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RESEARCH ARTICLE

CHEMICAL COMPOSITION, ANTIOXIDANT AND ANTIFUNGAL ACTIVITY OF ESSENTIAL OIL OF *TRACHYSPERMUMAMMI*SEEDS

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Abstract

The essential oil of locally available *Trachyspermumammi*(ajowan) seeds was obtained through hydrodistillation. Its chemical composition was analysed by gas chromatography mass spectrometry (GC-MS). Twenty - two compounds of EO were identified with the help of NIST library. Thymol (38.5%), ρ-cymene (27.2%) and γ-terpinene (26.8%) were identified as major components. Furthermore, its antioxidant power of IC_{50} value 65.3 µg/ml was determined by DPPH (2, 2-diphenyl-1-1-picrylhydrazyl radical) free radical scavenging activity. In addition, ethanolic suspension of EO exhibited strong inhibition of plant pathogen *Sclerotinia sclerotiorum*under *in-vitro*conditions having IC_{50} value of 691 ppm.

Introduction:-

Natural products have been recognised as a vital resource of novel chemicals. Plant essential oils (EO) have infinite potential to be applied in medicine, agriculture and food industries. Some of the well-known plants families producing EOs belong to Alliaceae, Apiaceae, Asteraceae, Lamiaceae, Myrtaceae, Poaceae and Rutaceae. EOs are complex mixtures containing low molecular weight compounds [1]. Most economical extraction procedure of essential oil is hydrodistillation through Clevenger's apparatus. Edapho-climatic factors such as genetic variety, soil type, seasonal changes have huge impact on chemical composition and biological activities of EOs so obtained. *Trachyspermumammi* L. (family: Apiaceae) is an annual herb and highly acclaimed for its medicinal utilities; known as Ajowan in Hindi. Most of the research documented on Ajowan plant has been related to biological activity and composition of its essential oil. It is a native plant of India, Pakistan, Iran, Egypt and Afghanistan. Seeds and fruits have been known to contain plethora of application in medicine and food industry [2]. Former studies have described the seeds of the plant to have antioxidant, antimicrobial and carminative activity. The EO of ajowan have been found be contained in many therapeutic formulations (e.g. tooth paste and cough syrup) [3].

Ajowan seeds (200 g) were purchased from local grocery shop in Delhi, India. 100 g of seeds were dissolved in distilled water (500 ml) using a Clevenger-type apparatus for about 4hrs. This procedure was repeated twice. The immiscible, upper essential oil (collected in collecting arm of Clevenger apparatus) was dried over anhydrous $Na_2SO_4$and kept at 4°C under refrigeration. The antioxidant activity of the *T. ammi* essential oil (EO) was investigated by DPPH free radical assay. For the same, stock solution of 1000 ppm of ethanolic EO was prepared. It was diluted in ethanol to make following different concentrations: 200, 100, 75, 50, 25 and 10 ppm. 100µl of each of the concentration was mixed with pipette in 100µl of 0.2 mM DPPH in ethanol in triplicate. After 25 mins of incubation in dark, absorption was measured at 518 nm spectrophotometrically. Ascorbic acid (≥ 98% pure, Sigma

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aldrich) was positive control while DPPH served as negative control, both dissolved in ethanol. Antioxidant activity was calculated by the equation:

$$(A0 - A1)/A0X100$$

where A0= absorbance of positive control and A1= absorbance of a sample and DPPH quenching ability was estimated as IC50 (µg/mL) by regression equation in Microsoft excel. Plant pathogen Sclerotinia sclerotiorum was obtained from ITCC, IARI Pusa, Delhi and was maintained on PDA. Each concentration of 1000, 750 and 500 ppm of ethanolic EO fraction was dissolved in 5ml of potato dextrose agar. 8mm of agar plug of freshly cultivated Sclerotinia sclerotiorum was placed onto the centre of each concentration in triplicate. Control set of experiment only had solvent (ethanol) dissolved in PDA medium. Antifungal activity was determined as IC_{50} (ppm) value by log probit analysis[4]. GC-MS analyses was done using Shimadzu GCMS-QP 2010 instrument utilising RTX-1 MS column having inert nitrogen as carrier gas. The instrument had a temperature program of 100 °C (2 min), 10 °C/min to 250 °C (5min), and 10 °C/min to 280 °C (15 min). Mass acquisition of programme ranged from 40-650 m/z. Mass spectra was interpreted with the aid of NIST library.

