ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 11, Issue 5, 2018

Online - 2455-3891
Print - 0974-2441

Research Article

EVALUATION OF *TRACHYSPERMUM AMMI* SEEDS FOR ANTIMICROBIAL ACTIVITY AND PHYTOCHEMICAL ANALYSIS

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Received: 26 December 2017, Revised and Accepted: 30 January 2018

ABSTRACT

Objective: The objective of this study is to identify medicinally important phytochemicals and evaluate the antimicrobial potential of *Trachyspermum ammi* seeds.

Methods: Four different extracts (methanol, acetone, chloroform, and water) were prepared using a soxhlet apparatus, antimicrobial activity was tested using agar well-diffusion technique.

Results: The results revealed the presence of flavonoids and saponins in all the extracts prepared. Similarly, alkaloids and phenols presence were obtained in methanol and aqueous extracts. Glycosides and carbohydrates in methanol, chloroform, and aqueous extracts. Further, proteins, terpenoids, and tannins presence were found in methanol, chloroform, and aqueous extracts, respectively. The maximum zone of inhibition was found in the methanolic extract (13.5 mm). Acetone, chloroform, and water extracts showed 9 mm, 10.5 mm, 11 mm respectively, while ciprofloxacin (control) showed 17.5 mm of the zone of inhibition.

Conclusion: *T. ammi* seeds exert biological properties due to the presence of various chemical constituents. Thus, it can be used to obtain novel antibacterial compounds for the treatment of infectious diseases in the future.

Keywords: *Trachyspermum ammi*, Seed extracts, Phytochemicals, Antimicrobial activity.

 DOI: http://dx.doi.org/10.22159/ajpcr.2018.v11i5.24430

INTRODUCTION

Since from past ages, medicinal plants, also known as medicinal herbs have been discovered and used in traditional medicine practices. The plants that are found naturally are capable to synthesize various chemical compounds for botanical functions that provide defense capability against the number of insects, fungi, diseases, and herbivorous mammals. There is a continuous need for the development of new effective antimicrobial drugs because of the emergence of new infectious diseases and drug resistance [1,2]. In the present scenario, herbal drugs and their formulations have become an alternative to the synthetic drugs [3]. The plant-derived natural products are the products of secondary metabolism; the compounds which are not essential for existence in laboratory conditions, but are certainly responsible for self-defense coordination in natural conditions [4]. Ajwain, *Trachyspermum ammi*, (L.) sprague ex-belonging to the family Apiaceae is also known as Ajowan caraway, Oomam in Tamil, bishop weeds or carom. *T. ammi* is mostly found throughout India and is cultivated in Rajasthan and Gujarat. The seeds of *T. ammi* are native of Egypt and are cultivated in different regions of Iraq, Afghanistan, and India. In India, the seeds are cultivated in Gujarat, Rajasthan, Madhya Pradesh, Uttar Pradesh, Maharashtra, Bihar, and West Bengal. The oil obtained from the seeds exhibits fungicidal [5] antimicrobial [6] and anti-aggregatory effects on humans [7]. It is an important source which acts as a remedial agent for flatulence (gas problem), atonic dyspepsia (indigestion), and diarrhea [8]. An essential oil obtained after the hydrodistillation of the fruits of the plant consists thymol, gamma-terpinene, and p-cymene as well as more than 20 trace compounds (predominately terpenoids) [9].

T. ammi has been found to possess the following properties:

- Antimicrobial [10].
- Hypolipidemic: Used for the treatment of high level of fats [11].
- Digestive stimulant [12].
- Antispasmodic: Used for smooth muscle relaxation.
- Bronchodilating: Provide relief from acute bronchoconstriction [13].
- Antihypertensive.
- Hepatoprotective.
- Diuretic: Increases the production of urine [14].
- Abortifacient [15].
- Anti-lithiasis.
- Galactogogic [16].
- Antiplatelet-aggregator [7].
- Anti-inflammatory [17].
- Antitussive: Suppress coughing [18].
- Anti-filarial [19].
- Gastroprotective [20].
- Nematicidal [21].
- Anthelmintic [22].
- Detoxification of aflatoxins [23].
- Ameliorative effects [24].

