



Research Article

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Antibacterial activity and chemical analysis of fruit oil of *Trachyspermum ammi* Linn. Sprague

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ABSTRACT

Background: In traditional medicine, the fruit of *Trachyspermum ammi* (Family: Umbelliferae), commonly known as Asamodagam in Sinhala; Omum in Tamil and Ajowan or Bishop's weed in English is used in gastro intestinal disorders (loss of appetite, dyspepsia, diarrhea, abdominal pains, abdominal distension and vomiting) and respiratory diseases (Bronchial asthma). The water distillate (omatheeneer or oma water or asamodagam spirit) is used for stomach problems in children. The fruits of this plant are used in different forms such as powder, decoction and oma water in adults also. It is reported that essential oil and the solvent extracts of fruits are active against *Salmonella typhi*, *Escherichia coli*, *Lactobacillus* sp. and *Bacillus licheniformis*. **Objective and Methodology:** An attempt was taken to investigate the (a) antimicrobial properties of essential oil of *T. ammi* fruits against 8 human pathogens: *Staphylococcus aureus* (NCTC 6571), *Escherichia coli* (NCTC 10418), *Pseudomonas aeruginosa* (NCTC 10662) and five wild strains of Methicillin resistant *Staphylococcus aureus* (MRSA) by disc diffusion method and (b) chemical composition of the essential oil of *T. ammi* fruits by Gas Chromatography (GC). **Results:** Essential oil of *T. ammi* showed inhibitory activity against all tested microorganisms. The zone of inhibition was ranged from 21–30 mm. The major compound present in the essential oil of *T. ammi* fruits was thymol (60.7%) followed by p-cymene (21.7%) and γ-terpinene (13.6%). **Conclusion:** Findings of the present study will be helpful to develop antiseptic creams or lotions from fruits of *T. ammi* essential oil.

Keywords: Antibacterial activity, Gas chromatography, *Trachyspermum ammi*.

INTRODUCTION

Trachyspermum ammi Linn. Sprague (Family: Umbelliferae), commonly known as Asamodagam in Sinhala; Omum in Tamil and Ajowan or Bishop's weed in English is an annual herb, 60–90 cm tall^[1]. Ajowan is native of Egypt and grows widely around Mediterranean Sea and in Southwest Asia. It is cultivated in India, Iran, Afghanistan, Pakistan and Iraq^[2]. Stem of *T. ammi* is much branched and striated. Inflorescence is compound umbel having 16 umbels each containing up to 16 flowers. Fruit is grayish brown, ovoid, consisting of two mericarps with prominent ridges and 2 mm long and 1.7 mm wide^[3]. Fruits are used in gastrointestinal disorders (loss of appetite, dyspepsia, diarrhea, abdominal pains, abdominal distension and vomiting) and respiratory diseases (Bronchial asthma). The fruits of this plant are used in different forms such as powder, decoction and oma water in adults also^[1]. In Siddha medicine, the oma water is prepared using special equipment called omathiravakam iyanthiram. This oma water is used to children for stomach problems. The water distillate of fruits contain thymol as the major ingredient and used to prepare oma water or asamodagam spirit. The dried ripe fruits of *T. ammi* contain 4–6 % of volatile oil and thymol is the major component. The oil has carminative, antispasmodic and anti-diarrheal^[4] properties. In addition, oil exhibits antimicrobial^[5], fungicidal^[6] and anti-aggregatory^[7] effects.

In the present study, an attempt was taken to investigate the (a) antimicrobial properties of essential oil of *T. ammi* fruits against 8 human pathogens: *Staphylococcus aureus* (NCTC 6571), *Escherichia coli* (NCTC 10418), *Pseudomonas aeruginosa* (NCTC 10662) and five wild strains of Methicillin resistant *Staphylococcus aureus* (MRSA) by disc diffusion method and (b) chemical composition of the essential oil

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of *T. ammi* fruits by Gas Chromatography (GC).

MATERIALS AND METHODS

Collection of seeds

The dried fruits of *T. ammi* were purchased from Western Province of Sri Lanka in between July and August 2011, identified and authenticated by Senior Scientist, Department of Botany, Bandaranayaka Memorial Ayurveda Research Institute, Navinna, Maharagama, Sri Lanka.

