Phytochemical characterization of Caraway *(Carum carvi)* seed extract and its use as a potent medicinal agent

Chauhan Vivek, Devi Sunita, Sharma Sonaxi and Kanwar Shamsher S.*
Department of Biotechnology, Himachal Pradesh University, Summer Hill, 171005, INDIA
*kanwarss2000@yahoo.com

Abstract
Carum carvi (Caraway) is a member of Apiaceae family which originated in Asia. Due to its economic importance, it is widely cultivated. Caraway is the only annual species, commonly present in arable land, moist meadows and on low lands to mountains. Caraway has different applications in pharmaceutical and food industries. Phytochemical screening of different medicinal plants is helpful in identifying new sources of industrially and therapeutically important compounds.

In this study, Caraway obtained from forest surrounding the village Goherman, Lahaul and Spiti, Himachal Pradesh (India) was used. Seed extract of caraway extracted in methanol (MSE) and distilled water (WSE) was used for phytochemical analysis to determine the constituents of caraway seeds. Further, caraway seeds extract was checked for antibacterial activity [Staphylococcus aureus (ATCC 6538), Salmonella typhimurium (NCTC 74), Escherichia coli (ATCC 25922) and Staphylococcus epidermidis (ATCC 12228)], antioxidant activity and acid neutralizing activity. After analysing the results, it can be said that caraway seeds possess many medicinal properties and it can be used in enhancing human health.

Keywords: Phytochemical characterization, TLC analysis, antimicrobial, antacid, antioxidant.

Introduction

Carum carvi (Caraway) a member of Apiaceae family which originated in Asia.[26] Caraway is the only annual species out of 25 species in *Carum* genus. It is commonly present in arable land, moist meadows and on low lands to mountains. Caraway has high economic value due to its applications in pharmaceutical and food industries. Caraway is extensively used in many food products on account of its pleasant flavour and preservative properties and also has a high medicinal value as its fruits can be used to cure pneumonia and indigestion and can be used as carminative, appetizer and galactagogue.[26,36]

Caraway fruits possess expectorant, antispasmodic and stimulant effects which allow their use to treat constipation, nausea and stomach aches. They help in increased secretion of gastric juice and promote high discharge of bile-pigments which in turn increase the appetite and results in digestive stimulation.[8]

About 5000 years ago, first evidence of caraway was found in Middle Eastern Asia.[37] Today, it is known to be cultivated in many countries like Russia, Holland, Poland, the Netherlands, Hungary, Denmark, Romania, Bulgaria, Morocco, Syria, the USA, Turkey and India. In India, the species is available in wild in the high-altitude regions of Jammu and Kashmir (Zanskar, Ladakh and Lungna), Himachal Pradesh (Lahaul and Spiti, Pangi-Bharmour and Kinnaur) and is cultivated in hills (as summer crop) and in plains (Northern India) as annual winter crop.[9]

Caraway seeds are rich source of dietary fibre and about 100 g of seeds can fulfil daily recommended intake (38%) of fibre. A pungent, anise-like aroma and flavour of caraway fruit is an attribute of essential oils mostly anethole, limonene and carvone. Due to abundance of essential oils caraway is frequently added to sauerkraut. Caraway fruits are used as remedy against pneumonia, indigestion and as appetizer and carminative in different traditional systems. The roots of this plant also serve as a food source and are cooked as vegetable like parsnips or carrots. In addition, leaves of caraway are consumed as herbs similar to parsley[44]. On account of this diverse spectrum of caraway applications, an attempt was made to study caraway seeds and test them for their medical benefits.

Material and Methods

Collection of sample: Collection of a sample (Caraway) was done from forest surrounding the village Goherman (32°.37.47. N; 76°.52.37. E), Lahaul and Spiti, Himachal Pradesh (India).

