Research Article

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PHYTOCHEMICAL SCREENING, QUANTITATIVE ESTIMATION OF TOTAL PHENOLIC, FLAVANOIDS AND ANTIMICROBIAL EVALUATION OF *TRACHYSPERMUM AMMI.*

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Abstract:

Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. They are nonessential nutrients, meaning that they are not required by the human body for sustaining life. It is well-known that plant produces these chemicals to protect themselves but recent research demonstrate that they can also protect humans against diseases. *TRACHYSPERMUM TRACHYSPERMUM AMMI* is also traditionally known as a digestive aid, a relief for abdominal discomfort due to indigestion and an antiseptic. The medicinal properties of the plant are due to the anti oxidants present in the plant. Present studies deals with the identification of phytochemicals and there quantitative estimation, antimicrobial activity of *TRACHYSPERMUM AMMI* and its important medicinal qualities provided one does not play around with poisonous plants they are totally free of harmful side effects - unlike the modern drug industry.

Key Words:

Trachyspermum ammi, Phytochemical screening, Anti oxidants, Anti microbial

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Introduction:

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

The use of local plants in folk medical practices has a long history. The resource base of the traditional medical practices prevalent in rural and tribal villages of India and abroad is mainly the plants [(0)]. Medicinal plants are used to maintain and promote healthy life, prevent disease and cure ailments. It has been estimated that even today, 80% of the world population rely on herbal traditional medicine for their primary health care (Absar A. Qureshi *et al.,* 2008). Traditional knowledge of medicinal plants has always guided the search for new cures. In spite of the advent of modern high throughput drug discovery and screening techniques, traditional knowledge systems have given clues to the discovery of valuable drugs (Buenz *et al.,* 2004). Traditional medicinal plants are often cheaper, locally available and easily consumable, raw or as simple medicinal preparations [(1)]. Nowadays, traditional medicinal practices form an integral part of complementary or alternative medicine. Although their efficacy and mechanism of action have not been tested scientifically in most cases, these simple medicinal preparations often mediate beneficial responses due to their chemical constituents (Park and Puzzutto, 2002). Nowadays, more emphasis is given on functional foods which are being consumed as part of a usual diet but are demonstrated to have physiological benefits and/ or reduce the risk of chronic disease beyond basic nutritional functions. Spices are being used as food additives since ancient times mostly for their organoleptic attributes. It is now understood that spices also exhibit several beneficial physiological effects in addition to enhancing taste and flavor of food (Chandrassekhara and Srinivasan, 1999). In this background, we have planned to review traditional knowledge, phytochemical constituents and scientific validation of traditional claims of a very well know culinary spice i.e. *Trachyspermum*

ammi which is commonly known as Ajowan. Among traditional potential herbs used as spice in day to day life, ajwain (*Trachyspermum ammi* L.) belonging to family Apiaceae, is widely used for curing various diseases in both humans and animals. Its other names in literature are, ajwan, ajowan, Bishop's wee, carom, or Ethiopean cumin. The most utilizes part of ajwain is the small caraway like fruit, which is particularly popular in Indian savory recipes, savory pastries, snacks and as spice (Anilkumar *et al.*, 2009). *Trachyspermum ammi* is a grassy, annual plant which grows in the east of India, Iran and Egypt with a white flower and small, brownish seeds.

Plant description:

An erect, glabrous or minutely pubescent, branched annual, up to 90 cm., tall, cultivated almost throughout India. Stems striate; leaves rather distant, 2-3 pinnately divided, segments linear, ultimate segments 1.0-2.5 cm. long; flowers in terminal or seemingly-lateral pedunculate, compounds umbels, white, small; fruits ovoid, muricate, aromatic cremocrps, 2-3 mm. long, grayish brown; mericarp compressed, with distinct ridges and tubercular surface, 1-seeded. Flowers and fruits from January-April (Asima Chatterjee, 1995 and Anonymous, 2003).

Materials and methods:

The seeds of *Trachyspermum ammi* are purchased from local market and are gown in a fertile soil with sufficient water and nutrient supply. The seeds are germinated in 5 days and are grown up to 25 days. The all the plant plant parts are collected, washed, shade dried and is made powder mechanically and the powder is used for the experimental procedure.

