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PHYTOCHEMICAL AND PHARMACOLOGICAL INVESTIGATION OF TRACHYSPERMUM AMMI-AN OVERVIEW

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Abstract: The use of plants as medicine is as old as human civilization. As herbs are free from side effects and safe in nature, they are used in various herbal formulations to cure ailments. The main objective of the present overview was an investigation of phytochemical and pharmacological properties of *Trachyspermum ammi* L. (Ajwain) of the family Apiaceae and it is highly cultivated in Egypt, India, Afghanistan Iraq, Iran and Pakistan. It plays a crucial role in human health such as carminative, laxative, antispasmodic an in treatment of piles, abdominal tumors, female genital disorders, etc. Thymol, the major phenolic compound present in ajwain (35-60%) and other chemical constituents are *p*-cymene, terpinene, carvacol, α-pinene, β-pinene, camphene, δ-carene, limonene, α-phellandrene, myrcene, etc. It possess various pharmacological activities like antimicrobial, anthelmintic, anticoccidial, antifungal, cytotoxic, antibacterial, repellant, antioxidant, larvicidal, spermicidal, etc.

Index Terms **- Ajwain, antibacterial, herbal medicines, pharmacological, thymol.**

INTRODUCTION

Plants have been a significant source of both nutrition and medicine since the beginning of human civilization[1]. According to religious and cultural traditions, medicinal herbs have long been thought of as a source of therapeutic cures[2]. Due to the numerous adverse effects of synthetic medications, plant substances are now used as drug sources. Because of this the researchers became more interested in herbal treatments[3]. In contrast to synthetic alternatives, compounds produced from plants are more widely accepted by the general public and are less restricted in international trade[4]. Despite the fact that herbs had been valued for their therapeutic, flavoring, and aromatic properties for ages, their significance was briefly eclipsed by modern synthetic products. But people are now turning back to natural products in the expectations of finding safety and security because their blind dependence on synthetics had ended[5]. As scientists examine plants for a full range of biological activities, from antibiotics to antitumor agents, the search for plants with therapeutic characteristics continues to receive attention[6]. As herbs are natural products they are free from adverse effects, relatively safe, environmentally friendly, and locally accessible[5].

According to the World Health Organization (WHO), traditional medicines, which include plant extracts or their active components, are used by 65-80% of the world's population for their basic medical needs[7], [8]. Herbal medicines have become more popular in treating a variety of diseases due to their significance in folk traditions. By taking into account the active plant ingredients in the preparation of various herbal medications, herbal-based formulations have recently become increasingly important in advancing the pharmaceutical business[3]. These pharmaceutical preparations frequently mediate a favorable response because of their chemical components, even if their efficacy and mechanism of action have typically not been scientifically evaluated[9]. The golden fact is that everyone can utilize herbal remedies, regardless of age or gender[5].

Ajwain, also known as Trachyspermum ammi (L.) Sprague, an herbaceous plant that is member of highly valued medicinally important family Apiaceae (Umbelliferae)[10], [11]. This family contains about 250 genera and more than 3300 species[1]. Trachyspermum ammi (L.) Sprague is a Greek word in which Trachy means rough and spermum means seeded, whereas ammi is the name of plant in Latin[12]. It is also referred as Bishop's weed or carom, which is primarily used in Indian cooking[13]. It is native of Egypt and is cultivated in India, Afghanistan Iraq, Iran and Pakistan. In India, it is cultivated in Maharashtra, Madhya Pradesh, West Bengal, Bihar, Uttar Pradesh, Rajasthan and Gujarat[14].

The seeds contain 2–4.4% brown colored oil called as ajwain oil[15]. Ajwain or ammi oil is used on neurological disorders, including paralysis, tremor, and continual pains[3]. Peoples used the Ajwain after a meal[16]. The main component of this oil is thymol, which is used in lack of appetite, bronchial problems and gastrointestinal ailments[17]. The antilithiatic, antidiuretic, nematicidal, hypertensive, antispasmodic, antibacterial, ant filarial and antihyperlipidemic properties of the low molecular weight compounds derived from methanolic or ethanolic extracts of ajwain, including thymol (major oil), limonine, carvone, terpenes and pinene, have received extensive research[18]. Ajwain grows well in all types of soil, both thrives in loam soil having a pH range of 6.5-8.2 at temperature 15- 250C, and relative humidity of between 67% and 70%[19]. Ajwain grow in October-November and

harvested in May-June[16]. According to pollination behaviour, the Trachyspermum ammi plant is a cross-pollinated crop with a somatic chromosomal number of 2n = 18[20].

