS analysis	(Dutta, Kundu <i>et al.</i> 2021).
Thymoquinol		Seeds	Methanolic extract	UPLC-MS analysis	(Dutta, Kundu <i>et al.</i> 2021).
Thymoquinol-5- <i>O</i> - $\beta$ -glucopyranoside		Fruits  Seeds	Methanol  Acetone, and EtOAc extracts	Column chromatography and NMR. FT-IR and NMR.	(Ishikawa, Sega <i>et al.</i> 2001).  (Khan, Jamila <i>et al.</i> 2020).

**Table 2: Cont.**

Name	Structure	Organ	Extract	Technique	Reference
Thymoquinol-2- <i>O</i> - $\beta$ -D-glucopyranoside		Fruits	Aqueous fraction of methanolic extract	Column chromatography and NMR	(Ishikawa, Sega <i>et al.</i> 2001).
7-Hydroxythymol 3- <i>O</i> - $\beta$ -D-glucopyranoside		Fruits	Aqueous fraction of methanolic extract	Column chromatography and NMR	(Ishikawa, Sega <i>et al.</i> 2001).
6-Hydroxycarvacrol 2- <i>O</i> - $\beta$ -D-glucopyranoside		Seeds	Ethanolic extract	Si gel Column chromatography and UV, IR, NMR	(Garg, Sharma <i>et al.</i> 1980, Chauhan, Kumar <i>et al.</i> 2011).

**Table 3: Examples of identified phenolic acids from *Trachyspermum ammi* L.**

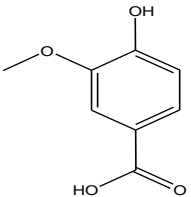
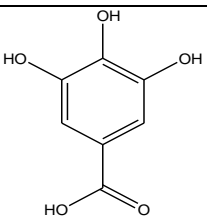
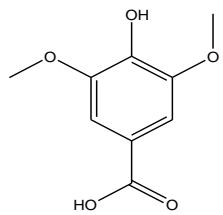
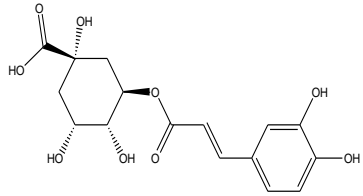
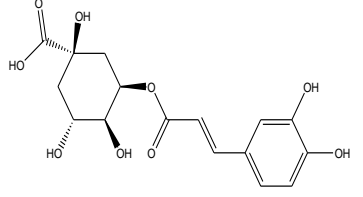
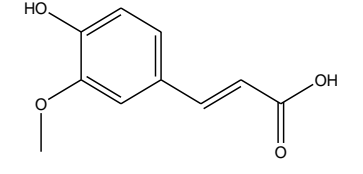
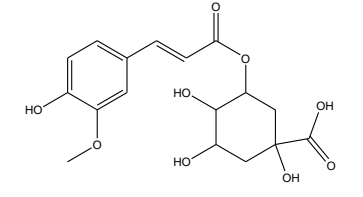
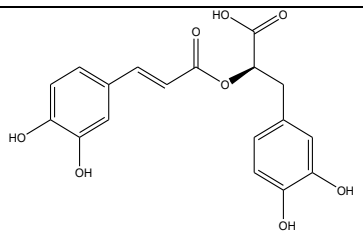
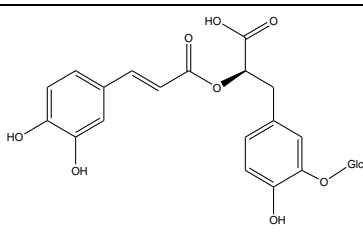
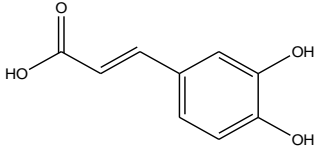
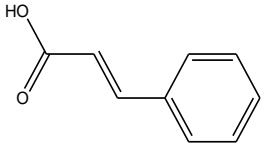
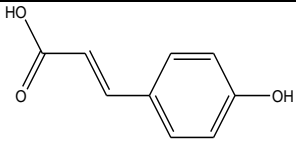
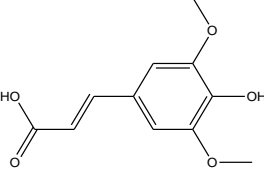
Name	Structure	Organ	Extract	Technique	References
Vanillic acid		Seeds	Methanol Hexane extract of fixed oil.	UHPLC HPLC	(Mueed, Shibli <i>et al.</i> 2023). (Daga, Vaishnav <i>et al.</i> 2022).
Gallic acid		Seeds	Methanol extract	UHPLC	(Mueed, Shibli <i>et al.</i> 2023).
Syringic acid		Seeds	Hexane extract of fixed oil. Methanolic extract.	HPLC UPLC-MS	(Daga, Vaishnav <i>et al.</i> 2022). (Dutta, Kundu <i>et al.</i> 2021).