The results are in accordance with previous studies conducted both within and outside India. Thymol has been found to be the chief component of essential oil of *T. ammi* seeds [5, 6, 7]. In a north-eastern Indian variety EO was found to be rich in carvone (46.2%) followed by limonene [8] whereas thymol (97.9% and 87.75%) was the main constituent of south Indian varieties of ajowan plant [9, 10]. Essential oil contents and yields have been to known to be affected by various environmental factors. Thymol is a monoterpenoid phenolic compound synthesised by hydroxylation of its precursor ρ-cymene [11]. The most common non-phenolic monoterpenoids detected in this study from *T. ammi*EO are: terpinen-4-ol (0.61%), myrcene (0.59%), limonene (0.55%), carvacrol (0.52%), α-thujene (0.47%) and 4-Thujanol (0.45%) amongst others (Table 1, Figure 1). Technically, antioxidant compounds have the capability of slowing down the oxidation process of an oxidizable substance when used in rather a modest quantity [12, 13]. The EO of *T. ammi* seeds exhibited a dose-dependent antioxidant activity by quenching DPPH free radical having an IC_{50} value of 65.34 µg/ml (Figure 2). In the present work, we investigated the inhibitory activity of EO of *T. ammi*against stem rot causing phytopathogenic fungus, *Sclerotinia sclerotiorum*. The ethanolic suspension of EO exerted strong growth inhibition on *S. sclerotiorum* through poisoned food bioassay technique carried out in potato dextrose agar medium having an having IC_{50} value of 691 ppm (Figure 3). Essential oils have been found to be potent against phytopathogenic and food-borne fungi due to the presence of several terpenes which have high binding affinity for fungal membrane sterols. In literature, *T. ammi* EO has been documented to have antagonistic activity against different *Aspergillus* sp. and thymol was the antifungal metabolite in the EO [14, 15]. However, this is the first report of antifungal activity of essential oil of *T. ammi* seeds against phytopathogen *Sclerotinia sclerotiorum*.The study recommends that *T. ammi* EO should be taken up for management of *S. sclerotiorum* further for carrying out in-planta studies.

Table 1:- List of metabolites detected by GC-MS analysis of T. ammi seed essential oil.

S.No.	Retention Time	Area%	IUPAC name of the compound	Molecular weight	Literature Retention Index values
1	23.892	38.47	Thymol	150.22	1264
2	11.657	27.19	p-cymene	134.21	1011
3	13.166	26.81	γ -terpinene	136.278	1055
4	9.649	3.09	β-pinene oxide	152.24	970
5	18.728	0.61	Terpinen-4-ol	154.25	1175
6	10.179	0.59	Myrcene	136.237	1173
7	11.896	0.55	limonene	136.24	1033
8	24.193	0.52	Carvacrol	150.22	1304
9	7.692	0.47	α-thujene	136.24	935
10	11.315	0.45	α-terpinene	136.238	1020
11	19.415	0.28	α-terpineol	154.253	1195
12	15.075	0.23	4-Thujanol	154.253	1068
13	7.953	0.21	Ocimene	136.238	1252
14	9.471	0.17	(+)-Sabinene	136.238	983
15	23.471	0.06	Thymol acetate	192.258	1364
16	20.969	0.06	Citronellol	156.27	1228
18	18.116	0.04	Hexa-2,4-dienyl butyrate	168.236	n.a.

