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Antimicrobial Activity and Phytochemical Analysis of Selected Indian Spices

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The present study was intended to assess the Phytochemicals screening and optimization of organic solvent for extraction of Phenolic compounds from five Indian spice extracts, namely Aniseed (*Pimpinella anisum*), Poppy seed (*Papaver somniferum*), cloves (*Syzygium aromaticum*), Fenugreek seeds *(Trigonella foenum-graecum)* and Ajwain *(Trachyspermum ammi*). All of these have been traditionally used in Ayurveda, and Unani medicine, and are still used in the alternative system of health care. The antimicrobial activity of these commonly used Indian spices was tested against food borne pathogenic bacteria and fungal, which are responsible for many health-related problems. These spice extracts were tested using DPPH method. The results showed that the extracts of clove and Ajwain had good antioxidant activity.

Key words: Indian spices, Phytochemicals, Phenolic compounds, Antioxidant activity, DPPH.

India is one of the largest producer, consumer and exporter of spices. India grows over fifty spices out of the eighty-six grown worldwide. Spices, which include leaves (coriander, mint), buds (clove), bulbs (garlic, onion), fruits (red chili, black pepper), stem (cinnamon), rhizomes (ginger) and other plant parts, have been defined as plant substances from indigenous or exotic origin, aromatic or with strong taste, used to enhance the taste of foods[1]. There is at present increasing interest both in the industry and in scientific research for spices and aromatic herbs because of their strong antioxidant and antimicrobial properties, which exceed many currently used natural and synthetic antioxidants. These properties are due to many substances, including some vitamins, flavonoids, terpenoids, carotenoids, phytoestrogens, minerals, etc. and render spices and some herbs or their antioxidant components as preservative agents in food[2]. The cloves are a commonly used household spice. Antioxidant, superoxide,dismutase, and catalase activities of Piper cubeba Linn. have been reported[3].

Antioxidants have a long history of use in the nutrition/health community and food industry. The traditional understanding has been that antioxidant chemicals promote health by removing reactive species that may otherwise exert harmful metabolic effects. In general, maximizing antioxidant concentrations is thought to minimize the risk for chronic disease[4]. Several studies have shown that spices are able to counteract oxidative stress in in vitro and in vivo systems[5,6]. In the present study, an attempt was made to evaluate the antioxidant and antimicrobial activities besides the Phytochemicals in five commonly used spices

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in Indian dishes with a view to evaluate their bioefficiency for their possible pharmaceutical applications.

MATERIALS AND METHODS

Spice materials

The following spices were used in this study and they were obtained from a local supermarket: Aniseed (*Pimpinella anisum*), Poppy seed (*Papaver somniferum*), Ajwain *(Trachyspermum ammi*), Fenugreek seeds *(Trigonella foenum-graecum)* and cloves (*Syzygium aromaticum*).

Preparation of extracts

A decoction method was used to obtain aqueous extracts of the spices. A total of 500 g of each spice was added to 2.5 L of boiling distilled water and left to simmer for 1 h. The solutions were allowed to cool, filtered using muslin cloth and stored at -20 °C when not in use. Some of the solutions obtained were freeze-dried and the powdered samples obtained were stored at 4 °C. And 25 g of the fine powder with Hexane, Ethanol, Methanol and Ethyl acetate separate by for extraction of Phytochemicals.

Phytochemical screening

A qualitative phytochemical test is performed to detect the presence of Phytosterols, Terpenoids, Flavanoids, Tannins, Saponin, Alkaloids, Carbohydrate and Cardiac Glycosides using standard procedures[7-10].

Test for terpenoids (Salkowski test)

To 0.5 g each of the extract was added 2 ml of chloroform. Concentrated H2S04 (3 ml) was carefully added to form a layer. A reddish brown coloration of the interface indicates the presence of terpenoids.

Test for flavonoids

Three methods were used to test for flavonoids. First, dilute ammonia (5 ml) was added to a portion of an aqueous filtrate of the extract. Concentrated sulphuric acid (1 ml) was added. A yellow colouration that disappears on standing indicates the presence of flavonoids. Second, a few drops of 1% Aluminium solution was added to a portion of the filtrate. A yellow coloration indicates the presence of flavonoids. Third, a portion of the extract was heated with 10 ml of ethyl acetate over a steam bath for 3 min. The mixture was filtered and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution. A yellow coloration indicates the presence of flavonoids.

