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Trachyspermum ammi: Antimicrobial activity and biochemical analysis against pathogens causing gastrointestinal disturbances

Harsha Upadhyay, Jaishree Sikka

Abstract

The present study is based on determining pharmacological uses through the antimicrobial activities of aqueous and ethanolic extracts of *Trachyspermum ammi* on selected gastric microbes. The microorganisms used in the study cause gastrointestinal disturbances such as *Vibro parahaemolyticus*, *Campylobacter jejuni*, and *Escherichia coli*. Antimicrobial activity of medicinal plant against selected microbes determined by disc diffusion method. *E. coli* was the most susceptible microorganism, while *C. jejuni* was found to be the least susceptible. The highest antimicrobial activity was observed for *E. coli* (9.6 mm) with both extracts of *T. ammi*, while the lowest activity was observed for *C. jejuni* (1.2 mm). Biochemical analysis reveals the presence of carbohydrates, flavonoids, alkaloids, resins, coumarins, phenolic compounds, tannins, etc., in both extracts of *T. ammi*.

Keywords: Antimicrobial activity, Biochemical analysis, Levofloxacin, Pathogens, *Trachyspermum ammi*

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Introduction

Over the centuries, nature has gifted many plants that have been the main sources of traditional medicine in various forms, which is still widely used all over the world in relieving disease. It is well known that medicinal plants have been used for many primary health issues for centuries, being a safe, low-toxic, economical, and reliable major natural resource of medicines all over the world. Many areas of the world still lack modern medical facilities, where medicinal plants are used only. According to the World Health Organization, most of the world's population relies on traditional medicine for primary health care. India has been rich in folklore and biodiversity since ancient times, making India one of the best in the world of ethnobotanical with over 2000 species of medicinal herbs and species. Over, 550 different indigenous communities use indigenous traditional knowledge of medicinal plants for people's healthcare and advancement of drugs by local people for the conservation of biodiversity and cultural practice. The ethnic knowledge of the medicinal plant of the Indian subcontinent is very ancient which was reported in several ancient manuscripts such as Rigveda, Atharvaveda, Upanishads, Mahabharata, Purana, Sushruta Samhita, and Charaka Samhita[1,2]. Ashtanga Hridaya Samhita mentions over 1200 herbal medicines.

Trachyspermum ammi commonly called Ajwain in Hindi belongs to the Apiaceae family.[3] *T. ammi* is widely cultivated throughout India. It is a dicotyledonous, annual, aromatic, and herbaceous plant. Its height is about 60–90 cm with deep branches covered with soft fine hairs. The leaves are wing-like, 2–3 finely divided.[4] The flowers are terminal and compound with linear segments. The fruit is a greyish-brown compressed mericarp, 2–3 mm in length, with five distinct ridges and a tubercular surface, small, ovate, muricate, and surrounding cremocarps.[5]

The bioactive components of plants are important to keep the body healthy and disease-free. The most important of these is fiber, carbohydrates, tannins, glycosides, proteins, fats, saponins, flavones, minerals, thymol, -terpene, p-cymine, α- and β-pinenes, α-thujen, myrcene, α-pinenes, 1, 8-cineole, and carvacrol.[6]

T. ammi is widely used as an antimicrobial,[7] hypolipidaemic,[8] digestive stimulant,[9] antihypertensive, hepatoprotective, antispasmodic, bronchodilating,[10] antilithiasis, diuretic,[11] abortifacient,[12] antioxidant, anti-inflammatory,[13] antifilarial,[14] and gastroprotective.[15] The study aims to examine the antimicrobial activity and biochemical analysis of ethanol and aqueous extracts from leaves of *T. ammi*.

Department of Botany, P. M. B. Gujarati Science College, Indore, Madhya Pradesh, India

Corresponding Author: Harsha Upadhyay, Department of Botany, P.M.B. Gujarati Science College, Indore M.P India. E-mail: harshadubey2008@gmail.com

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Materials and Methods

Plant Material

The plant (*T. ammi*) was cultivated from seed in a laboratory garden [Figure 1]. The plant materials were authenticated by botanist in the National Botanical Research Institute, Lucknow.