Table 3: Cont.

Name	Structure	Organ	Extract	Technique	References
Chlorogenic acid		Seeds	Methanolic extract	UHPLC	(Mueed, Shibli <i>et al.</i> 2023).
Neo-chlorogenic acid		Seeds	Methanolic extract	UHPLC	(Mueed, Shibli <i>et al.</i> 2023).
Ferulic acid		Seeds	Methanolic extract	HPLC	(Mirniyam, Rahimmalek <i>et al.</i> 2022).
Feruloyl-quinic acid		Seeds	Methanolic Extract	UPLC-MS	(Dutta, Kundu <i>et al.</i> 2021).
Rosmarinic acid		Seeds	Methanolic extract	HPLC	(Mirniyam, Rahimmalek <i>et al.</i> 2022).
Rosmarinyl-3-O-glucoside		Seeds	Methanolic extract	UPLC-MS	(Dutta, Kundu <i>et al.</i> 2021).

**Table 3: Cont.**

Caffeic acid		Seeds	Methanolic extract	HPLC	(Mirniyam, Rahimmalek <i>et al.</i> 2022).
Trans-Cinnamic acid		Seeds	Hexane extract of fixed oil	HPLC	(Daga, Vaishnav <i>et al.</i> 2022).
<i>p</i> -Coumaric acid		Seeds	Methanolic extract	UHPLC	(Mueed, Shibli <i>et al.</i> 2023).
Sinapic acid		Seeds	Methanolic extract	UPLC-MS	(Dutta, Kundu <i>et al.</i> 2021).

**Table 4: Identified flavonoids from *Trachyspermum ammi* L.**

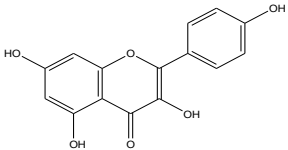
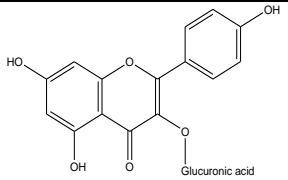
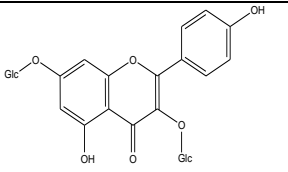
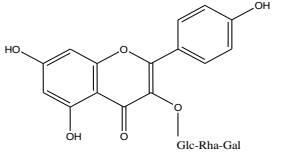
Name	Structure	Organ	Extract	Technique	References
Kaempferol		Seeds	Methanol  Hexane extract of fixed oil.	UHPLC  HPLC	(Mueed, Shibli <i>et al.</i> 2023).  (Daga, Vaishnav <i>et al.</i> 2022).
Kaempferol-3- <i>O</i> -glucuronide		Seeds	Methanolic extract	UPLC-MS	(Dutta, Kundu <i>et al.</i> 2021).
Kempferol-3,7- <i>O</i> -diglucoside		Seeds	Methanolic extract	UPLC-MS	(Dutta, Kundu <i>et al.</i> 2021).
Kaempferol-3- <i>O</i> -glucosyl-rhamnosyl-galactoside		Seeds	Methanolic extract	UPLC-MS	(Dutta, Kundu <i>et al.</i> 2021).

Table 4: Cont.