Moisture content estimation: The moisture content of Caraway seeds was determined according to the Standard method of AOAC (1999). The sample was weighed and placed in a hot-air oven maintained at 37± 1ºC for 4 days. The material was then cooled to room temperature using a desiccator and the weight loss in percentage was reported as moisture content of the seeds.

Preparation of Caraway Seeds extract(s): Dried plant seeds were washed under running tap water, air dried and then homogenized to a fine powder. The powered preparations were stored in airtight glass vials. For preparing seeds extract(s), 1.0 g of dried powder extract was placed in 10.0 mL of different solvents (methanol, water, ethanol, chloroform and petroleum) in a conical flask, closed with

cotton plug and kept on a rotary shaker (200 rpm) for 48 h. The extract was filtered using a Whatmann filter paper no. 1 and the filtrate was centrifuged at 10,000 X g at 4ºC for 15 min. The supernatant was collected and left at room temperature for complete evaporation.[5]

The dried extract was stored in labeled sterile screw-capped bottles at 5ºC in the refrigerator until when required to use.[17] The leftover extract residue was dissolved in PBS (0.05 M Phosphate buffer saline, pH 7.2) to reach a final concentration. The extract was then sterilized by filtration using 0.22 µm Millipore filter(s). The filter-sterilized seed extract obtained was stored in a freezer -20°C in airtight vials to be used for further studies.[33] The yield of the extract in each of the solvents (methanol, water, ethanol, chloroform and petroleum) was determined.

Phytochemical analysis of plant extracts

Qualitative phytochemical investigation: The seed extracts prepared were used for screening of phytochemicals and other biologically active compounds.[46] Phytochemical analysis was carried out according to the previously reported methods (Table 1). The caraway seeds extracts prepared in different solvents were screened for the presence of terpenoids, flavonoids, steroids, carbohydrates, protein, phenolic compounds and tannins (Table 1).

Quantitative phytochemical analysis: Different tests were conducted to check for the quantitative analysis of phytochemical content of flavonoid, phenol, tannin and terpenoid present in the caraway seed extract (Table 2).

Thin Layer Chromatography (TLC) analysis of the phytochemical content of caraway seeds: The crude of caraway seeds extract was spotted on the baseline draw about 1.0 cm from the bottom of the TLC plate by using the 0.5 µL micropipette tip[39] and standard for each test was also spotted on the same line at a distance. Spots were dried and plate was developed in a chromatographic tank saturated with vapours of the mobile phase.[28]

A mobile phase was used for the movement of components of caraway seeds extract. The fully developed TLC plate was sprayed with various sprays (Table 3). R_f value was calculated by using standard. R_f value is "retardation factor" or "ratio to front" which could be calculated as:[48]

$$R_f = \frac{\text{Distance traveled by the compound from the origin}}{\text{Distance traveled by solvent from origin}}$$

These R_f values were calculated by observing discretely resolved spots on TLC plates.

Table 1
Test conducted for qualitative estimation of phytochemical content in caraway

S.N.	Phytochemicals	Chemical Tests
1	Terpenoids[16]	Salkowaski Test
2	Flavonoids[24]	Sodium Hydroxide Test
3	Tannins[10]	Ferric Chloride Test
4	Steroids[18]	Lieberman Burchard's Test
5	Carbohydrates[38]	Molisch Test
6	Phenols[10]	Ferric Chloride Test
7	Alkaloids[45]	Wagner's Test

Table 2
Quantitative analysis of phytochemicals of caraway seeds

S.N.	Phytoconstituent Estimation	Standard used
1	Total flavonoid content[41]	Quercetin
2	Phenol content[40]	Tannic acid
3	Tannin content[31]	Tannic acid
4	Terpenoid content[4]	Linalool

Table 3
Different solvents used for the TLC

Phytochemical	Solvent system (v:v)	Confirmatory test
Flavonoids	Chloroform: Methanol 1:1	$AlCl_3$ spray
Phenols	Chloroform: Methanol 1:1	3.0% $FeCl_3$ spray
Tannins	Chloroform: Water 6:4	5.0% $FeCl_3$ spray
Terpenoids	Chloroform: Methanol 1:1	Methanol: Sulphuric acid 95.0 mL:5.0 mL