Distillation of oil

The fruits were washed, dried under shade for 5 days and used for oil distillation. The dried fruits (400 g) were crushed and soaked in water (6 L) overnight in a 10 L round bottomed flask and the oil was hydro-distilled for 6 hours using a Clevenger apparatus. The distillate (3 L) was collected. The distillate was allowed to settle and the separated oil used for antibacterial assay.

Test Microorganisms

The fruit oil was screened for antibacterial activity against bacterial isolates which were obtained from the Department of Microbiology, Faculty of Medicine, University of Peradeniya. The bacteria included *S. aureus* NCTC 6571, *E. coli* NCTC 10418, *P. aeruginosa* NCTC 10662 and five wild strains of Methicillin resistant *S. aureus* (MRSA).

Preparation of bacterial inocula-MacFarland 0.5 Series

Each isolated bacterial colony was taken separately to a sterile cotton wool plug. It was smeared on the inner wall of sterile universal bottle, containing approximately 2 mL of sterile normal saline. Subsequently, the bottle was capped and vortexed for five seconds to dissolve the bacterial culture well. The turbidity of the liquid culture obtained was made similar to that of the MacFarland 0.5 standard solution by dissolving more of the colony or diluting with more normal saline^[5].

Assessment of antimicrobial activity by disc diffusion method

Screening for antimicrobial activity of oil was carried out using the disc diffusion method. A broth suspension with turbidity equivalent to 0.5 McFarland standard was prepared from a pure culture of each of the test organisms. A Mueller Hinton Agar (MHA) plate was inoculated with 1 mL of the broth suspension and the plate rotated to allow even spreading of the inoculum. After removal of the excess fluid, the plate was allowed to dry at 37 °C for 15 minutes. Sterile blank discs (6 mm) placed on the seeded plate were impregnated with 5 µL of oil and left on the bench for 30 minutes for absorption of oil. The plates were incubated at 37 °C for 24 h. After incubation the diameters of the zones of inhibition were measured.

For the control test, sterile blank discs (6 mm) were placed on the seeded plate, impregnated with 5 µL of distilled water, left on the bench for 30 minutes for absorption of water and same procedure was followed.

Gas Chromatographic analysis

The essential oil of the fruit was analyzed using Shimadzu 2010 Gas Chromatography (GC) (ISO 7609:1985 (E) normalization method) with the help of Flame Ionization Detector (FID). The capillary column Rt^x-Wax (Crossbond[®], Carbowax[®], Polyethylene glycol) 30 m×0.25 mm i.d × 0.25 µm film thickness (RESTEK) with a temperature program: 60 - 225 °C at 5 °C/min. gradient using Argon carrier gas with split ratio: 1:50 and injection volume: 0.3 µL was used in this analysis.

RESULTS AND DISCUSSION

The yield of the essential oil obtained from *T. ammi* fruits by hydrodistillation was 4.2 %.The oil was pale yellowish in colour with a typical ajwan aroma. In the present study, essential oil of *T. ammi* showed inhibitory activity against all tested microorganisms. The zone of inhibition was ranged from 21–30 mm (Table 1). In Ayurveda medicine, the fruits, fruit oil and the distillate obtained during essential oil distillation are used to treat diseases of the gastrointestinal disorders and respiratory problems. Further *E. coli* is the commonest microorganism for causing diarrhea and *S. aureus* is the commonest organism for causing skin diseases. Therefore, present study, validating the claims in Ayurveda medicine. Similar experiment was carried out by Aggarwal and Goyal^[5] using essential oil and solvent extracts of *T. ammi* against *S. typhi*, *E. coli*, *Lactobacillus* sp. and *B. licheniformis*. According to the results, essential oil exhibited better antimicrobial activity compared to that of solvent extracts. Furthermore, all the non polar solvent extracts including chloroform, petroleum ether and benzene extracts showed antimicrobial activities against *S. typhi*, *E. coli*, *Lactobacillus* sp. However, methanolic extract did not show any antimicrobial activity. Therefore, compound/s that is responsible for antimicrobial activity may accumulate to either in essential oil or low polar solvent extracts.

