

The chemistry of the various extracted materials from the seed of *Trachyspermum copticum* and their antibacterial activity

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Abstract : Seeing the important medicinal value of *Trachyspermum copticum* medicinal, nutritive and antibacterial activity of the crude extracts of the seed is determined. The seed is rich in dietary fiber (5.2%), protein (24.26%) and minerals (4.9% ash). The seed has high nutritive value (305 Kcal/100gm). Chemical examination were performed with methanol, ethanol and water extracts and shown the presence of alkaloids, falavanoids, steroids, saponins and tannins. Antibacterial activity of different extracts of seed of *Trachyspermum copticum* is studied against *Escherichia coli* & *Staphylococcus aureus*. Inhibition zone of methanol, ethanol and water extract is determined and compared.

(Keywords : Antibacterial, *Trachyspermum copticum*, *Escherichia coli* and *Staphylococcus aureus*.)

Introduction

Trachyspermum copticum belonging to family Apiaceae is a highly valued medicinally important seed.¹ The common name of this plant is Ajwain. Its seeds are used extensively as a food in India and therapeutically effective. It is traditional herb and is widely used for curing various diseases in humans. The seed possesses stimulant, antispasmodic and carminative properties. It is an important remedial agent for flatulence, atonic dyspepsia and diarrhoea². The seed of ajwain is bitter, pungent and it acts as anthelmintic, carminative, laxative. It also cures abdominal tumours, abdominal pains and piles³ and bears anti-inflammatory and antioxidant activity⁴.

Experimental

The seed of collected from the local market. The sample was washed and drained off in order to clean the dust particles. The powdered sample was kept in air tight container before further analysis. The sample was analyzed for moisture, protein, fat, ash, fiber and carbohydrate by the methods of AOAC (2003)⁷.

Moisture was determined by oven dry method. 2.0 gm. The sample was weighed accurately in clean dried crucible (W₁). The crucible was allowed in an oven at 100-105°C for 6-12 hours until a constant weight was obtained. Then the crucible was placed in the desiccators for 30 minutes to cool. After cooling it was weighed again. (w₂) The percentage of moisture was calculated by following formula.

$$\% \text{ moisture} = \frac{W_1 - W_2}{\text{weight of sample}} \times 100$$

Where,

w₁ = Initial weight of crucible + sample

w₂ = final weight of crucible + sample

For the determination of ash clean empty crucible was placed in muffle furnace at 600°C for an hour, cooled in desiccators, and then weight of empty crucible was noted (w₁). 2.0 gram of each sample was taken in crucible (w₂). The sample was kept in muffle furnace at 600°C for six hours. The appearing of dry white ash indicates the complete

oxidation of all organic matter in the sample. After ashing the muffle furnace was switched off. The crucible was cooled and weighted (w_3). The percentage of ash was calculated by following formula

$$\% \text{ of ash} = \frac{\text{difference in weight of ash}}{\text{weight of sample}} \times 100$$

$$\text{Difference in weight of ash} = w_3 - w_1$$

Crude fat was determined by ether extract method by using soxlet apparatus. In this method, first all the apparatus were rinsed with petroleum ether, drain, dried in oven at 102°C , for 30 minutes, then cooled in desiccators. A piece of cotton wool was placed in the bottom of a 100 ml beaker. Put a plug of cotton wool in the bottom of an extraction thimble and stand the extraction thimble in the beaker. Then 5.0 grams of fat free sample was taken into the thimble 1.5 gram of sand was also added to it and mixed with a glass rod. Take the piece of cotton wool from the bottom of the beaker and place it in the top of the thimble. Insert the thimble in soxlet apparatus. Accurately weigh a clean dry 150 ml round bottom flask and put about 90 ml of petroleum ether in it. Placed the extraction unit on electric heating mantle. Heated the flask at 50°C for 6 hours. After 6 hours the extract was distilled by using Rota vapor. Residue was placed in an oven at 102°C and the contents were dried and weigh subsequently percentage of crude fat was calculated by using the following formula -

$$\% \text{ off crude fat} = \frac{W_2 - w_1}{\text{weight of sample}} \times 100$$

W_1 = weight of empty flask,

W_2 = weight of extracted fat

For the determination of Crude fiber 5.0 gram of moisture free and ether extracted sample was weighed and transferred to a crucible (w_0). The sample was first digested with dilute sulphuric acid and then with dilute KOH solution, filtered and washed with hot distilled water. The samples were kept in an oven at 150°C for 1 hour. The samples were cooled in desiccators and weighed (w_1). Kept the samples in muffle furnace at 55°C for 4 hours. The sample was ignited and the ash

was weighed (W_3). Loss in the weigh was calculated by following formula -

$$\% \text{ of crude fiber} = \frac{W_1 - W_2}{W_0} \times 100 \text{ where,}$$

W_0 = weight of crucible,

W_1 = weight of sample

W_2 = weight of crucible + weight of sample,

w_3 = weight of ash

Crude protein was determined by Kjeldahl method. 1.0 gram of sample was weighed into a digestion flask and digested with concentrated sulphuric acid (H_2SO_4) in Kjeldahl digestion flask in presence of CuSO_4 and K_2SO_4 in the ratio of 9:1 respectively. Some pieces of pumic stone were added in order to prevent bumping. The Kjeldal method is conveniently divided into three steps - digestion, neutralization and titration.