The main goal of this mini-review is to survey the chemical constituents and the most important pharmacological activities of Ajwain[21].

PLANT DESCRIPTION

The herb is reportedly grown extensively in dry and semi-arid areas when the soil contains significant amounts of salt[22]. Ajwain is an erect, glabrous, or minutely hairy, branching, annual spice herb that grows up to 60-90 cm tall[1]. The stem is striated; the flowers are actinomorphic, white male, and bisexual; and the inflorescence is composed of 16 umbellets; corolla 5, 5 stamens alternating with petals, inferior ovary, knobby stigma; innate, with a terminal and 7 pairs of lateral leaflets [14].

This plant has an annual growth rate of up to 3 feet and their seeds have a light brown color and have green, thin leaves. Flowers are terminal or seemingly lateral pedunculate, compound umbels, white, tiny, pedicels (0.5-4mm), and uneven[11]. They are also delicate to the touch[23]. The ajwain seeds have a strong flavor, are hot and dry, and have a mildly bitter after taste[24]. The oil is known as water of the Oman in India[25].

Vernacular names[17]

Sanskrit: Yamini, Yaminiki, Yaviniki
Marathi: Onva
English: Bishop's weed
Hindi: Ajwain, Jevain
Bengali: Yamani, Yavan, Yavani
Gujarati: Ajma, Ajmo, Yavan, Javain
Kannada: Oma, Yom, Omu
Malayalam: Oman, Ayanodakan

Taxonomical classification[8]

Kingdom: Plantae
Subkingdom: Tracheobionta
Super-division: Spermatophyta
Division: Magnoliophyta
Class: Magnoliopsida
Subclass: Rosidae
Order: Apiales
Family: Apiaceae
Genus: *Trachyspermum*
Species: *ammi*

Fig.1: *T. ammi* seeds

Fig. 2: *T. ammi* leaves

Phytoconstituents

Trachyspermum ammi, Ajwain is a rich in vitamins and minerals, it is also concentrated in health promoting phytonutrients such as carotenoids (beta-carotene, and lutein) and flavonoids to provide powerful antioxidant protection[26]. The main chemical constituents present in the essential oil are thymol, *p*-cymene, terpinene, carvacol, α-pinene, β-pinene, camphene, δ-carene, limonene, α-phellandrene, myrcene, pulegene, terpin-4-ol, α-terpineol, thujylalcohol, *p*-cuminol-7-ol, *cis*-myrtenol, *p*-mentha-1,3,8-triene, dipentene, linoleic acid, oleic acid, riboflavin, flavone, phenlolic glycoside, phenolic galactosidase, cadienen, camphor, isothymol, elemol, muurolol, α-cadinol, β-cudesmol, β-elemene, α-humulene, longifolene, δ-cadinane, caryophyllene, β-methylgalactosidase, 3-galactosyloxy-5-hydroxytoluene, 2-methyl-3- glucosyloxy-5-isopropylphenol[27]. Thymol, the major phenolic compound present in ajwain (35-60%), has been reported to be a germicide, antispasmodic and antifungal agent[8].

The dark green leaves of *T. ammi* contain various nutrients, especially the antioxidant carotenoids, lutein, and zeaxanthin and are used in green vegetable salads[28]. *Trachyspermum ammi* leaves contains phytochemicals such as carbohydrates (24.6%), tannins, glycosides, moisture (8.9%), protein(17.1%), saponins, flavones and various other components(7.1%) involving metals[29]. The constituents of seed of *T. ammi* includes fiber (11.9%), moisture (8.9%), fat (18.1%), carbohydrates (38.6%), protein (15.4%), glycosides, saponins, flavones, limonene (38%), carvone (46%), dillapiole (9%) and mineral matter (7.1%) containing calcium, phosphorous, iron, cobalt, copper, iodine, manganese, thiamine, riboflavin, and nicotinic acid[30], [31]. The dried ripe fruits of *T. ammi* contain 4–6 % of volatile oil with thymol as the major constituent (35% to 60%)[32]. The nonthymol fraction (thymene) contains para-cymene, γ-terpenine, α- and β-pinenes, dipentene, α-terpinene, and carvacol[33]. From the fruits, an yellow, crystalline flavone and a steroid-like substance has been isolated and it also contains 6-O-β-glucopyranosyloxythymolglucoside and yields 25% oleoresin containing 12% volatile oil (thymol, γ-terpinene, para-cymene, and α- and β-pinene)[34]. Minute amounts of