Pre-treatment of Plant Sample

The young leaves of the plant were collected and 3 times washed with clean tap water and then 2 times with distilled water. After this, the leaves were finely chopped and dried by spreading them on blotting paper at room temperature for 15 days in sterile conditions.[16]

Preparation of Crude Extracts

The plant-dried test sample was ground and sieved. Thereafter, the fine powder plant sample was divided into two portions,

Figure 1: *Trachyspermum ammi*

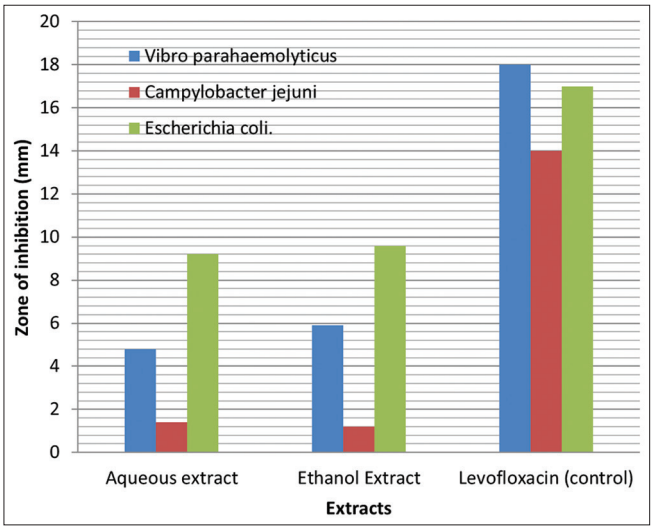

Figure 2: Zone of inhibition of *Trachyspermum ammi* against selected pathogens

each weighing 25 g; the two portions were separately soaked in 100 ml 90% ethanol and distilled water for 24 h. Reflux and steam distillation methods were used to prepare the plant extract of *T. ammi*. All extracts were concentrated by a rotary vacuum evaporator. The obtained extracts were stored in sterile and tinted glass bottles that were pre-washed with distilled water and dried for further use.[15]

Microbial Strains

The test organisms used for the study were *Vibro parahaemolyticus*, *Campylobacter jejuni*, and *Escherichia coli*. These organisms were collected from Hi-Media Pvt. The organisms were sub-cultured and maintained at 4°C.

Antimicrobial Assay

Antimicrobial assay of aqueous and ethanolic extracts of *T. ammi* was performed by disc diffusion method on Muller Hinton agar medium. The spread plate method was used for plates preparation. The plates were inoculated with a 0.1 ml volume of the inoculum of the test organisms used in the study. Hi-Media Sterile Sensitivity Test Discs were saturated with 100 µl of test compounds in a sterile environment, allowed to dry, and then mounted on inoculated agar plates. The plates were incubated overnight at 37°C in the incubator. The zone of inhibition of the plant extract was observed and recorded by measuring the diameter (mm) using a transparent scale surrounding the disc. Levofloxacin was used as a positive control.[17,18]

Preliminary Phytochemical Analysis of Extracts

Plant extracts were examined to test for the presence of different groups of secondary metabolites, including carbohydrates, alkaloids, flavonoids, resins, phenolic compounds, coumarins, and tannins, using previously described methods.[19]

Results and Discussion

The compounds produced by plants are called phytochemicals where phyto refers to the plant. These organic compounds are biological in functions protecting reagents and can be termed secondary metabolites. Intermediates products of metabolism are metabolites. The plants secondary metabolites are safe and effective even when synthetic drugs fail, which can be used as a single compound or as a mixture. Medicinal plants have several secondary metabolites such as carbohydrates, alkaloids, tannins, terpenoids, essential oils, steroids, saponins, and flavonoids, which play an important role in humans, animals, and the plant itself.[20] Katasani *et al*. reported in the study that glycosides, fixed oils, steroids, terpenes, sugars, tannins, amino acids, proteins are found in methanol, acetone, chloroform, and hexane extracts of the seeds.[21] Aqueous and ethanolic extracts of freshly prepared *T. ammi* leaves were subjected to elementary phytochemical screening of various components [Table 1]. The presence of carbohydrates, alkaloids, flavonoids, resins, phenolic compounds, coumarins, tannins, proteins, amino acids, organic, and inorganic acids were found in both extracts of *T. ammi* leaves by phytochemical screening. Convertionally, *T. ammi* whole plant has been used in Indian medicine for centuries. The identification of this family of compounds supports claims for *T. ammi* use as a traditional medicine that these valuable compounds can be used as amoebicidal, antifungal, and febrile conditions.[10,22] It is also used to treat inflammatory disorders, indigestion, etc. Studies have shown that alkaloids, steroids, saponins, and tannins have therapeutic activity against many pathogens.[13] Phenolic compounds such as tannins present in plants are potent inhibitors of proteolytic macerating enzymes that fall under the category of hydrolytic enzymes used by many plant pathogens.