During the digestion process nitrogen was converted into ammonia and other organic matter into CO_2 and H_2O . Acid was boiled for 40 minutes and the color of the mixture turned to dirty green which was indicative of complete digestion of the sample. The clear digested sample was transferred into 100 ml volumetric flask and made to the mark by repeated washing with distilled water after cooling at room temperature. Some Pieces of pumice stones were added in order to prevent bumping. Distillation was carried out by using steam distillation process. The distillate was collected in 20 mL boric acid (2% w/v) The tip of the condenser was completely immersed boric acid solution in order to prevent loss of ammonia. Ammonia liberated during the distillation was collected by the boric acid solution turning it into bluish green. The distillation was continued for 5 minutes after the solution in the conical flask has changed to bluish green. The distillate was titrated with 0.1 N HCl and the end point or titer was recorded. Methyl red was used as indicator to detect the end point.

The titer values obtained were used to calculate the total nitrogen content of the Samples

The percentage of crude protein was calculated by multiplying the value of nitrogen with an appropriate conversion factor $100/x$, where x is the percentage of nitrogen in the food protein.

$$\% \text{ crude protein} = \% \text{ total nitrogen} \times 6.25$$

$$\% \text{ of nitrogen} = \frac{100 \times (V_A - V_B) \times N_A \times 0.014}{\text{weight of sample}} \times 100$$

Where,

V_A = Volume in ml of standard acid used in titration of the sample

V_B = Volume in ml of the standard acid used in the titration of the blank

N = Normality of the acid used

0.0140 = mille equivalent weight of nitrogen

Carbohydrate content was determined by difference of total contents of ash, fat, moisture and protein from 100

$$\text{Total carbohydrate} = 100 - (\% \text{ of ash} + \% \text{ of moisture} + \% \text{ of fat} + \% \text{ of protein})$$

Nutritive value is finally determined by the following formula

$$\text{Nutritive value} = 4 \times \text{percentage of protein} + 9 \times \text{percentage of Fat} + 4 \times \text{percentage of carbohydrate}$$

Determination of sodium, potassium, calcium, chlorine, fluoride, Iodine and Iron by flame photometer.⁸

Principle - The flame photometer measures the emission of radiant energy when atoms of an element return to their extraction by the high temperature of the flame. The degree of emission are related to the concentration of the element in the solution.

Method- Na, K and Ca analysis of the samples was done by the method of flame photometry. Standard solutions of 20 were used for Ca analysis where as standard solutions of

20 ppm were used for the determination of Na and K, Cl, F, I, Fe

Preparation of the extracts for antibacterial screening:

The extracts were prepared in three solvents, i.e., methanol, ethanol and distilled water by using soxhlet apparatus. Then the extracts were concentrated under reduced pressure by using Rota Vapor at 40⁰-45⁰C. Concentrated extracts were centrifuged in centrifuged machine at 2500 rpm and sediment was air dried to obtain the powder. The presence of various natural products as alkaloids, protein, flavanoids, carbohydrates, steroids, tannins etc was tested by usual methods⁹.

Preparation of discs for antibacterial screening :

100 mg powder was dissolved in 100 ml distilled water and shaken well to emulsify. Emulsified sample was placed on sensitive disc made by Whatman no. 1, filter paper and left for one week to air dry. Now the sample powder was spread well on sensitive disc.

Preparation of media bacteria culture:

Taken 2.5gm of Mac Conkey agar powder and boil in 50 ml of distilled water for 15 min and placed in petri dishes and allowed to cool. Placed in an incubator at 37⁰C. Now the collected samples of bacteria were placed on Mac Conkey media with the help of Platinum spreader and kept in an incubator for 24 hour. Growth of bacteria was seen and gram stained.

Preparation of the growth media (Nutrient agar media):

34.0 gm of nutrient agar was dissolved in 1000 ml of distilled water then heated to boiling. Liquid agar was poured in several flasks (400ml) which were then closed with sterile cotton & aluminum foil. The flasks were autoclaved for 45 min. 28-30 ml of autoclaved nutrient agar was added into a 100 ml diameter Petri plate to obtain media from 5-6mm in depth. The plates were kept at room temperature to solidify.