camphene, myrcene, and α-3-carene also have been found in the plant. Alcoholic extracts contain a highly hygroscopic saponin[33].

Traditional applications

It is common to treat diarrhoea using ajwain-ka-arak (aqueous extract) and exhibits anti-inflammatory effects in rat model systems[18], [35]. Additionally, ajwain oil-cake is utilized as fish farm feed[36]. The active constituents of the oil- phenolic compound, thymol and carvacol are reported to be responsible for antimicrobial activity[37]. It is also reported the use of the fumigation form of *T. ammi* seeds in female genital disorders[38]. It was believed to be beneficial for dissolving the calculi and stones if taken with wine[33]. As a cosmetic agent, local administration of ajwain as a paint results in yellowish complexion on the skin[22].

Table 1: Almost all the parts of Ajwain show different pharmacological actions[39].

Sr no.	Parts of plant	Applications	References
1.	Seeds	It shows its action as laxative, carminative, anthelmintic, stomachic, nematicidal and antiulcer. It also cures piles, abdominal tumors and abdominal pains. Seeds also have excellent aphrodisiac properties and it is also reported that tinctures made by using ajwain seeds are effective in inhibition of acne causing bacteria. The seeds are also used in traditional ethno-veterinary for treating indigestion, diarrhoea and constipation in animals. In case of weakness and to boost milk production, it is given orally to animals.	[29], [34] [17], [37] [40]
2.	Fruits	The small fruit similar to caraway, which has a bitter and pungent taste, is the most beneficial components of ajwain and is particularly adored in Indian recipes, flavorful baked products, and snacks. Due to its carminative, stimulant, antispasmodic and tonic characteristics, it is used in Ayurvedic medicine as a restorative herb as well as being used for diseases of the liver and spleen.	[41], [42] [41], [43]
3.	Roots	Diuretic	[17]
4.	Seed oil	It is antibacterial, antifungal, anti-infectious, antiseptic, anti-nausea, antiviral, anti-parasitic, and tonic and also used for the treatment of dyspepsia, indigestion, colitis and flatulence. The oil is also preferred as deodorant in tooth pastes, mouth washes and gargles and as a flavoring agent for disinfectant soaps.	[44], [45] [37]

MICROSCOPIC CHARACTERISTICS

Two hexagonal structures are visible in transverse fruit sections under a microscope, and they are connected by carpophores. The mesocarp has slightly thicker walls and polygonal tangentially elongated cells with five ridges and six occasional vittae, which divide five primary ridges whereas the epicarp is formed of a single layer of tangentially elongated tabular cells[14], [34]. Fruiting bodies and vascular bundles are composed of clusters of thick-walled, elongated cells, whereas the endosperm is made up of thin-walled, polygonal cells and small, circular oil globules and the powder shows clusters of endosperm cells and oil globules[40]. Fruit is made up of two mericarps that are greyish brown, compressed, ovoid, and about 2 mm long and 1.7 mm wide[14].

PHYSICOCHEMICAL STUDY

It's detailed explanation of the physicochemical properties of the active ingredient in the drug *Trachyspermum ammi* L. (ajwain) is given below.

Loss on drying:

This parameter determines the amount of moisture as well as the volatile components present. Place the powdered drug sample (10 g) on a tared evaporating dish, dry at 105◦C for 6 hours and weigh. Continue the drying was until two successive reading matches each other or the difference between two successive weighing is not more than 0.25% of constant weight. Record the loss on drying as moisture[46].