Preparation of extracts:

The powdered material was weighed in a selected quantity and is subjected to soxhelt extraction using Methanol, Acetone , chloroform and Hexane in successive mode respectively for 48 h. The solvent was then recovered using Rotary Vacuum Evaporator and the concentrated extract was preserved in an airtight bottle. The crude extracts thus obtained were used for further investigation for of Phytochemical screening, and Anti microbial Evaluation

Phytochemical screening[3,4,5,6]**:**

The extracts of the dry powdered leaves of *Trachyspermum ammi* were analyzed for the presence of various phytoconstituents like carbohydrates, reducing sugars, monosaccharide (Evans, 1996), Tannins (Evans, 1996), Saponnins (Evans, 1996), Flavonoids(Shinoda's Test), Terpenes /steroids (Liebermann - Burchard's Test), Alkaloids(Evans, 1996), Anthraquinones (Borntrager's test), cardiac glucosides (sodium nitro proside method) proteins (copper sulphate and Folin Ciocalteau solution) and amino acids(Ninhydrin) were

identified using standard phytochemical procedures.

The results of the phytochemical identification is shown in table A.

Quantitative estimation of total phenolic and flavonoid compounds:[7,8]

Total phenols determination:

The total phenolics content in different solvent extracts was determined with the Folin- Ciocalteu's reagent (FCR)**.** In the procedure, different concentrations of the extracts were mixed with 0.4 ml FCR (diluted 1:10 v/v). After 5 min 4 ml of sodium carbonate solution was added. The final volume of the tubes were made upto 10 ml with distilled water and allowed to stand for 90 min at room temperature. Absorbance of sample was measured against the blank at 750 nm using a spectrophotometer (Shimadzu UV-1609, Japan). A calibration curve was constructed using catechol solutions as standard and total phenolic content of the extract was expressed in terms of milligrams of catechol per gram of dry weight and the standard graph was shone in figure -1 and results are shown in table B.

Total flavanoid determination

Total flavonoid content was determined by Aluminium chloride method [11] using catechin as a standard. 1ml of test sample and 4 ml of water were added to a volumetric flask (10 ml volume). 5 min after adding 0.3 ml of 5 % Sodium nitrite, 0.3 ml of 10% Aluminium chloride was added. After 6 min incubation at room temperature, 2 ml of 1 M Sodium hydroxide was added to the reaction mixture. Immediately the final volume was made up to 10 ml with distilled water. The absorbance of the reaction mixture was measured at 510 nm against a blank spectrophotometrically (Shimadzu UV-1609, Japan). Results were expressed as catechin equivalents (mg catechin/g dried

Anti microbial activity[9]**:**

After complete solvent evaporation, each of these solvent (Methanol, Acetone , chloroform and Hexane) extract was weighed and preserved at 5°C in airtight bottles until further use. One gm of each solvent residue was dissolved in 5 ml of Methanol, which served as the test extracts for antibacterial activity assay.

Plant pathogenic bacterial cultures:

Authentic pure cultures of bacteria namely *Bacillus subtilis* , *staphylococcus* , *Pseudomonas aeruginosa*, *Enterobacter cloacae* isolated from different spoiled fruits and soil samples and Fungi like *Aspergillus niger*, *Candida*, are obtained from bread samples are cultured in selected culture media.

Anti-bacterial activity assay[10]**:**

Antibacterial activity of aqueous extract, solvent extracts and isolated constituents was determined by cup diffusion method on nutrient agar medium (Anon, 1996). Cups

were made in nutrient agar plate using sterile cork borer (5 mm) 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 each of all aqueous, solvent extracts and isolated constituents were placed in the cups made in inoculated plates. The treatments also included 50 µl of sterilized distilled water and Methanol separately which served as control. one anti bacterial and one and one anti fungal tablets were purchased from local market and concentrations were prepared same as extract at their respective recommended dosage were tested for comparative efficacy. The plates were incubated for 24 h. at 37°C and zone of inhibition if any around the wells were measured in mm (millimeter). For each treatment six replicates were maintained. The data was subjected to statistical analysis, results can be shown in table C.

Results:

All the 4 solvents i.e. Methanol, Acetone, chloroform and Hexane extraction of *Trachyspermum ammi* shows the positive result for few phyto chemicals, some chemical components can't extract some solvents. The results are shown in table A.

Discussion:

Methanolic extracts if the plant show positive results for many number of tests, indicates that methanol is used as a best solvent for extraction of phytochemicals. Many chemical components can not show +ve results for other solvents like Chloroform, Acetone and Hexane, which are less commonly used for the extraction of phytochemicals.

All the solvents(methanol, Chloroform, Acetone and Hexane) show antimicrobial growth inhibition in cup diffusion method, indicates that the solvent extracts show resistant against the growth of microorganisms. Methanolic extracts shows best results against the growth of Bacillus subtilis and the minimum inhibition in Hexane extract of *Aspergillus niger*.