The major compound present in the essential oil of *T. ammi* fruits was thymol (60.7%) followed by *p*-cymene (21.7%) and *γ*-terpinene (13.6%). Gas chromatogram of essential oil of *T. ammi* fruits is shown in Figure 1. Similarly, in the previously reported data, thymol was found to be the major component of essential oil of *T. ammi* fruits^[8,9,10]. However, cymene (76.3%) was identified as the major compound in essential oil of *T. ammi* grown in India. In this study, thymol (13.3%), was second major compound followed by D-limonene (3.2%)^[11]. Such differences may be due to the ecological and geographical conditions, seasons, maturity of the plant and time of harvesting^[12].

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CONCLUSION

The essential oil of *T. ammi* fruit was rich in thymol and exhibited antimicrobial effect against the tested gram positive and gram negative bacteria. Therefore, findings of the present study will be helpful to develop antiseptic creams or lotions from fruits of *T. ammi* essential oil

Table 1: Antibacterial activity of essential oil of *Trachyspermum ammi* fruits

Microorganisms	Inhibition zone (mm)
<i>Staphylococcus aureus</i> NCTC 6571	30.33 ± 0.57
<i>E. coli</i> NCTC 10418	28.33 ± 0.57
<i>P. aeruginosa</i> NCTC 10662	21.33 ± 0.57
MRSA – Strain 1	29.33 ± 0.57
MRSA – Strain 2	29.33 ± 0.57
MRSA – Strain 3	29.33 ± 0.57
MRSA – Strain 4	29.66 ± 0.57
MRSA – Strain 5	29.66 ± 0.57
Control	0.00 ± 0.00

Data represented as Mean±SD of three independent experiments in triplicate (n=3)

Table 2. Major compounds of essential oil of *Trachyspermum ammi* identified by Gas Chromatography

Compounds	Retention time (in min.)	Percentage (%) in oil
Thymol	29.3	60.7
p-Cymene	8.3	21.7
γ-Terpinene	7.8	13.6

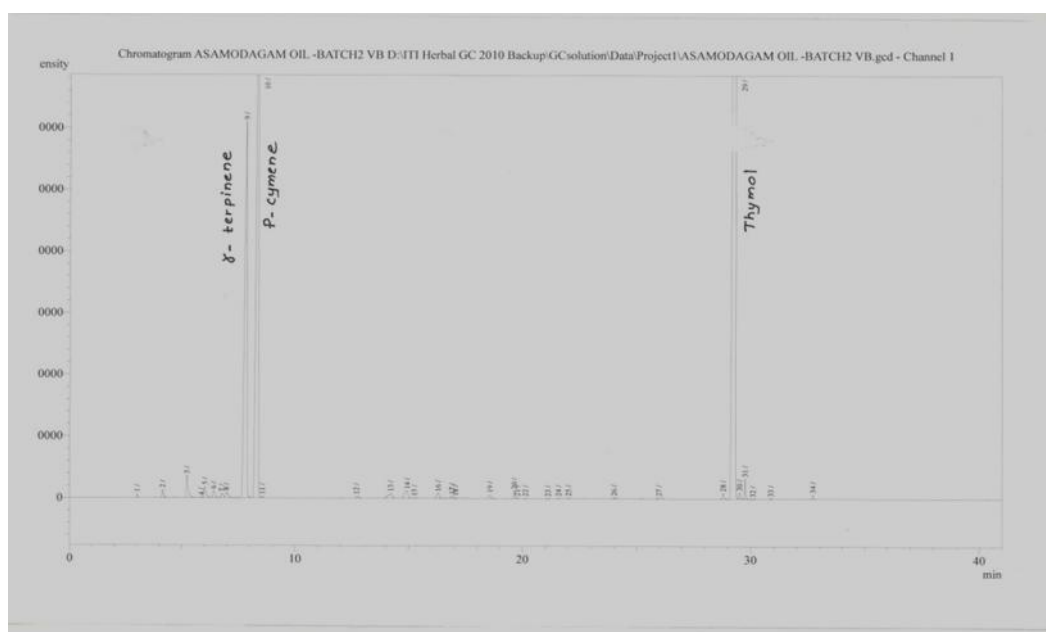


Fig 1: Gas Chromatography of essential oil of *Trachyspermum ammi* fruits

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