Determination of ash value:

This parameter is used for determination of inorganic materials, e.g., silicates, oxalates, phosphates and carbonates.

- Total ash:
  Incinerate the ground drug (2 g) in a silica crucible at a temperature not exceeding that 450◦C until free from carbon. Then cool it and weigh to get the total ash content.
- Acid insoluble ash:
  Boil the ash with 25 ml dilute HCl (6N) for 5 minutes. Collect the insoluble matter on ash-less filter paper, wash with hot water and ignite at a temperature not exceeding 450◦C to a constant weight.
- Water soluble ash:
  Dissolve the ash in distilled water. Collect the insoluble part on an ash-less filter paper and ignite at 450◦C to a constant weight[46].

Determination of extractive values:

- Water soluble extractive:
  Weigh accurately about 4 g of coarsely powdered drug into a 250 ml conical flask with stopper. Add 100 ml of chloroform water. Shake the flask frequently during first 6 hr. Keep it aside without disturbing for 18 hr. and then filter. Pipette out 25 ml of the filtrate and evaporate to dryness in a weighed shallow flat-bottomed dish on a water bath. Then dry the residue at 105°C for 6 hrs to a constant weight. Cool in a desiccator for 30 minutes and weigh immediately. Calculate the percentage w/w of water-soluble extractive.
- Alcohol soluble extractive:
  Weigh about 4 g of the coarsely powdered drug in a weighing bottle and transfer it to a dry 250 ml conical flask. Fill 100 ml graduated flask to the delivery mark with the solvent (90 %) alcohol. Wash out the weighing bottle and pour the washings, together with the remainder of the solvent into the conical flask. Cork the flask and set aside for 24 hours shaking frequently. Filter into a 50 ml cylinder .When sufficient filtrate has collected, transfer 25 ml of the filtrate to a weighed thin porcelain dish as used for the ash value determinations. Evaporate to dryness on a water bath and complete the drying in an oven at 105°C for 6 hrs. Cool in a desiccator for 30 min and weigh immediately. Calculate the percentage w/w of extractive with reference to air dried drug.[47]

Determination of tapped density (ρT):

This parameter is useful to calculate the compressibility index and Hausner Ratio of powder. It is determined by placing a graduated cylinder containing a known mass of drug or formulation on a mechanical tapper apparatus, which is operated for fixed number of taps (~1000) until the powder bed volume has reached a minimum. It is denoted as g/ml.

Tapped density (ρT) = Mass of powder (M)/ Tapped volume (V_t)

Determination of bulk density (ρB):

This parameter gives an understanding of powder flowability and compressibility. It is determined by pouring presieved (40 mesh) bulk into a graduated cylinder via a large funnel and measuring the volume and weight.

Bulk density (ρB) = Mass of powder (M)/ Bulk volume (V_b)

Determination of angle of repose (θ):

A funnel is secured with its tip at a given height H, above graph paper that is placed on a flat horizontal surface. Powder or granulation is carefully poured through the funnel until the apex of the conical pile just touches the top of the funnel. The diameter of the base of conical pile is then determined to calculate the angle of repose.

Angle of repose (θ) = $\tan^{-1}(h/r)$

Hausner ratio:

Depending on the material, the compressibility index can be determined using V_{10} instead of V_0. If V_{10} is used, it is clearly stated in the results.

Hausner ratio = Tapped density/ Bulk density

Carr's index:

The Carr index (Carr's index or Carr's Compressibility Index) is an indication of the compressibility of a powder. It is named after the scientist Ralph J. Carr, Jr. The Carr index is calculated by the formula,

Carr's index $=100[\rho T - \rho B/\rho B]$

Where ρB is the freely settled bulk density of the powder, and ρT is the tapped bulk density of the powder after "tapping down". It can also be expressed as $C=100[1-\rho B/\rho T]$[48]

PHYTOCHEMICAL STUDY

Crude drug powder shows the presence of (Glycosides, Fixed Oils, Steroids, Terpenes). Ethanol Extract of seeds shows the presence of (Reducing sugar, Tannins, Glycoside). Ethanol and Ether Extract shows the presence of (Alkaloid, Amino acids, Proteins, Sterols, Terpenes, Glycosides). A detail Phytochemical study by on Methanol, Acetone, Chloroform and Hexane extract of seed is given in Table 4[17].