Assay for antibacterial activity of Caraway seeds by well diffusion method: Antibacterial activities of the caraway seed extracts were tested using the well-diffusion method. A total of 4 bacterial strains that included *Staphylococcus aureus* (ATCC 6538), *Salmonella typhimurium* (NCTC 74), *Escherichia coli* (ATCC 25922) and *Staphylococcus epidermidis* (ATCC 12228) were employed to determine the antimicrobial activities of the extracts. Petri plates containing Mueller-Hinton (MH) agar were inoculated with selected bacterial strains using surface spreading method. With a sterile borer, uniform diameter (0.5 cm) wells were created in the MH-agar plates. The seeds extracts were poured into the well using a sterile auto-pipette. Plates were incubated at 35ºC± 0.5ºC, 24 h for the bacterial growth to appear.

The plates were observed for the zone clearance around the wells. The zones of inhibition(s) around the well (in mm) including the well diameter were recorded. The observations were taken in triplicate from three different directions and the average values were tabulated for the same. Values less than 8mm for the tested caraway seeds extract were considered as not active against microorganisms.[32]

Minimum inhibitory concentration (MIC) against selected bacterial strains: Dilution methods are best suited to determine the MIC of different antimicrobial agents. In dilution tests; selected microorganisms were tested for their ability to produce visible growth in 96-wells micro-plate containing broth (broth micro-dilution) and serial dilutions of the antimicrobial agent i.e. caraway seeds extract. The lowest concentration of caraway seed extract agent that was able to inhibit the visible growth of microorganisms was referred to its MIC against the selected strain.[2] To detect the presence or absence of microbial growth, resazurin dye was used which changes colour from blue to pink in presence of microbial growth.

Antioxidant activity

Free radical scavenging activity: The free radical scavenging effect of the caraway seed extract was done by a previously reported method.[40,43] As DPPH is light sensitive, its solution was kept in a vial wrapped with an aluminium foil. The DPPH radical scavenging capacity was estimated from the difference in absorbance for the sample and blank and expressed as a percentage of DPPH scavenging activity.[11] Activity was measured after which the A_{517} values of the reaction mixture were recorded.

$$\text{Scavenging of extracts (\%)} = \frac{(A_{control} - A_{sample})}{A_{control}} \times 100$$

The A_{517} value(s) decreased due to colour change from purple to yellow. This change occurs as radical is taken by antioxidants through and a stable DPPH molecule is formed.

Determination of acid-neutralizing capacity of Caraway seed extract(s): Two different methods were employed for analysis of antacid properties [6] of MSE and WSE of caraway seeds.

Acid neutralising capacity: To specified volume of juice (20.0 mL), an equivalent of minimum dosage was added to a beaker and the content was mixed for 1 minute over magnetic stirrer. Pipette out 8.57 mL of 1.0 N HCl into the preparation while continuing to stir on a magnetic stirrer. After addition of the acid, titration was done for excess hydrochloric acid with 0.5 N NaOH to attain stable pH of 3.5. The number of mEq of acid consumed was calculated using the following equation:[12]

$$\text{Total mEq} = (8.57 \times N_{HCl}) - (V_{NaOH} \times N_{NaOH})$$

where N_{HCl} and N_{NaOH} are the normalities of HCl acid and NaOH respectively and V_{NaOH} was the volume of NaOH used for titration.

Determination of the neutralization capacity *in vitro* using the titration method: Fordtran's model of titration was used to determine neutralization capacity of the caraway seeds extract.[22,35] Each test solution (20.0 mL) was placed in a beaker and is warmed to 37ºC. The stomach movement was imitated by continuously running the magnetic stirrer at 30 rpm. Artificial gastric juice was used to titre the test solution to the endpoint of pH 3 and its consumed volume (V) was measured for each test solution. Using the value of V, the H^+ consumed for each test solution was calculated:

H^+ consumed (mmol) = 0.063096×V (mL).