Inoculation with activated bacterial strain:

The agar plates were inoculated by touch a sterile Platinum spreader swab to one mature colony from the activated culture plate (its age 24h) and evenly streaking the swab in three directions over the entire surface of the plates, which were then allowed to dry. The impregnated disc and the controls were placed in the medium suitably apart, sterile disc each containing 20 mg of the different agents were placed on the surface of a streaked nutrient agar plate inoculated with each bacterium were placed on the surface of the streaked nutrient agar plate inoculated with *E. coli* and *S. aureus* the plates were incubated at 5°C for 1 hour to permit good diffusion then transferred to an incubator at 37°C with reading taking after 24 hour. The anti bacterial activities of the crude samples were determined by the inhibition zone diameters formed by these samples against the bacterial strain. Erosion seen in the sensitivity disc indicates the sensitivity of sample towards bacteria.

Results and Discussion

Results of nutrition related value are given in Table – I, which are the average of three runs carried out each. The results of concentration of some biologically active micro nutrients are given in Table- II, the results of the presence of specific natural products are summarized in Table-III and the results of antibacterial activity of different extracts are given in Table IV.

**Table-I,
Proximate analysis of nutrients present in
100gm *Trachyspermum copticum*.**

	I	II	III	Mean
Carbohydrate	42.9	42.7	43.01	42.87
Moisture	7.9	7.7	7.7	7.7
Crude fat	5.4	5.1	5.1	5.2
Crude fibre	5.6	5.5	5.6	5.56
Crude protein	24.2	24.3	24.3	24.26
Ash	4.5	5.1	5.1	4.9

From the data presented in table - II, it was found that *Trachyspermum-copticum*. contains 7.7% moisture, 24.26%protein, 5.2% fat, 5.56% fibre and 4.9% ash. Protein supports the immune system, repairs damaged tissues and are needed to form blood cells⁵.

Since, the data presented in table –III, confirms the presence of various secondary metabolites (e.g. alkaloids, flavanoids, tepenoids, saponins and tannins) in *Trachyspermum-copticum*., it supports the use of plant in the treatment of various diseases. The nutritive value of *Trachyspermum copticum* was calculated 305 Kcal/ 100gm which supports its use as food and good source of various important nutrients for live stock.

From the data presented in table -IV, it reveals that ethanol extract is strongly sensitive towards *E. coli* and *S. aureus* both, where as methanol extract shows moderate activity towards *E. coli* and *S. aureus*, on the other hand water extract shows negligible activity. The overall study reveals that ethanol extract of the seed is found to be significant in antimicrobial activity.

In the present study highest recorded activity of ethanolic extract of *Trachyspermum-copticum*. may be due to the fact that different solvents have different degree of solubility for different phytochemicals⁶. And it may be anticipated that more and more phytochemicals

Micro nutrients

Na	105
K	0.0
Ca	192
Cl	3.03
F	2.88
I	141.27
Fe	2.33

like alkaloids, flavanoids, steroids, saponins, terpenoids and tannins are present in this extract and hence the solvent is responsible to increase the potentiality of the biologically active constituents of *Trachyspermum-copticum*.

This study provides the information regarding the nutritional potential and justify the

use of plant in different diseases associated with deficiency of various nutrients. The results of this study highlight the importance of the plant in term of rich carbohydrates, protein, crude fiber, mineral composition. The value of nutrients from the sample has an importance to improve the benefits for health and therapeutic potential by targeting the compounds present in this seed.

Table-II
Concentration of some biologically active elements in ppm
Water Extract of *Trachyspermum copticum*

CHEMICAL CONSTITUENTS	TEST	WATER EXTRACT	ETHANOL EXTRACT
Carbohydrates	Fehling's test	+	+
Alkaloids	Mayer's test	+	+
	Wagner's test	-	-
Test for glycoside linkage	H ₂ SO ₄ test	+	+
	Legal's test	-	-
	Killer killani test	-	-
Test for amino acid	Ninhydrin test	+	+
	NaOH test	+	-
Test for saponins	Foam test	-	+
Test for tannins	lead acetate test	+	+
Test for steroids	Salkowski test	-	+

Table III - Phytochemical analysis of *Trachyspermum copticum*

Extract	Concentration of sample	Gram positive bacteria	Gram negative bacteria
Methanol	20 mg	++	+++
Ethanol	20 mg	++	++
Water	20 mg	+	+++
Streptomycin(10mg)	20 mg	+	+++

Table – IV -
Antibacterial screening of *Trachyspermum copticum*. Key to the symbols

Inhibition zone >9 mm = +++ (highly sensitive) 6-9 mm = ++ (moderate sensitive)
3-6 mm = + (slightly sensitive), <3 mm = - (no activity)

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