Test for carbohydrates

Molisch test:

To 2-3 ml aqueous extract, add few drops of alpha-naphthol solution in alcohol, shake and add conc. H_2SO_4 from sides of the test tube. Violet ring is formed at the junction of two liquids.

Test for reducing sugar:

Fehling's test:

Mix 1 ml Fehling's A and B solutions, boil for one minute. Add equal volume of test solution. Heat in boiling water bath for 5-10 minutes. First yellow and then brick red ppt. is observed.

Test for monosaccharides:

Barfoed's test:

Mix equal volume of Barfoed's reagent and test solution. Heat 1-2 min. in boiling water bath and cool. Red ppt. is observed.

Test for tannins:

5% $FeCl_3$ solution:

To 2-3 ml of aqueous or alcoholic extract, add few drops of ferric chloride solution. Deep blue-black colour is observed.

Test for saponins:

Frothing test:

The test-tube was stoppered and shaken vigorously for about 5 min, it was allowed to stand for 30 min and observed for honeycomb froth.

Test for steroids/ terpenes:

Liebermann-Burchard reaction:

Mix 2 ml extract with chloroform. Add 1-2 ml acetic anhydride and 2 drops conc. H_2SO_4 from the side of test tube. First red, then blue and finally green colour appears.

Test for alkaloids:

Mayer's test:

3 ml filtrate with few drops of Mayer's reagent gives yellowish or white ppt.

Test for cardiac glycosides:

Legal's test:

To aqueous extract, add 1ml pyridine and 1 ml of sodium nitroprusside. Pink to red colour appears.

Test for proteins:

Million's test:

Mix 3 ml test solution with 5 ml Million's reagent. White ppt. is observed. Warm it. It turns into brick red ppt.

Test for amino acids:

Ninhydrin test:

Heat 3 ml test solution and 3 drops 5% Ninhydrin solution in boiling water bath for 10 min. Purple or bluish colour appears.

Test for anthraquinone glycosides:

Borntrager's test:

To 3 ml extract, add dil. H_2SO_4. Boil and filter. To cold filtrate, add equal volume benzene or chloroform. Shake well and separate the organic solvent. Add ammonia. Ammoniacal layer turns pink or red.[47]

Table 2: Phytochemical studies[17]

S. No.	Sec. Metabolites	Test names	Methanol	Acetone	Chloroform	Hexane
1.	Carbohydrates	Molisch test	+	+	+	_
2.	Reducing sugars	Fehling test	+	+	_	_
3.	Monosaccharide	Barfoed's test	+	_	_	_
4.	Tannins	Ferric chloride	+	+	_	+
5.	Saponins	Frothing test	_	_	_	_
6.	Terpenes/steroids	Liebermann-Burchard test	+	_	+	+
7.	Alkaloids	Mayer's reagent	+	+	_	+
8.	Cardiac Glycosides	Legal's test	+	+	_	_
9.	Proteins	Million's test	_	_	_	_
10.	Amino acids	Ninhydrin test	_	_	_	_
11.	Anthraquinones	Borntrager's test	+	+	_	_

PHARMACOLOGICAL STUDY

Antibacterial activity:

More specifically, the compound thymol, which was isolated from the oil distilled from the seeds of the plant *T. ammi*, is effective against bacteria that are multi-drug resistant and resistant to even the most common third generation antibiotics, making it a plant-based fourth generation herbal antibiotic. The Gram-positive bacteria such as *Staphylococcus aureus, Bacillus subtilis, Bacillus cereus* and *Listeria monocytogenes* show good inhibition action than Gram-negative bacteria (such as *Pseudomonas aeruginosa* and *Escherichia coli*)[49].