Statistical analysis: All the experiments were done in triplicate and the data was presented as mean± standard deviation using IBM SPSS software.

Results and Discussion

Moisture Content: The moisture content of caraway seeds after 4 days of complete drying was 3.9 g. The initial weight of caraway seeds was 94.53 g and their weight after incubation at 37± 1ºC for 4 days was 90.63 g. Thus a weight loss in percentage was recorded to be 4.12% (w/v).

Physiochemical analysis of Caraway seeds extract(s): Chemical tests were employed in the preliminary phytochemical screening of the caraway seed extract(s) in various solvents (methanol seed extract [MSE], water seed extract [WSE], ethanol seed extract [ESE], chloroform seed extract [CSE] and petroleum seed extract [PSE]) for various secondary metabolites such as terpenoids, flavonoids, steroids, carbohydrates, phenols, tannins and alkaloids (Table 4).[42,47] The phytochemical analysis revealed the excellent presence of phenolic compounds, terpenoids, tannins and flavonoids in MSE and WSE (Table 4). Thus, for further analysis, WSE and MSE of Caraway seeds were used. The quantitative assays for detection of prominent biochemical constituents in MSE and WSE for Caraway seeds were performed and results were recorded (Table 4).

Phytochemical screening of medicinal plants helps in identifying new sources of industrially and therapeutically important compounds.[19] The presence of compounds like terpenoids, flavonoid and other phytochemicals may attribute antibacterial activity of plants.[15] For example, phytochemicals such as terpenoids, flavonoids, tannins and steroids have anti-inflammatory effects.[1,27] Tannins may have potential values as cytotoxic agents.[20] Insecticidal and antimicrobial activities in many plants are due to their phenolic content.[25]

In some animal studies, terpenoids helped in decreasing blood sugar level.[14] Steroids possess analgesic properties[13] and also help in improving certain central nervous system activities. Flavonoid content was found to be 19.20µg/mg for MSE and 13.77µg/mg for WSE (Table 5); this was in accordance to a previous study which showed caraway to have about 25% content of flavonoids.[7] Previous studies also showed that caraway seed had high tannin content.[29]

Table 4
Qualitative phytochemical analysis of Caraway seeds extract

Phytochemical Tested	WSE	ESE	MSE	CSE	PSE
Terpenoids	++	-	++	+	-
Flavonoids	++	++	++	+	-
Steroids	+	+	+	-	-
Alkaloid	-	+	+	-	-
Carbohydrates	++	-	+	++	-
Phenols	++	+	++	-	-
Tannins	++	+	++	-	-

+: Indicates presence of tested phytochemicals.
++: Indicates excellent presence of tested phytochemicals.
-: Indicates absence of tested phytochemicals