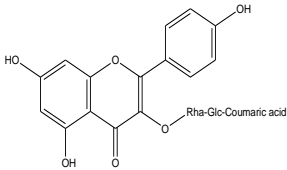
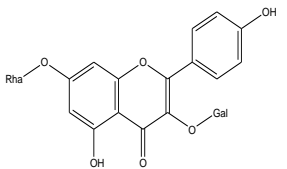
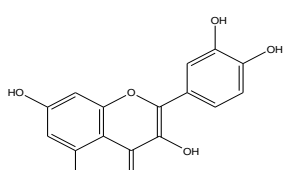
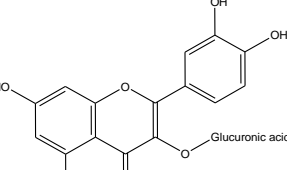
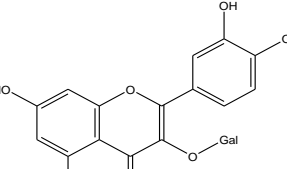
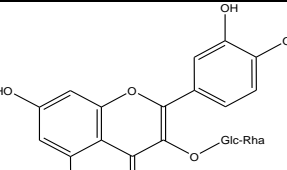
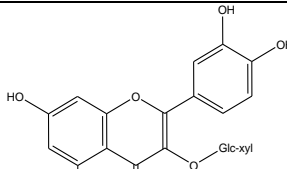
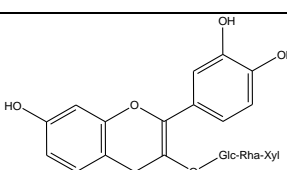
Kaempferol-3-(6-coumaroyl glucosyl)-rhamnoside		Seeds	Methanolic extract	UPLC-MS	(Dutta, Kundu <i>et al.</i> 2021).
Kaempferol-3-O-galactoside-7-O-rhamnoside		Seeds	Methanolic extract	UPLC-MS analysis	(Dutta, Kundu <i>et al.</i> 2021).
Quercetin		Seeds	Methanol  Hexane extract of fixed oil.	UHPLC  HPLC	(Mueed, Shibli <i>et al.</i> 2023).  (Daga, Vaishnav <i>et al.</i> 2022).
Quercetin-3-O-glucuronide		Seeds	Methanolic extract	UPLC-MS	(Dutta, Kundu <i>et al.</i> 2021).
Quercetin-3-O-galactoside		Seeds	Methanolic extract	UPLC-MS	(Dutta, Kundu <i>et al.</i> 2021).
Rutin		Seeds	Methanolic extract	HPLC	(Mirniyam, Rahimmalek <i>et al.</i> 2022).
Quercetin 3-O-glucosyl-xyloside		Seeds	Methanolic extract	UPLC-MS	(Dutta, Kundu <i>et al.</i> 2021).
Quercetin-3-O-xylosyl-rutinoside		Seeds	Methanolic extract	UPLC-MS	(Dutta, Kundu <i>et al.</i> 2021).

Table 4: Cont.

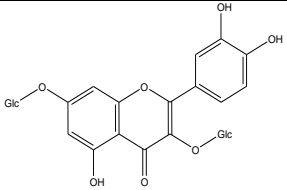
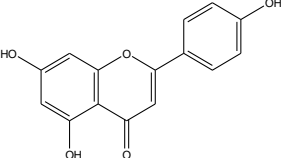
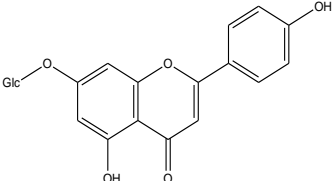
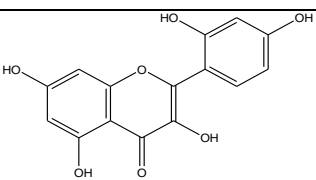
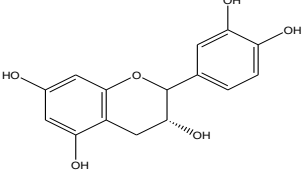
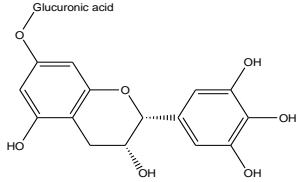
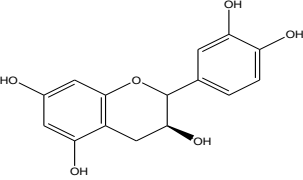
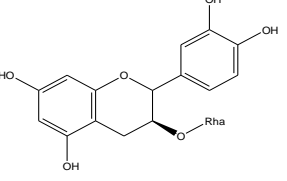
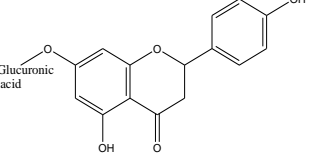
Quercetin-3,7- <i>O</i> -diglucoside		Seeds	Methanolic extract	UHPLC analysis	(Mueed, Shibli <i>et al.</i> 2023).
Apigenin		Seeds	Methanolic extract	HPLC	(Mirniyam, Rahimmalek <i>et al.</i> 2022).
Apigenin-7- <i>O</i> -glucoside		Seeds	Methanolic extract	UPLC-MS	(Dutta, Kundu <i>et al.</i> 2021).
Morin		Seeds	Methanolic Extract	UPLC-MS	(Dutta, Kundu <i>et al.</i> 2021).
Epicatechin		Seeds	Hexane extract of fixed oil	HPLC	(Daga, Vaishnav <i>et al.</i> 2022).
Epigallocatechin-7- <i>O</i> -glucuronide		Seeds	Methanolic extract	UPLC-MS	(Dutta, Kundu <i>et al.</i> 2021).
Catechin		Seeds	Methanolic extract	UHPLC	(Mueed, Shibli <i>et al.</i> 2023).
Catechin-3- <i>O</i> -rhamnoside		Seeds	Methanolic Extract	UPLC-MS	(Dutta, Kundu <i>et al.</i> 2021).
Naringenin-7- <i>O</i> -glucuronide		Seeds	Methanolic Extract	UPLC-MS	(Dutta, Kundu <i>et al.</i> 2021).