By using the cup diffusion method on nutritional agar medium, the antibacterial activity of aqueous extract, solvent extracts, and isolated ingredients was evaluated. Using a sterile corkborer (5 mm), cups were created in nutrient agar plates, and inoculums containing 106 CFU/ml of bacteria were spread on the solid plates with a sterile swab moistened with the bacterial suspension. Then, 50 µl of each of the aqueous, solvent extracts, and isolated ingredients were added to the cups prepared in the inoculated plates. As a control, 50 µl of sterile distilled water and Methanol were also included in the treatments. One antibacterial and one

antifungal pills each were obtained from the local market, and concentrations made in the same way as extract at the appropriate dosage were tested for comparative efficacy. The plates were incubated at 37°C for 24 hours, and any zone of inhibition around the wells was measured in millimeters (millimeter). Six duplicates were kept for each treatment. A statistical analysis of the data was performed[9].

Antifungal activity:

Thymol and carvacol together are responsible for the antifungal properties of ajwain oil. Thymol damages cell membrane integrity by rupturing vesicles and cell membranes and impairing ergosterol production in *Candida* strains. Thymol also causes cell lysis in *S. cerevisiae*, changing the cell's internal structure and halting cell growth. Similar to thymol, carvacol has antifungal properties that affect Ca^{2+} and H^{+} homeostasis, gene transcription levels that are up-regulated and down-regulated, membrane integrity is disrupted, and ergosterol production is impaired in *Candida* strains[40].

The ajwain fruit was powdered (to an 800 mesh size) using a household model mixi and hydrodistilled in a Clevenger-style device for 6 hours. The result was yellow oil with a distinctive odour and bitter flavor (yield: 2.2%). It was dried over anhydrous sodium sulfate to get rid of any moisture, and then it was kept in the refrigerator at 4°C in the dark until use.

- Isolation of the Acetone Extract:

The powdered fruits were dried at 25°C following the separation of the essential oil.20 g of dried fruits were extracted in 900 mL of acetone for 6 hours in a Soxhlet system to obtain the extract. Up to 20 mL of the extract was concentrated. The samples were put in a vacuum dryer at a low pressure to evaporate any leftover acetone. To determine the antifungal efficacy of the volatile oil and its extract, the pathogenic fungus *Aspergillus niger* (AN), *Aspergillus oryzae* (AO), *Aspergillus flavus* (AF), *Aspergillus ochraceus* (AO′), *Pencillium madriti* (PM), *Pencillium citrium* (PC), *Penicillium viridicatum* (PV), *Fusarium monoliforme* (FM), *Fusarium graminearum* (FG), and *Curvularia lunata* (CL) were undertaken. These fungi were separated from food materials such as onion, curd, vegetable waste, wheat straw, vegetable, decaying vegetation, fruits of *Musa* species, sweet potato, and cheese, respectively. Slants were kept at 5°C while cultures of each of the fungi were kept on Czapek (DOX) agar media with the pH adjusted to 6.0-6.5. Using inverted petriplate and food poisoning procedures, the antifungal activity of the volatile oil and acetone extract against fungus was investigated. The necessary doses (2, 4, and 6 L) of the undiluted sample were mixed with the 20 mL of culture medium in the food poison technique, whereas the necessary doses (2, 4, and 6 L) of the undiluted sample were soaked on a small piece of Whatman No. 1 filter paper (diameter 12 mm) and kept on the lid of the inverted petriplate in the inverted petriplate method. Each test was run three times with three different concentrations (2, 4, and 6 L)[50].

***Antioxidant activity*:**

Strong antioxidant activity against DPPH is present in the methanol extract of *T. ammi*[49]. In comparison to aqueous and methanol extract of ajwain seeds, acetone extract has the greatest FRAP value (2270.27 0.05 mol/l) at 1 mg/ml. This extract provides a highly significant bio-resource of antioxidants that can be used in food and pharmaceuticals as well as in our daily lives[31]. A dried powdered sample of *T. ammi* (100.0 g) was sequentially treated with n-hexane, chloroform, ethyl acetate, acetone, and methanol in a Soxhlet extraction procedure, filtered, concentrated by rotatory evaporation, and dried with oxygen-free nitrogen gas. In other extraction method, 100.0 g of *T. ammi* were extracted directly by maceration in both cold and hot distilled water for 8 hours, yielding the aqueous extracts "a" (at room temperature) and "b" (in hot water), respectively. DPPH, ABTS, and FRAP tests were used to measure the antioxidant activity. The FRAP results were represented in micromole trolox equivalent per gram (mM TE/g) of extracts, whilst the results for the DPPH and ABTS radical scavenging were expressed as the IC50[28].