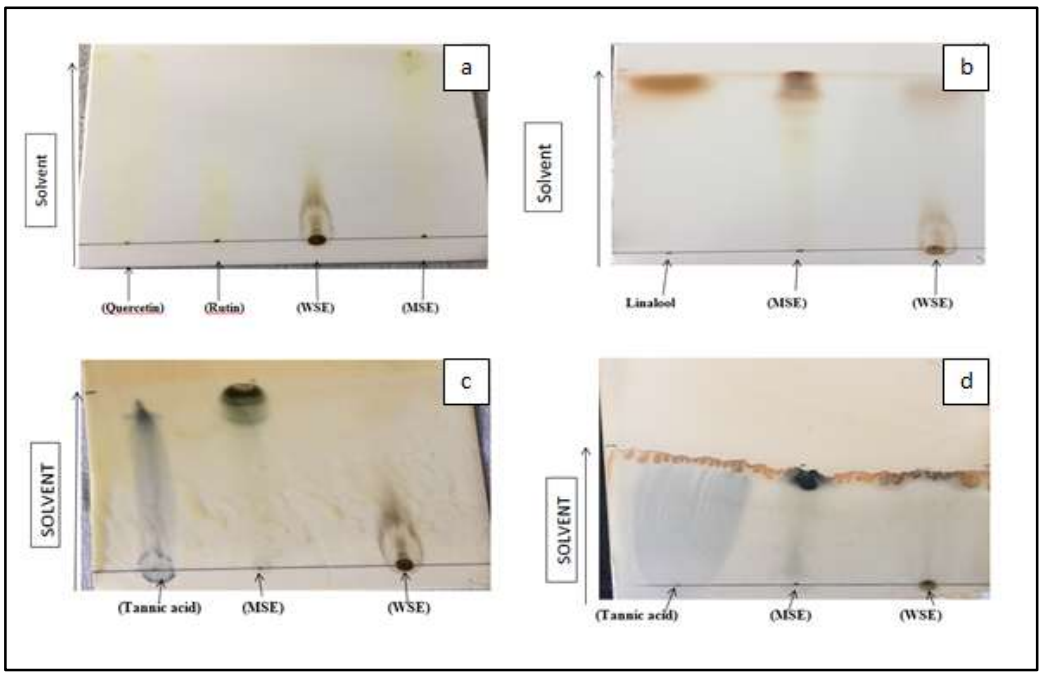

Figure 1: TLC analysis of caraway seeds extract(s) for a. flavonoids b. terpenoids c. phenols and d. tannins

Table 5
Quantitative phytochemical analysis of Caraway seeds extract

Phytochemical	MSE(µg/mg)	WSE (µg/mg)
Flavonoid	19.20± 1.34	13.77± 0.45
Phenol	3.45± 0.67	3.08± 0.57
Tannin	50.20± 0.87	43.80± 0.60
Terpenoid	17.40± 0.81	13.40± 0.55

Presence of the different constituents of the extracts was further analysed by TLC. WSE and MSE were checked for different biochemical constituents by comparing against different standards (Figure 1) and the Rf value was calculated for the same (Table 6). Extracting plant material in alcohol-water mixtures is most common method for preparation of plant extract for TLC. On comparing the Rf value with different standards (Table 6), the presence of different phytochemicals in caraway seeds was confirmed.

Assay for antibacterial activity in MSE and WSE: Activities of different seed extracts (MSE and WSE) against the test organisms were expressed as zone of inhibition (in mm) (Table 7). Zone of inhibition (mm) on MH-medium was checked for two different concentrations of the seed extract (5.0 mg/mL and 10.0 mg/mL). Further MIC was done using 96- vial titre plate to check for the minimum concentration of the seed extract required to inhibit growth of selected pathogenic microbes (Figure 2) (Table 8).

The seed extract of caraway was tested towards 4 pathogenic bacterial strains involved in various diseases in human beings and other animals. Only MSE preparations were effective against all the pathogenic bacterial strains (Table 7) that are causative agent(s) of human diseases like paratyphoid (*S. paratyphi*), Traveler's diarrhea (*E. coli*) and urinary tract infections (UTIs) or exist as common skin microflora (*S. aureus* and *S. epidermidis*).

Best antibacterial activity (maximum zone of inhibition) was observed for MSE against *S. aureus.* The least antibacterial activity was observed towards *S. epidermidis* and *E. coli*. This capability of MSE could be compared with the results of previous studies.[21,34] The antibacterial activity of *Carum carvi* can be attributed to the presence of terpenoids like linalool (Table 7) which inhibits the growth of gram-positive and gram-negative bacteria and also many fungi like *Aspergillus parasiticus* and yeasts.[29]

Many previous studies have found caraway to be effective in performing medium antimicrobial activity against many pathogens as *Escherichia coli, Staphylococcus aureus, Salmonella typhi, Mycobacterium tuberculosis* and *Vibrio cholerae*.[30]