Conclusion:

Plants are the best source of chemical compounds that are show resistance against the number of micro organisms that cause disease. By the use of these chemicals can cure the diseases without any side effects, unlike the modern drugs that cause many side effects.

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Tables and Figures:

S.No	*Sec.* Metabolites	Test names	Methanol	Acetone	chloroform	Hexane
1	Carbohydrates	Molisch's test	+	+	+	-
2	reducing sugars	Fehling test	+	+	-	-
3	monosaccharide	Barfoed's test	+	-	-	-
4	Tannins	1.Ferric chloride 2. lead sub acetate	+	+	-	+
5	Saponnins	Frothing test	-	-	-	-
6	Flavonoids	Shinoda's Test	+	+	-	+
7	Terpenes/steroids	Liebermann - Burchard's Test	+	-	+	+
8	Alkaloids	1.Mayer's Wagner's reagent 2.with KI	+	+	-	+
9	cardiac glucosides	sodium nitroproside	+	+	-	-
10	Proteins	copper sulphate and Folin Ciocalteau solution	-	-	-	-
11	amino acids	Ninhydrin	-	-	-	-
12	Anthraquinones	Borntrager's test	+	+	-	-

Table A: Results of the phytochemical screening of *Trachyspermum ammi*

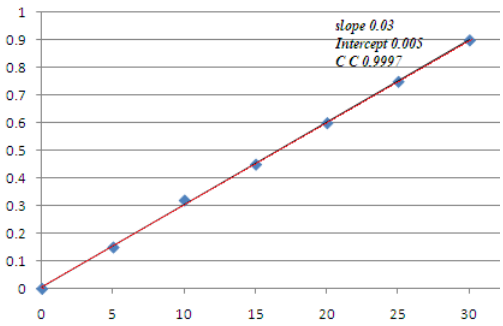

Fig 1: standard graph of total phenolic compounds

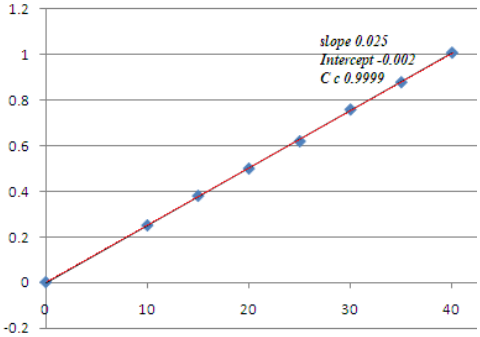

Fig 2: standard graph of total flavanoids compound

Extract	Total phenolic content. Mg of Catechin equivalents /mg dried extracts.	Total flavanoids content. Mg of Catechol equivalents /mg dried extracts.
Methanol	11.51	15.32
Acetone	7.37	6.47
chloroform	9.24	12.38
Hexane	7.59	7.25

Table B: total phenolic and flavanoids content of different extacts of *Trachyspermum ammi*

Statistical analysis:

S.No	Micro organism	Mean zone of inhibition of microbial grwth(mm)				
		Methanol	Acetone	chloroform	Hexane	Blank(water)
1	*Bacillus subtilis*	15.38	12.35	9.37	9.25	NA
2	*staphylococcus*	14.24	11.21	10.3	8.39	NA
3	*Pseudomonas aeruginosa*	12.37	8.37	9.52	10.39	NA
4	*Enterobacter cloacae*	13.91	14.37	8.27	7.32	NA
5	*Aspergillus niger*	10.32	8.39	6.21	5.39	NA
6	*Candida*	9.37	8.81	7.39	6.39	NA

Table C: Anti-bacterial activity assay

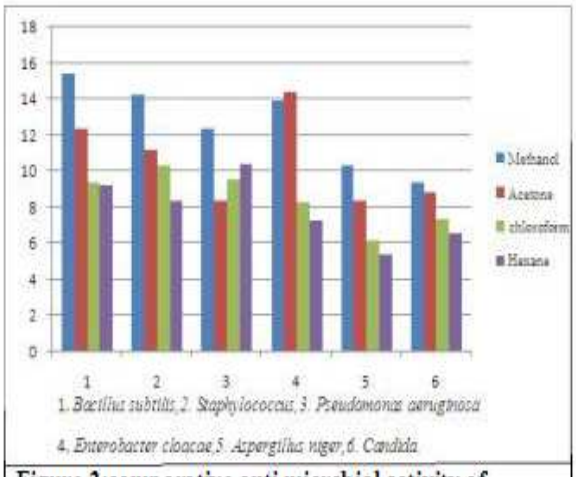

Figure 3:comparative anti microbial activity of different extracts of *Trachyspermum ammi*

Figure 4: anti microbial zone inhibition By well diffusion method