Therapeutic uses of *T. ammi* fruits include stomachic, expectorant and carminative [25], antiseptic and amoebiasis, and antimicrobial. Although a lot of work has been performed to evaluate the phytochemicals and pharmacological action of these seeds, from the point of ethnomedical claims, nothing has been discovered with regard to *T. ammi* action over *Escherichia coli*. Therefore, the current study was aimed to carry out the phytoconstituents testing and to analyze antibacterial activity against *E. coli* using the extracts prepared in the laboratory.

Taxonomic classification [26]

- Kingdom: Plantae, Plant
- Subkingdom: Tracheobionta, Vascular plants

- Super division: Spermatophyta, Seed plants
- Division: Magnoliophyta, Flowering plants
- Class: Magnoliopsida, Dicotyledons
- Order: Apiales
- Family: Apiaceae
- Genus: *Trachyspermum*
- Species: *Ammi.*

METHODS

Plant material collection and authentication

Ajwain (*T. ammi*) was obtained from the local market and field of Greater Noida, India. The seeds were verified by Associate Professor Dr. Avijit Guha in the Department of Biotechnology, IILM College of Engineering and Technology. The seeds were dried using an oven and powdered using an electric grinder. The study of plant morphology was done using a simple determination technique, the shape, size, color, and odor.

Preparation of crude extracts

About 3 g of coarse seed powder sample in each 4-conical flask (200 ml) was soxhlet with distilled water (50 ml), methanol and water (7:3, v/v), chloroform, and acetone (70%) for 48 h in the successive mode using a soxhlet apparatus.

Rotary evaporator (Rotavap, Heidolph Labortechnik VV 2000) was taken for further concentration of crude extracts along with the water bath set at 55°C. The dried extracts obtained was weighed and percentage extracted was calculated which was then transferred to airtight jars and stored at 4°C in the refrigerator for the future use. The crude extracts obtained were taken for further investigation of phytochemicals and antimicrobial evaluation.

Equipment's sterilization

The petri dishes and pipettes packed into metal canisters were appropriately sterilized in the hot air oven at 170°C for 1 h at each occasion. Laminar air flow was cleaned with 70% ethanol before starting the culturing of microbes.

Maintenance of test organisms

The *E. coli* sample was maintained weekly by subculturing on agar slants. Before starting the experiment, the cells were activated by successive subculturing and incubation.

PHYTOCONSTITUENTS ANALYSIS

The phytochemical tests were carried out for four different extracts as mentioned above using the standard methods [27-30].

Test for alkaloids

Dragendorff's test

About 1 ml of aqueous extract was taken and stirred properly with the addition of 1 ml of the Dragendorff's reagent. A reddish-brown precipitate confirms that test as positive.

Test for carbohydrates

Benedict's test

1 ml of Benedict's solution was added to the concentrated and filtrate aqueous extract obtained by mixing 1 mg of seed extract in 2.5 ml of water and boiled for 5 min. The presence of carbohydrates was identified by the formation of a brick red precipitate.

Test for saponins

Froth test

About 0.5 mg of the dried seed extracts was added to 3 ml of distilled water and concentrated. The mixture was shaken vigorously for a few minutes. Saponin presence was identified by the formation of foam across the surface for a few minutes.

Test for flavonoids

Alkaline reagent test

About 3 ml of aqueous seed extract was added with a few drops of sodium hydroxide solution. Flavonoids presence was identified by the formation of yellow color which in addition of dilute acetic acid disappears.

Test of proteins

Biuret test

About l ml of the seed extract solution was taken and 4% NaOH solution and 1% CuSO4 solution were added. Proteins presence was identified by the formation of violet color accordingly.