Table 4: Cont.

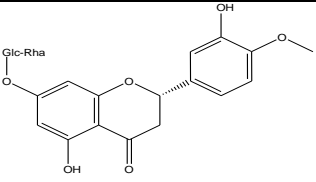
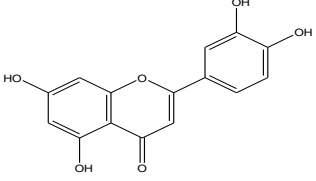
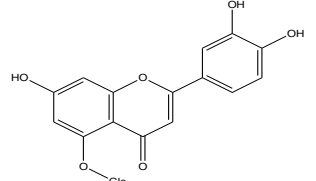
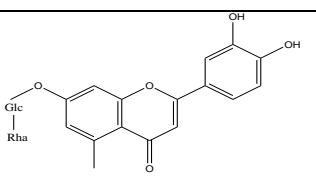
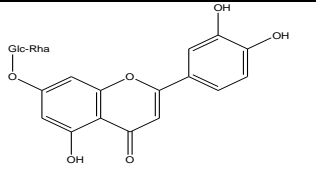
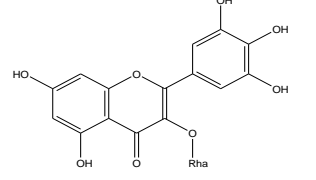
Hesperidin		Seeds	Methanolic Extract	UHPLC	(Mueed, Shibli <i>et al.</i> 2023).
Luteolin		Seeds	Methanolic extract	UPLC-MS	(Dutta, Kundu <i>et al.</i> 2021).
Luteolin-5-O-glucoside		Seeds	Methanolic extract	UHPLC	(Mueed, Shibli <i>et al.</i> 2023).
Luteolin-7-O-neohesperidoside		Seeds	Methanolic extract	UHPLC	(Mueed, Shibli <i>et al.</i> 2023).
Luteolin-7-O-rutinoside		Seeds	Methanolic extract	UPLC-MS	(Dutta, Kundu <i>et al.</i> 2021).
Myricetin-3-O-rhamnoside		Seeds	Methanolic extract	UPLC-MS analysis	(Dutta, Kundu <i>et al.</i> 2021).

Table 5: Other isolated phenolic, aromatic and alkyl glycosides.

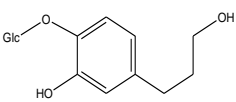
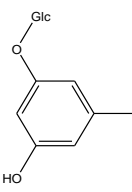
Name	Structure	Organ	Extract	Technique	Reference
3,4 Dihydroxyphenyl propanol-3-O- $\beta$ -D-glucopyranoside		Fruits	Aqueous fraction of methanolic extract	Column chromatography and NMR	(Ishikawa, Segal <i>et al.</i> 2001).
3,5-Dihydroxytoluene 3-O- $\beta$ -D-galactopyranoside		Seeds	Ethanol extract	Si gel column chromatography and UV, IR, NMR	(Garg, Sharma <i>et al.</i> 1980, Chauhan, Kumar <i>et al.</i> 2011).