The use of the antibacterial properties of the plant leaves extracts can be of great importance in treatment. Many researchers have reported that various parts of many plants such as flowers, bark, stems, leaves, and roots have antimicrobial properties.[23] Various antibacterial investigations were carried out from aqueous and ethanolic extracts obtained from *T. ammi* seed to investigate the antimicrobial potential.[24] Bashyal and Guha reported that the maximum zone of inhibition against *E. coli* was found in methanolic seed extract (13.5 mm). Seed extracts of water, chloroform, and acetone were found to be 11 mm, 10.5 mm, and 9 mm, respectively, while the inhibition of ciprofloxacin was found to be 17.5 mm in the region where it was used as a control.[25] Concerning this research, our study suggests that the antimicrobial activity of *T. ammi* leaves extract showed different results with the solvents used for extraction [Table 2]. The organisms in the test showed inhibition activity in the region of 1–20 mm. The inhibition zone was found to be higher in the ethanolic extract than in the

Table 1: Qualitative analysis of aqueous and ethanolic leaves extracts of *Trachyspermum ammi*

S. No.	Phytoconstituents	Aqueous extract	Ethanol extract
1	Carbohydrate		
	Molisch's test	+	+
	Benedict's test	+	+
	Fehling's test (free reducing sugars)	+	+
	Fehling's test (reducing sugars)	+	+
	Barfoed's test	+	+
	Anthrone test	+	+
2	Alkaloids		
	Dragendorff's test	+	+
	Hager's test	+	+
	Mayer's test	+	+
3	Flavonoids		
	Shinoda's test	+	+
	Ferric chloride test	+	+
	Alkaline reagent test	+	+
4	Resins	+	+
5	Phenolic compounds		
	Lead acetate test	+	+
	Ferric chloride test	+	+
6	Coumarins	+	+
7	Tannins		
	Lead acetate test	+	+
	Ferric chloride test	+	+
8	Protein		
	Biuret test	+	+
	Millons test	+	+
9	Amino acids		
	Millons test	+	+
	Ninhydrin test	+	+
10	Oils and fats	+	+
11	Phlobatanins	-	-
12	Anthraquinones	-	-
13	Glycosides		
	Borntrager's test	+	+
	Keller killaini's test	+	+
14	Inorganic acids		
	Sulfate test	+	+
	Carbonate test	+	+
15	Organic acids		
	Malic acid Test	+	+
	Oxalic acid Test	+	+

Keywords: +Indicates presence, –Indicates absence

Table 2: Antimicrobial activity of aqueous and ethanolic leaves extracts of *Trachyspermum ammi*

Pathogens	Aqueous extract (mm)	Ethanolic extract (mm)	Levofloxacin (control) (mm)
Vibro parahaemolyticus	4.8	5.9	18
Campylobacter jejuni	1.4	1.2	14
Escherichia coli	9.2	9.6	17

aqueous extract. The inhibition zones for aqueous extracts were 4.8 mm, 1.4 mm, and 9.2 mm for *V. parahaemolyticus*, *C. jejuni*, and *E. coli*, respectively. Zones of inhibition for ethanolic extracts were 5.9 mm, 1.2 mm, and 9.6 mm for *V. parahaemolyticus*, *C. jejuni*, and *E. coli,* respectively. The most susceptible microorganism in this study was found to be *E. coli* while the least susceptible organism was *C. jejuni*. Levofloxacin was used as a positive control, which showed 17.5 mm an average zone of inhibition. The graphical representation was shown in Figure 2. Although test organisms were more sensitive to levofloxacin, there was a reasonably good inhibitory response in both types of leaf extracts of *T. ammi*. In the previous studies, the most of the work was done on *T. ammi* seeds only, in which the highest antimicrobial activity was found against *E. coli*.[24,25] However, in this study, it was observed that the leaf extracts of *T. ammi* showed antimicrobial activity against not only *E. coli* but also showed antimicrobial activity against *V. parahaemolyticus*, and *C. jejuni*. In this study, we observed that ethanolic extracts of *T. ammi* leaves showed superior antimicrobial activities against all bacterial strains used in the test, in contrast to aqueous extracts, which may be due to the ability of the organic nature of ethanol to dissolve more organic and active antimicrobial compounds.

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