Test of tannins

Ferric chloride test

A volume of 3 ml of seed extracts were taken and a few drops of 0.1% ferric chloride solution were added and allowed to stand for a few minutes. Tannins presence was identified by the formation of brownish-green or blue-black color.

Tests for steroids and terpenoids

Salkowski test

1 ml of each aqueous extract was treated in chloroform with a few drops of concentrated H_2SO_4, shaken well and left to stand for a few minutes. After few minutes, steroids presence was identified by formation of red color at the lower layer and terpenoids by the formation of a yellow-colored lower layer.

Tests for glycosides

Borntrager's test

About 3 ml of seed aqueous extract was shaken vigorously. Further, 1 ml of benzene and 1 ml of dilute ammonia solution were added. Glycosides presence was identified by the formation of reddish pink color.

EVALUATION OF ANTIMICROBIAL ACTIVITY

Test microorganisms and control

The extracts of the seeds of *T. ammi* were tested against *E. coli*. The clinical site was the major source used for the isolation of *E. coli* cells. The medium for isolation and subculture was sterilized using the wet heat sterilization method. The isolated culture in the nutrient agar medium was subcultured in a nutrient broth and was taken for the incubation, which was kept at the temperature of 37°C for 24 h for. Ciprofloxacin was taken as the control for *E. coli* cells. Moreover, the zone formation was compared with the control along with the measurement of the length (mm).

Antimicrobial assay

Agar well-diffusion method was used to determine the antimicrobial activity. The *E. coli* suspension was seeded on two Muller Hinton Agar plates which were maintained in the sterilized condition. Using the sterilized corn borer, two wells were punched in each plate. 30 mg of dried crude extract (each) was dissolved in 1 ml of 20% dimethyl sulfoxide and from the stock solution, using a micropipette 70 µl of water extract and control (100 µg/well) was loaded in the first plate (well 1 and 2) and again, the same concentration of acetone, chloroform, and methanol extract was loaded in the second plate in respective numbered wells. Then, plates were incubated for 24 h at the temperature of 37°C.

The antimicrobial activity was analyzed using the diameter measurement method of inhibition zone formed around well. The effects were compared with that of the standard antibiotic ciprofloxacin.

RESULTS

Phytoconstituents screening

Phytochemical test of four different extracts prepared using an Soxhlet apparatus (Fig. 1) is shown in Table 1. Flavonoids and saponins presence was found in all extracts. Alkaloids and phenols presence

were seen in methanol and aqueous extracts. Alkaloids show a potent antioxidant property. An antioxidant is an important property by which living organisms can neutralize the toxic and cell-damaging the molecules called free radicals, which are produced during various metabolic reactions in the body [31]. Carbohydrates and glycosides presencewere seen in methanol, chloroform, and aqueous extracts. Further, proteins, terpenoids, and tannins presence were found in methanol, chloroform, and aqueous extracts, respectively. Plant terpenoids are used extensively for their aromatic qualities and play a role in traditional herbal remedies [32].

Antimicrobial activity

After incubation for 24 h from the time of loading of extracts, inhibition zones were measured. From this process, we came to know that different forms of extracts have different antimicrobial potential. The controlled region showed inhibition zone of 17.5 mm. At a concentration of 70 µl taken from the stock solution, the methanolic, acetone, chloroform, and aqueous extracts showed inhibition zone of 13.5 mm, 9 mm, 10.5 mm, and 11 mm (Table 2). A maximum zone of inhibition was found in the methanolic extract.