Table 5: Cont.

Name	Structure	Organ	Extract	Technique	Reference
1'-(3-hydroxy-4,5-dimethoxy-phenyl) propane-2'-3'-diol		Fruits	Aqueous fraction of methanolic extract	Column chromatography and NMR	(Ishikawa, Sega <i>et al.</i> 2001).
1'-(3-hydroxy-4,5-dimethoxyphenyl)-propane-2',3'-diol 3'-O-β-D-glucopyranoside		Fruits	Aqueous fraction of methanolic extract	Column chromatography and NMR	(Ishikawa, Sega <i>et al.</i> 2001).
Piceatannol		Seeds	Methanolic extract	UPLC-MS	(Dutta, Kundu <i>et al.</i> 2021).
Dillapiole		Seeds	Essential oil Hydro-distillation	GC-MS	(Choudhury, Ahmed <i>et al.</i> 1998, Khan, Jamila <i>et al.</i> 2020).
Myristicin		Seeds	Essential oil Hydro-distillation	GC-MS	(Choudhury, Ahmed <i>et al.</i> 1998, Khan, Jamila <i>et al.</i> 2020).
(4aS, 5R, 8aS) 5, 8a-di 1-propyl-octahydronaphthalen-1-(2H)-one		Seeds	Petroleum ether extract	Si gel column chromatography and FTIR, MS NMR	(Khan, Zakir <i>et al.</i> 2010).
benzyl β-D-glucopyranoside		Fruits	Aqueous fraction of methanolic extract	Column chromatography and NMR	(Ishikawa, Sega <i>et al.</i> 2001).
2-methyl-3-buten 2-ol-β-D-glucopyranoside		Fruits	Aqueous fraction of methanolic extract	Column chromatography and NMR	(Ishikawa, Sega <i>et al.</i> 2001).

### 3. Biological activities

*Carum copticum* (ajwain) has been traditionally used for treating a wide range of ailments, including respiratory disorders, cough, cold, headache, menstrual pain, GI disturbances, neurological conditions, cholera, helminths, and kidney stones (Zarshenas, Moein *et al.* 2014, Goyal, Chaturvedi *et al.* 2022). Previous studies have shown that the *T. ammi* enhances digestion by stimulating digestive enzyme secretions, increasing the bile flow rate and biliary excretions, and by reducing the food transit time. Additionally, the antiulcer

activity of ajwain is mainly attributable to its antioxidant, anti-inflammatory activities and its inhibitory effect on gastric acid secretions. Furthermore, *T. ammi* is known for a variety of other pharmacological effects, including carminative, antispasmodic (Bentley and Trimen 1880), antimicrobial (Bonjar 2004), nematocidal, anti-inflammatory (Thangam and Dhananjayan 2003), hepatoprotective, antihypertensive, antihyperlipidemic, and broncho-dilating effects (Boskabady, Jandaghi *et al.* 2005). The most prominent pharmacological activities are summarized in Table 6.

**Table 6: The most often documented biological activities from *Trachyspermum ammi*.**

Organ / extract	Assay method	Results	References
<b>I. Digestive stimulant activity</b>			
Herb Suspension in 0.9% saline	In vitro assay using digestive enzymes of isolated pancreas and small intestine from rats.	Ajwain at 0.1 and 1 mg/ml concentrations exhibited significant increase of digestive enzymes.	(Ramakrishna Rao, Platel <i>et al.</i> 2003)
Herb	Calculating the food transit time in experimental rats.	<i>Trachyspermum ammi</i> (0.2 g%) accelerated the food transit time, so improved digestion.	Platel and Srinivasan (2001)
Herb	Assessing the effect of ajwain on bile flow rate (by measuring the bile volume collected for 2.5 hours) and bile solid content in experimental rats.	<i>T. ammi</i> caused a remarkable increase in bile secretions and the bile flow rate which aid in food digestion.	Platel and Srinivasan (2000)
<b>II. Gastroprotective activity</b>			
Fruits Essential oil	Studying the effect of essential oil and its major volatile compounds on treating the peptic ulcer induced by ethanol in experimental rats.	Essential oil inhibited the peptic ulcer and diminished the lesions in a dose-dependent manner. Thymol exhibited potent inhibitory effect compared to <i>p</i> -cymene and $\gamma$ -terpinene.	(Eftekhari, Hoseinsalari <i>et al.</i> 2019)

Table 6: Cont.