Table 6
Rf value (cm) obtained for MSE and WSE in TLC analysis

Phytochemical	Standard used	Rf value (cm)		
		Standard	WSE	MSE
Flavonoid	Quercetin	0.94	0.96	0.35
	Rutin	0.37		
Phenol	Tannic acid	0.98	0.37	0.97
Tannin	Tannic acid	0.98	0.95	0.97
Terpenoid	Linalool	0.98	0.86	0.92

Table 7
Antibacterial activity of Caraway seeds extract

Test organism	Zone of inhibition (mm) M_1 (5.0 mg/mL)	Zone of inhibition (mm) M_2 (10.0 mg/mL)
Staphylococcus aureus (ATCC 6538)	20.3± 0.89	24.0± 1.22
Salmonella typhimurium (NCTC 74)	-	14.6± 1.66
Escherichia coli (ATCC 25922)	11.0± 1.23	13.0± 0.78
Staphylococcus epidermidis (ATCC 12228)	11.0± 0.76	14.3± 0.98

Table 8
MIC of MSE of *Carum carvi* against selected pathogenic bacterial strains

Microorganisms	MSE tested concentration (mg/mL)									
	10	5	2.5	1.25	0.625	0.312	0.156	0.078	0.039	MIC (mg/mL)
S. aureus (ATCC 6538)	-	-	-	-	-	-	+	+	+	0.312
S. typhimurium (NCTC 74)	-	-	-	+	+	+	+	+	+	2.5
E. coli (ATCC 25922)	-	-	-	-	-	+	+	+	+	0.625
S. epidermidis (ATCC 12228)	-	-	-	-	+	+	+	+	+	1.25

-Indicates no bacterial growth.
+Indicates turbidity/ growth of selected bacterial strains

The 2, 2-diphenyl picryl-1-picrylhydrazyl (DPPH) radical scavenging activities in both MSE and WSE of Caraway seeds were measured and recorded. The radical scavenging activity was measured as a decrease in the absorbance of DPPH or increase (%) in activity. Hence, best scavenging (%) activity (Figure 3) was observed in MSE of *Carum carvi* (99.6%). On the other hand, the WSE of *Carum carvi* showed 98% scavenging activity. The DPPH is stable free radical that can readily undergo scavenging by an antioxidant with maximum absorbance at 517 nm.