% inhibition= [(A DPPH - A Extract) / A DPPH] ×100 [45]

Spermicidal activity:

Using a Clevenger-style apparatus, 250 g of the crushed fruits of *T. ammi* were hydrodistilled for three hours. The oil was kept at 48◦C in a sealed vial after being dried over anhydrous Na_2SO_4. Semen samples were obtained through masturbation from 10 prescreened healthy fertile candidates between the ages of 25 and 30 who had more than 60% normal morphology, 40% normal motility, and more than 70% viable sperm. As per usual procedures, semen samples were taken after 72–96 hours of not having any sexual activity. In a 1:1 volumetric ratio, various essential oil concentrations (25, 50, 75, 100, 125, and 150 mg/mL) were combined with human ejaculate. In the control experiment, the ejaculates were combined in a volumetric ratio of 1:1 with physiological saline (pH 7.4) containing 5% DMSO that had been prewarmed to 37◦C. A phase-contrast microscope was used to count 200 sperms under 400x magnification after seeing at least 10 fields of 200 sperms on a prewarmed slide using 10 mL of the mixture for the assessment of sperm motility. Following that, the mixture was incubated at 37◦C.Sperm motility was measured in accordance with the established methodology within 20 seconds, five minutes, ten minutes, and thirty minutes of incubation. The motility experiment was also used to measure the effective concentration of the essential oil (EC50) that immobilizes highly mobile cells by 50%. Following the motility experiment, the spermatozoa were twice washed in physiological saline and then incubated one more in 5% DMSO at 37◦C for 30 minutes to watch the sperm motility reverse[51].

***Cytotoxic activity*:**

The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) colorimetric test was used to measure the antiproliferative effect of *T. ammi* extract on human acute lymphoblastic leukemia (ALL) cancer cell line (Nalm-6) cells. Viable cells were able to use mitochondrial dehydrogenase to convert yellow, water-soluble MTT into purple, water-insoluble formazan crystals. A 96-well plate with 5×104cells per well was seeded with cells in the log-phase of development in a humid environment at 37°C and 5% CO_2. The cells were exposed to 5 µL of extracts in triplicate at final concentrations of 31, 62.5, 125, 250, and 500 g/mL for 48 hours after being incubated for the previous night. The positive and negative controls, respectively, were etoposide and DMSO or ethanol. Centrifugation was followed by the removal of the medium and the addition of 200 µL of phenol red-free media containing MTT at a final concentration of 1 mg/mL to the wells, which was then incubated for 4 hours. Each well received 100 µL of DMSO to help dissolve purple formazan crystals after the medium had been removed. After 30 minutes of shaking, the absorbance of each well was determined using a microplate reader at 492 nm wavelengths. Regression analysis was used to get the IC50 value for each extract using concentration response curves[45].

Anthelmintic activity:

- Preparation of extract:

The fresh leaves of *T. ammi* were collected and crushed. With the help of the Soxhlet Apparatus and a continuous hot extraction procedure, the leaves were extracted for 72 hours using ethyl acetate, chloroform, and petroleum ether, respectively. Ethyl acetate had a yield of 1.7%, chloroform was at 1.1%, and petroleum ether was at 0.82%. To verify that there were no solvent traces remaining in the extracts that could interfere with the activity, all of the resulting extracts were completely dried, concentrated, and filtered.

- Standard drug:

Albendazole was used as a standard drug. For the activity, different concentrations of albendazole were produced in 1% gum acacia in ordinary saline solution, ranging from 20 mg/ml to 100 mg/ml.

- Preparation of extract for the activity:

For the activity, a series of concentrations of Ethyl Acetate Extract, Chloroform Extract, and Petroleum Ether Extract were produced in 1% gum acacia in ordinary saline solution, ranging from 100 mg/ml to 20 mg/ml.