Organ/ Extract	Assay method	Results	References
Fruits Aqueous extract	Examined the antisecretory and anti-peptic ulcer activity in albino rats with ulcerations or gastric lesions induced by various reagents	Ajwain at both doses (250 and 500 mg/Kg) exhibited notable reduction in gastric secretions, and ulcer indices. Furthermore, higher doses exhibited protective effect against gastric mucosal damage and increased the mucosal content.	(Alqasoumi 2011)
<b>III. <u>Antioxidant activity</u></b>			
Fruits Essential oil	ABTS and Linoleic acid/ $\beta$ -carotene bleaching assays. Determining the main volatile components with potent activity via TLC-bioautography method.	Essential oil showed considerable inhibition of lipid peroxidation and potent antioxidant activity. Thymol was assessed as the most powerful antioxidant compound.	(Nickavar, Adeli <i>et al.</i> 2014)
Seeds Methanolic extract	Superoxide radical, Hydroxyl radical, and Nitric oxide scavenging activity assays.	Ajwain seeds exhibited significant antioxidant potential in all performed assays.	(Mueed, Shibli <i>et al.</i> 2023)
Seeds Essential oil	DPPH, FRAP, and $H_2O_2$ scavenging assay methods.	The results of all performed assays established the potent antioxidant activity of ajwain.	Chatterjee, Goswami <i>et al.</i> (2013)
Seeds <u>Hydrodistilled</u> Essential oil at 70 °C, 80 °C, 90 °C	$H_2O_2$ radical scavenging assay method	Essential oil at 90 °C showed the highest $H_2O_2$ scavenging activity compared to essential oil at 70 °C and 80 °C which was mainly attributed to the greatest content of thymol at 90 °C <u>hydrodistilled</u> oil.	(Paliwal, Dwivedi <i>et al.</i> 2023)

Table 6: Cont.

Organ/ Extract	Assay method	Results	References
Seeds <i>n</i> -hexane, chloroform, ethyl acetate, acetone, methanol, and aqueous extracts	Determining the total phenolics and flavonoid contents. and DPPH, ABTS, and FRAP assay methods.	<u>EtOAc</u> , acetone, <u>MeOH</u> and aqueous extracts exhibited potent antioxidant activity with the greatest radical inhibitory effect in <u>EtOAc</u> extract which was correlated to highest phenolic and flavonoid contents.	(Khan, Jamila <i>et al.</i> 2020)
Leaves (fresh and frozen) Methanolic extract	DPPH assay	The results proved that frozen leaves have a higher antioxidant activity than fresh leaves due to their higher carotenoids and phenolic contents.	(Raza, Shukla <i>et al.</i> 2015)
Seeds Ethanol extract	In vivo antioxidant activity assay using (HCH) hexachlorocyclohexane-induced lipid peroxidation and oxidative stress in Wistar rats.	Ajwain extract enhanced the activity of liver antioxidant enzymes and increased the glutathione level which aids in scavenging oxygen free radicals and preventing lipid peroxidation.	(Anilakumar, Saritha <i>et al.</i> 2009)
<b>IV. <u>Hepatoprotective activity</u></b>			
seeds 70% methanolic extract	Monitoring the mortality rate in rats after administrating a fatal dose of paracetamol and Assaying the hepatoprotective effect in rats with hepatic injury through measuring liver enzymes spectrophotometrically.	Treating rats with <i>T. ammi</i> extract (500 mg/kg) caused 80% protection against the fatal dose of paracetamol. Also, it caused significant reduction in plasma levels of ALT, AST, and ALP enzymes which increased in both paracetamol and CCl <sub>4</sub> -induced hepatotoxicity.	(Gilani, Jabeen <i>et al.</i> 2005)

Table 6: Cont.