19	11.975	0.04	1,8-cineole	154.249	1024
21	14.374	0.03	Terpinolene	136.238	1078
22	16.153	0.03	Caryophyllenediepoxide	220.350	1581

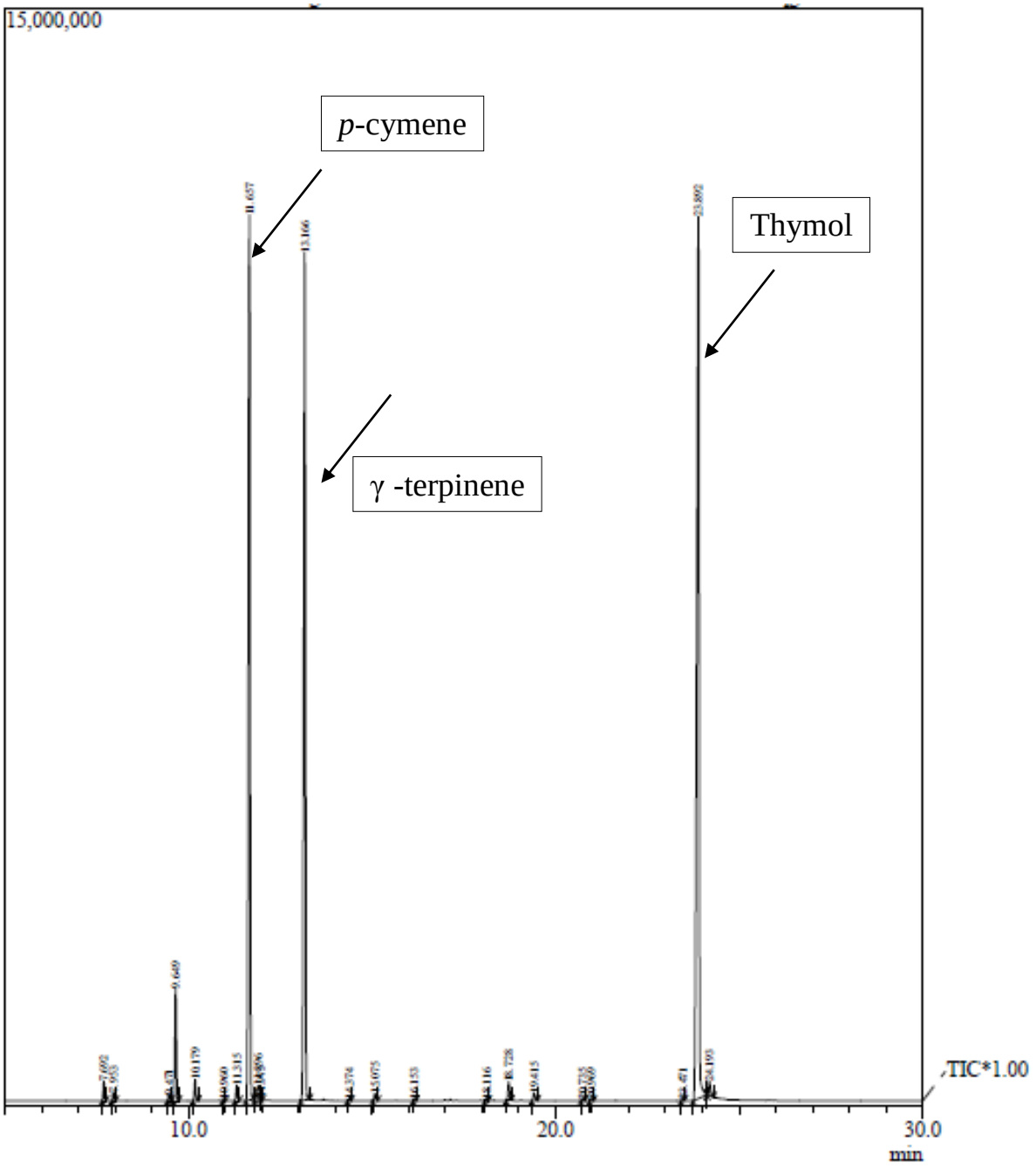

Figure 1:- GC-MS analysis of essential oil of *Trachyspermumammi*seeds.