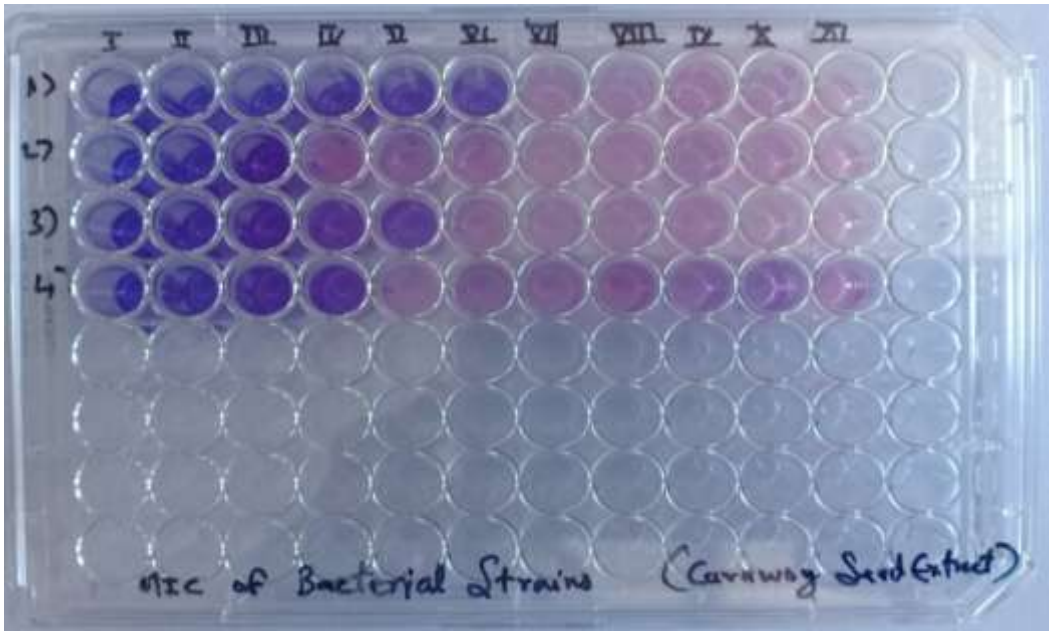

Figure 2: Minimum inhibitory concentration of MSE of *Carum carvi* against the selected pathogenic bacterial strains [1. *S. aureus* (ATCC 6538) 2. *S. typhimurium* (NCTC 74) 3. *E. coli* (ATCC 25922) 4. *S. epidermidis* (ATCC 12228)] and 5 Free radical scavenging (Antioxidant) activities of Caraway seeds extract (DPPH activity)]