Organ/ Extract	Assay method	Results	References
<b>V. Anti-inflammatory activity</b>			
Seeds Aqueous and alcoholic extracts	Using experimental rats with carrageenan induced rat paw oedema and cotton pellet induced granuloma.	Both extracts (100mg/kg) exhibited significant anti-inflammatory activity in both rat models.	(Thangam and Dhananjayan ۲۰۰۲)
Seeds Methanol, chloroform, and hexane extracts	Measuring the inhibitory percentage of paw edema in Wistar rats with carrageenan induced edema.	<i>T. ammi</i> seeds have remarkable anti-inflammatory activity and their hexane extract showed more efficacy than other extracts.	Mohammad, Siddiqui <i>et al.</i> (2020)
<b>VI. Immunomodulatory activity</b>			
Seeds Different extracts in hexane, chloroform, and methanol solvents	<u>delayed-type</u> hypersensitivity assay method by measuring skin thickness in experimental rats after topical administration of dinitrochlorobenzene.	<i>T. ammi</i> extracts showed considerable stimulation of immunity and the highest activity was observed in methanolic extract.	(Siddiqui, Aslam <i>et al.</i> 2019)
<b>VII. Antimicrobial Activity</b>			
Fruits Essential oil	Both microdilution and paper disc diffusion methods were performed against <i>Staph. Aureus</i> , <i>Enterococcus faecalis</i> , <i>E. coli</i> , <i>C. albicans</i> , and <i>Pseudomonas aeruginosa</i> .	Essential oil showed substantial inhibition of gram-positive bacteria particularly against <i>Staph. aureus</i> and good activity against <i>E. coli</i> and <i>C. albicans</i> , but it exhibited insignificant inhibition of <i>Pseudomonas aeruginosa</i> .	(Vitali, Beghelli <i>et al.</i> 2016)

Table 6: Cont.

Organ/ Extract	Assay method	Results	References
Herb Aqueous and ethanolic extracts	Measuring the diameter of inhibition zones and calculating the MIC and MBC/MFC values against <i>S. aureus</i> , <i>E. coli</i> , <i>Entérobacter</i> , <i>K. Pneumonia</i> , <i>S. typhimurium</i> , <i>C. albicans</i> , and <i>Pseudomonas aeruginosa</i> .	Ethanolic extract displayed superior antimicrobial activity compared to aqueous extract against all microbes except for <i>Pseudomonas aeruginosa</i> which showed greater sensitivity to aqueous extract.	(Oueslati, Rigane <i>et al.</i> 2016)
Seeds Petroleum ether, ethyl acetate, acetone, chloroform, and alcoholic extracts	Studying the sensitivity of some multidrug resistant strains of <i>Candida albicans</i> , <i>C. krusei</i> , <i>C. tropicalis</i> , <i>C. glabrata</i> , <i>E. coli</i> , <i>S. mutans</i> and <i>S. bovis</i> .	Petroleum ether extract showed higher antimicrobial activity with lower MIC and MBC/MFC values than other extracts against all tested microorganisms.	(Khan, Zakir <i>et al.</i> 2010)
Seeds (70%) Ethanolic extract	Calculating the MIC and MBC values against different strains of <i>Helicobacter pylori</i> bacteria.	Ajwain extract showed considerable anti- <i>Helicobacter pylori</i> properties.	(Zaidi, Yamada <i>et al.</i> 2009)
Seeds Essential oil and cream containing 5% EO	The bacterial species studied were <i>S. aureus</i> , <i>E. coli</i> , <i>A. oryzae</i> , <i>A. niger</i> , <i>C. utilis</i> , <i>M. luteus</i> , <i>L. acidophilus</i> , <i>P. digitatum</i> , and <i>Mucor</i> . Measuring wound contraction in rabbits after applying the ajwain cream.	The essential oil exerted significant activity against all microorganisms, particularly <i>E. coli</i> and <i>A. oryzae</i> and the cream showed a 99.68% wound area contraction on the 15 <sup>th</sup> day.	(Gilani, Mahmood <i>et al.</i> 2013)
Seeds Essential oil and different solvent extract	Four types of bacteria, namely <i>Salmonella typhi</i> , <i>E. coli</i> , <i>Lactobacillus</i> , and <i>B. licheniformis</i> were used for this assay.	Ajwain EO exhibited the highest antimicrobial activity, followed by chloroform extract, whereas there was no antibacterial activity in methanol extract.	(Aggarwal and Goyal 2012)

Table 6: Cont.