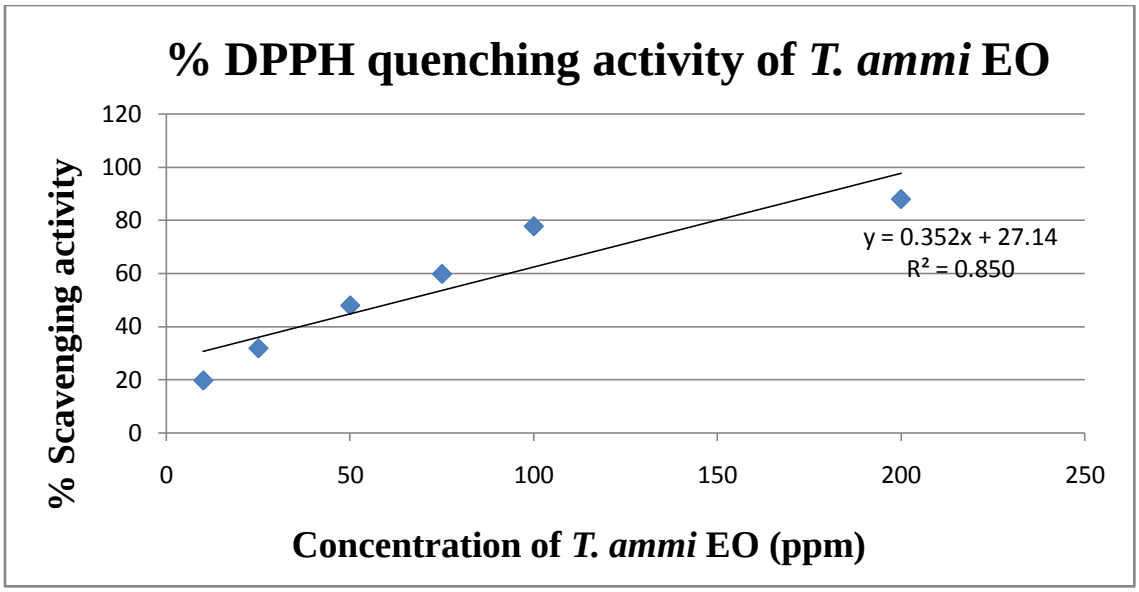

Figure 2:- Antioxidant activity of EO *Trachyspermumammi*seeds.

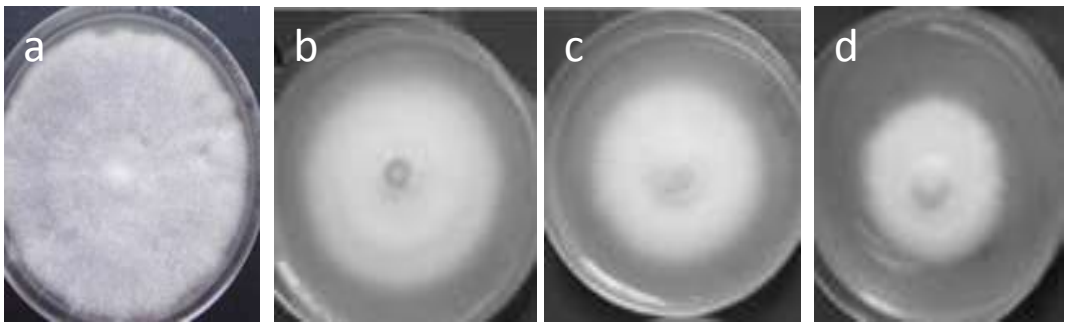

Figure 3:- Antifungal activity of EO *Trachyspermumammi* seeds against *Sclerotinia sclerotiorum*.

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