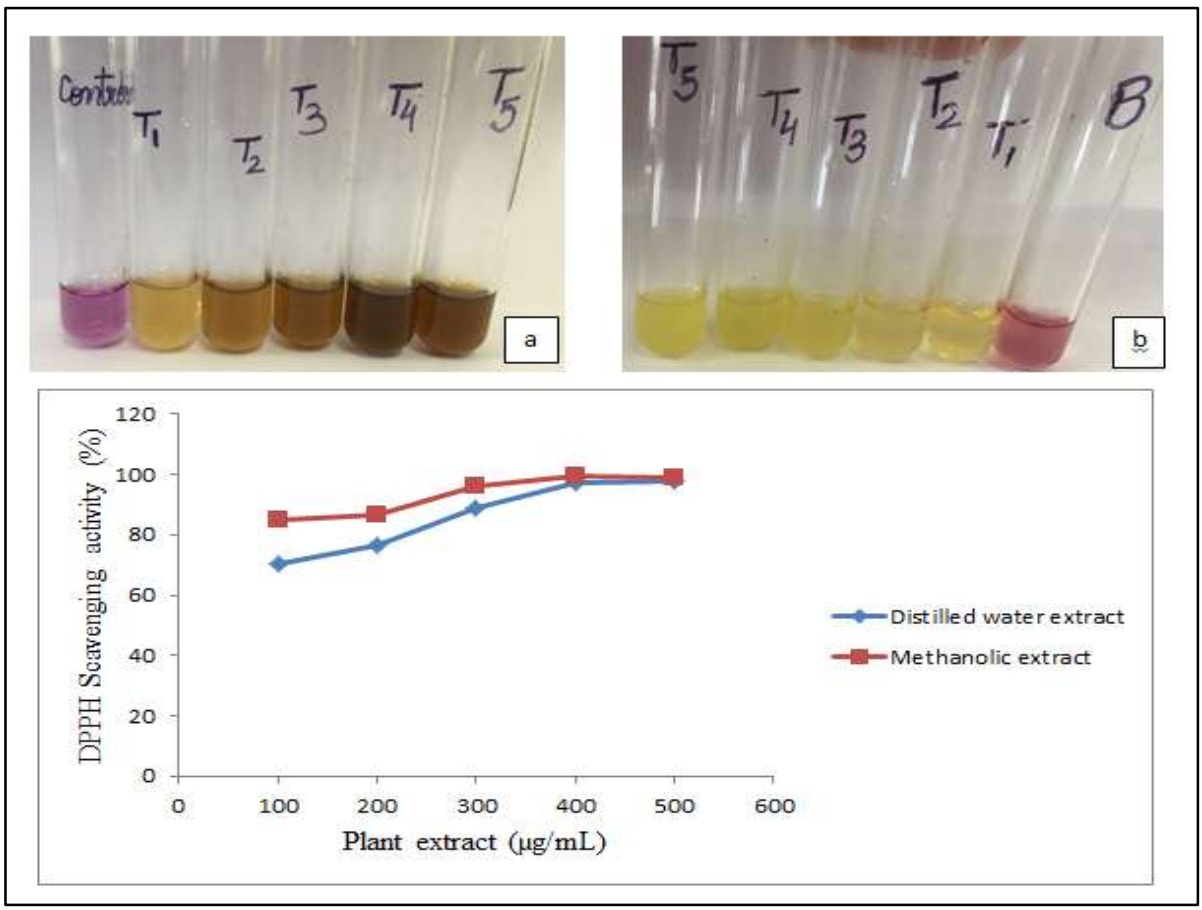

Figure 3: DPPH free radicals scavenging activity in Caraway seed in a. WSE and b. MSE.

Table 9
Quantity of NaOH required for neutralization

	MSE	WSE	ENO
Volume of 0.5 N NaOH used (mL)	10.0	6.0	5.00
mEq	5.57	3.57	6.07

Table 10
Acid-neutralization activity of MSE and WSE in an *in vitro* system

	WSE	MSE	ENO antacid (mL)
Consumed volume of gastric acid used (mL)	27.15± 0.34	4.50± 0.67	65.00± 0.115
mmol of H^+	1.70± 0.04	0.28± 0.02	4.10± 0.08

Caraway showed antioxidant activity in streptozotocin induced diabetic rats where it served as a natural hypoglycaemic antioxidant compound.[3] Many different studies have shown that plant derivatives can act as antioxidants by terminating the free radical thus preventing body from oxidative damage.[23] Antioxidants thus help in an extended protection against neurodegenerative, cardiovascular and chronic diseases. A potent scavenger of free radicals may serve as a possible preventative intervention for the microbial diseases.[23]

Both the extracts of caraway seeds in this study exhibited a potent antioxidant activity. Hence, the isolation and purification of therapeutic potential compounds from *Carum carvi* seeds could be used as an effective approach for management of many bacterial diseases in humans. The present study is in accordance to many previous works which provides the evidence that the extract of *Carum carvi* seeds is a potential source of natural antioxidant and antimicrobial compounds.[23]

Antacid property of Caraway seeds extract

Determination of acid-neutralising capacity (ANC): The acid-neutralization activity of both MSE and WSE of caraway seeds was tested and data were recorded (Table 9). A higher acid-neutralization activity of a compound/extract will give a faster relief against hyperacidity. It was observed that WSE had a high value of mEq (5.57) as compared to MSE mEq (3.57).

Determination of *in vitro* neutralization capacity of Caraway seeds extract by a titration method: Higher volume of use of gastric juice used indicated a higher mmol of H^+ indicating good neutralizing capacity, so WSE (27.0 mL) shows the maximum use of gastric acid as compared to MSE (4.5 mL) which suggests its greater acid neutralizing activity (Table 10).

Antacid properties[6] of the MSE and WSE were also studied by using two methods i.e. acid neutralising capacity[12] and Fordtran's Mode[22,35] respectively; all test solutions consumed significantly higher volumes of gastric acid which reveal the higher mmol of H^+ indicating their good acid neutralizing capacities. The acid in gastric juices kills different bacterial growth and also provides a suitable pH for initiation of digestion via pepsin. Increase in the acidic content may result in diseases as peptic ulcer, gastro oesophageal reflux and gastritis. Such diseases are mostly treated with H2 receptor antagonists, antacids and proton pump inhibitors.[22]

Conclusion

In this study, seed extract of *Carum carvi* was subjected to quite extensive phytochemical and clinical investigations. Different qualitative and quantitative testes for phytochemicals helped in determining the chemical constituents of the caraway seeds. Further, through experimental studies, anti-bacterial, antioxidant and antacid properties of the caraway seed extract(s) (MSE, WSE) were demonstrated. This study helped in depicting medical benefits of the caraway plant which can be a starting step towards a detailed clinical research for exploring its full therapeutic potential.

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