Table 1: Phytochemicals present/absent in seed extracts of *T. ammi*

S.No.	Phytochemicals	Methanol	Acetone	Chloroform	Water
1.	Alkaloids	+	−	−	−
2.	Carbohydrates	+	−	+	+
3.	Saponins	+	+	+	+
4.	Flavonoids	+	+	+	+
5.	Proteins	+	−	−	+
6.	Tannins	−	−	−	+
7.	Steroids	−	−	−	−
8.	Terpenoids	−	−	+	−
9.	Glycosides	+	−	+	+

(+): Indicates the presence of secondary metabolite, (−): Indicates the absence of secondary metabolite. *T. ammi: Trachyspermum ammi*

Table 2: Antibacterial activity of seed extracts of *T. ammi*

Solvent extract	Zone of inhibition (mm)
Methanolic	13.5±0.42
Acetone	9±0.34
Chloroform	10.5±0.20
Aqueous	11±1.2
Control (ciprofloxacin)	17.5±1.33

Inhibitory zones in mm, represented as mean±SD values (n=4).
T. ammi: Trachyspermum ammi, SD: Standard deviation

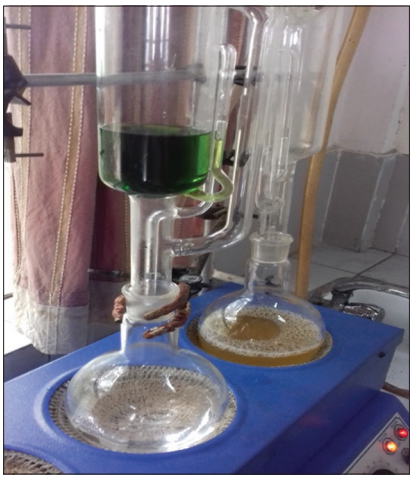

Fig. 1: Soxhlet apparatus

DISCUSSION

The study reported that most of the constituents required for the purpose of medicinal treatment were found in *T. ammi* seeds. Ghosh *et al.* [33] and Kavitha *et al.* [34] also performed the similar study through which they detected terpenoids, flavonoids, saponins, tannins, and phenolic compounds in different extracts of *Polyalthia longifolia,* which has a high content of pharmaceutically important plant-based chemicals. Bashyal *et al.* also performed the phytochemical study on leaves of *Calotropis procera* where they were able to find constituents such as carbohydrates, saponins, alkaloids, flavonoids, proteins, and tannins [35].

Nowadays, many plants have been discovered having the reservoir of the phytochemicals in the form of secondary metabolites. Various researchers are interested in formulating new drugs using these medicinal sources. Bharathi *et al.* [36] studied how the medicinal plants have affected the HIV-related opportunistic bacterial and fungal pathogens. From the analysis phytochemicals such as alkaloids, carbohydrates, flavonoids, saponins, tannins, terpenoids, and glycosides were found.

Many studies have pointed to the antioxidant properties of the medicinal plants. Flavonoid has the capability of acting as antioxidant property. Alkaloids bear the anticancer property. The seed extracts were also revealed to have saponins, which are known to produce the effect on inflammation [37]. Plant-source rich in phytochemicals not only show antibacterial action to few bacteria's but to a large number of Gram-positive and Gram-negative bacteria. A recent study shows that *T. ammi* seeds have the potential insecticidal activity [38] to *Tribolium castaneum*. Other plants that show similar actions to the *T. ammi* seeds are *Anethum graveolens* and *Nigella sativa*.

CONCLUSION

The study revealed that the seeds of *T. ammi* have potent antimicrobial activity and can be used for pharmacological evaluation, drug discovery, and treatment of various infectious diseases. We found that the seeds contain alkaloids, carbohydrates, glycosides, flavonoids, proteins, terpenoids, tannins, and phenols which have the high medicinal purpose. The high zone of inhibition was seen in the methanolic extract which signifies the high antimicrobial action than other three extracts. This medicinal plant needs a scientific exploration of the hidden curative and therapeutic potential.

ACKNOWLEDGMENT

The authors are thankful for the Head, Department of Biotechnology, IILM College of Engineering and Technology, Greater Noida, for providing necessary laboratory facilities to conduct this research work.

AUTHORS CONTRIBUTION

Sagar Bashyal carried out the experiment, wrote the manuscript along with the support and supervision of Associate Professor Dr. Avijit Guha. Both authors conceived the original idea.

CONFLICT OF INTEREST

The authors declare that no conflict of interest occurred during the work.

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