- Anthelmintic investigation:

The investigation was conducted using 25 groups of earthworms that were roughly comparable in size and contained six earthworms each group. Albendazole, Ethyl Acetate Extract, Chloroform Extract, and Petroleum Ether Extract (at doses of 100 mg/ml, 80 mg/ml, 60 mg/ml, 40 mg/ml, and 20 mg/ml, respectively) were administered to all groups. The control group contained 1% gum acacia in ordinary saline. The length of time it took for each individual worm to become paralyzed and die was observed. The worms were claimed to become paralyzed when they failed to resurrect even in normal saline. When worms lost their ability to move, followed by a fading of their body color, death was declared[52].

Larvicidal activity:

- Insects Rearing:

The insects were reared in a lab environment with a photoperiod of 8:16 L: D hr at 26±2 °C and 65±5% R.H. On fresh castor leaves, the larvae were fed (*Ricinus communis*). The insecticidal potential of *T. ammi* essential oil was examined using *S. littoralis* larvae in their fourth instar.

- Toxicity Assay:

Five repetitions of each concentration of *T. ammi* against the fourth larval instar of *S. littoralis* were used to analyze the lethal concentration values (LC30; LC50; and LC90). There were five *T. ammi* water concentrations used: 10, 15, 20, 30, and 40%. Each cup received ten starved larvae that were allowed to eat both the treated and untreated leaves. After 24 hours post-treatment, mortality numbers were calculated[53].

***Repellent activity*:**

By using a preferred bioassay method, the repulsive properties of ajwain oil, its polar and non-polar components, thymol, and its derivatives were examined. Five grams of rice were treated with four different concentrations of ajwain oil, its polar and non-polar constituents separately, and allowed to dry. As a control, acetone was used to treat the rice grains exclusively. In a petri dish, the grains that had been treated and those that hadn't were positioned next to one another with some space between them. The solvent was subsequently entirely ejected from both the treated and control halves by air drying. In the center of petri plates, ten adults of *S. oryzae* were positioned. Each treatment had three replications. After one hour and up to five hours, the number of insects in treated and control grains were counted. Repulsion is represented by positive values, whereas attraction is represented by negative numbers.

The results from all treatments were converted into percentage repulsion (PR) using the following formula:

$$PR\ (\%) = (Nc - 50) \times 2$$

Where Nc is the percentage of *S. oryzae* present on the control side[54].

Anticoccidial activity:

- Preparation of *Trachyspermum ammi* extract:

Using the Soxhlet device, an aqueous methanol extract of *T. ammi* seeds was prepared and this prepared *T. ammi* extract was kept at 4°C pending use.

- Collection of *Eimeria* oocysts:

Four different types of *Eimeria* oocysts were obtained from the caeca of infected intestine that was obtained from several poultry sale shops. Based on the shape of the *Eimeria* oocysts and the isolation site in the chickens' gastrointestinal tracts, different *Eimeria* species oocysts were identified. In potassium dichromate solution (2.5%), collected oocysts were stored and sporulated.

- Experimental design:

The sporulation inhibition assay was used to assess the in vitro effectiveness of *T. ammi* extract. In five-centimeter petri dishes, unsporulated oocysts of the four *Eimeria* species such as *E. tenella*, *E. brunetti*, *E. necatrix*, and *E. mitis* were exposed to a range of *T. ammi* concentrations (w/v; 10, 5, 2.5, 1.25, 0.625, and 0.31%) in 10% DMSO by performing twofold serial dilutions. Control groups included DMSO and potassium dichromate solution ($K_2Cr_2O_7$). *Eimeria* oocysts were incubated for 48 hours at a temperature of 27–29°C and 60% relative humidity. Each concentration underwent three replications. *Eimeria* oocyst sporulation was examined at a magnification of 40x using a light microscope. *Eimeria* oocyst damage and sporulation inhibition (SI) were measured and expressed as percentages[55].

CONCLUSION

It is concluded that developing drugs from endemic plants from traditional system of medicine are now getting full attention because they are believed to be safe. *Trachyspermum ammi* is a significant medicinal plant with both therapeutic and nutritional value and it also has a great potential for further research. A scientific investigation is required to explore the hidden curative and therapeutic potential of this medicinal plant. *T. ammi* shows various pharmacological activities like antibacterial, spermicidal, anthelmintic, anticoccidial, etc. It also exhibits different chemical constituents such as thymol, *p*-cymene, terpinene, carvacol, α-pinene, β-pinene, camphene, etc.

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