Organ/ Extract	Assay method	Results	References
Seeds Petroleum ether extract Isolated compound known as (4aS, 5R, 8aS) 5, 8a-di 1-propyl-octahydronaphthalen-1-(2H)-one	Determining MIC, MBC values and evaluating the antibiofilm and anti-adherence activities against <i>S. mutans</i> .	Petroleum ether fraction exhibited the significant activity against biofilm formation and naphthalene derivative showed <u>remakable antiadherence</u> effect (at 39.06 $\mu\text{g/mL}$ ), and <u>antibiofilm</u> activity at 78.13 $\mu\text{g/mL}$ with MIC and MBC values equaled to 156.25 and 312.50 $\mu\text{g/mL}$ , respectively.	(Khan, Zakir <i>et al.</i> 2010)
Seeds Essential oil	For assaying the antifungal activity, the most common harmful fungi were used in both disk diffusion and broth microdilution methods.	<i>T. ammi</i> was found to be effective against all tested fungal strains with the highest activity against <i>C. albicans</i> and <i>C. glabrata</i> .	(Shokri, Sharifzadeh <i>et al.</i> 2016)
Roots, stems, seeds, and leaves. With different extracts	The fungal species studied were <i>Aspergillus niger</i> , <i>A.s flavus</i> , <i>Alternaria alternata</i> , <i>Helminthosporium</i> and <i>Fusarium salami</i> .	Ethanollic, benzene, and petroleum extracts of ajwain seeds have moderate antifungal activity, while the other seed extracts were inactive. Roots, stems, and leaves in all extracts didn't exhibit any activity.	(Rizki, Fatima <i>et al.</i> 1997)
Fruits Methanolic extract	Evaluating the antiviral activity on Hepatitis C Virus (HCV) protease enzyme	<i>T. ammi</i> has a strong inhibitory impact on the Hepatitis C Virus (HCV) protease enzyme	(Hussein, Miyashiro <i>et al.</i> 2000)

Table 6: Cont.

Organ/ Extract	Assay method	Results	References
Seeds Essential oil	Plaque reduction neutralization test (PRNT) was used for examining the antiviral potential against Japanese encephalitis virus (JEF).	There was significant inhibition of virus plaques introduced by ajwain essential oil. 1 mg/ml essential oil caused 100% viral inhibition with no plaque formation.	Roy, Chaurvedi <i>et al.</i> (2015)
VIII. <u>Antitussive and bronchodilatory effects</u>			
Seeds Essential oil	Studying the <u>bronchodilatory</u> effect through inhibiting isolated guinea-pig tracheal chains' histamine receptors.	<i>T. ammi</i> competitively antagonized histamine (H1) receptors, in addition to its proposed $\beta$ -adrenergic stimulatory effect and anticholinergic properties.	(Boskabady and Shaikhi 2000)
Fruits Ethanolic extract and hexane, aqueous, CHCl <sub>3</sub> , and EtOAc fractions. Three isolated monoterpenes (thymol and two <u>menthene</u> derivatives).	Examining the <u>bronchodilating</u> effects on isolated Guinea-pig tracheal muscles with carbachol induced contractions.	Total ethanolic extract and all fractions showed significant <u>bronchodilating</u> effects, except for aqueous fraction. Additionally, the isolated compounds exhibited considerable dilating effects with the highest activity in thymol.	(Saeedan, Rehman <i>et al.</i> 2024)

Table 6: Cont.

Organ/ Extract	Assay method	Results	References
Aqueous extracts by maceration and soxhlation. Ajwain primary component (carvacrol).	Evaluating the antitussive effects of aerosols containing extracts and carvacrol using guinea pigs	The findings indicated a potent antitussive activity for both ajwain extracts which was not attributed to carvacrol as it didn't show appreciable decrease in cough numbers.	(Boskabady, Jandaghi <i>et al.</i> 2005)
<b>IX. Antihypertensive activity</b>			
Seeds 70% methanolic extract	Monitoring the fluctuations in blood pressure after injecting experimental rats with the plant extract.	Ajwain lowered the mean arterial blood pressure in a dose-dependent manner (3:100 mg/kg) which may be attributed to its potential cholinergic activity.	(Gilani, Jabeen <i>et al.</i> 2005)

## 4. Conclusion

This review highlighted the most prevalent biological activities of *Trachyspermum ammi* L. which are primarily attributed to its essential oil contents and its notable flavonoids and phenolics. Ajwain is clearly a prolific source of bioactive secondary metabolites. However, further research is still required to elucidate the detailed metabolic profile of this plant, particularly concerning its phenolic compounds.

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