Test for Saponins

To 0.5 g of extract was added 5 ml of distilled water in a test tube. The solution was shaken vigourously.and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigourously after which it was observed for the formation of an emulsion.

Test for tannins

About 0.5 g of the extract was boiled in 10 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black colouration

Test for alkaloids

0.5 g of extract was diluted to 10 ml with acid alcohol, boiled and filtered. To 5 ml of the filtrate was added 2 ml of dilute ammonia. 5 ml of chloroform was added and shaken gently to extract the alkaloidal base. The chloroform layer was extracted with 10 ml of acetic acid. This was divided into two portions. Mayer's reagent was added to one portion and Draggendorff's reagent to the other. The formation of a cream (with Mayer's reagent) or reddish brown precipitate (with Draggendorff's reagent) was regarded as positive for the presence of alkaloids.

Test for cardiac glycosides (Keller-Killiani test)

To 0.5 g of extract diluted to 5 ml in water was added with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlayed with 1 ml of concentrated sulphuric acid. A brown ring at the interface indicated the presence of a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer a greenish ring may form just above the brown ring and gradually spread throughout this layer.

Determination of total phenolic content

The total phenolic content of plant extract was determined using Folin-Ciocalteu reagent[11]. In brief, to 250 µl of Folin-Ciocalteu's reagent, 10 µl of sample was added, followed by 3.5 ml of deionised water. After 3 min, 1 ml of 20% sodium carbonate was added.The mixture was vortexed and incubated at 40 °C for 40 min. It was

allowed to cool in the dark. The absorbance was measured at 685 nm and all determinations were carried out in duplicates. A standard curve was obtained using various concentrations of Gallic acid. The results were expressed as mg Gallic acid equivalent/g dry weight of material.

Antioxidant activity (DPPH free radical scavenging activity) of methanolic extract

The antioxidant activity of the plant extracts and the standard was assessed on the basis of the radical scavenging effect of the stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH)-free radical activity by modified method[12]. The diluted working solutions of the test extracts were prepared in methanol. Ascorbic acid was used as standard in 1-100 µg/ml solution. 0.002% of DPPH was prepared in methanol and 1 ml of this solution was mixed with 1 ml of sample solution and standard solution separately. These solution mixtures were kept in dark for 30 min and optical density was measured at 517 nm using Cecil-Elect Spectrophotometer. Methanol (1 ml) with DPPH solution (0.002%, 1 ml) was used as blank. The optical density was recorded and % inhibition was calculated using the formula given below[13].

Percent (%) inhibition of DPPH activity=A-B×100/A

Antimicrobial activity by Paper disc diffusion technique (Kirby Bauer Technique)

The antimicrobial activity was evaluated using the agar diffusion method proposed by[14]. In order to produce an appropriate inoculums, an overnight culture (grown at 37ºC ±1.0) of Bacillus cereus (MTCC 430), Staphylococcous aureus (MTCC 3160),Eschericia .coli (MTCC 40) and Salmonella typhi (MTCC 3224) in Nutrient agar plates; Aspergillus niger (MTCC 281), Aspergillus oryzae (MTCC 624), Aspergillus Flavus (MTCC 227) and Peniciliium chrusogenum (MTCC 6795) in Rose Bengal agar plates. One ml of standardized suspensions of the microorganisms was deposited in Petri dishes (diameter 90 mm) and 18 ml of Rose Bengal Agar at 45ºC was added. Aliquots of 20 ml of the ethanol extract of filtered sample were applied to paper disks (6mm in diameter, Whatman No.1), which resulted in disks containing 200 µg of the sample. After evaporation of the loading solvent, each disk was placed at the centre of the Petri dishes containing previously inoculated Rose Bengal agar medium & Nutrient agar medium plates and incubated at 37ºC for 24 h. At the end of the incubation time, the diameter of microbial growth inhibition zone was measured in millimeters.

RESULTS AND DISCUSSION

Optimization of extraction solvent for Phytochemicals

Different organic solvents such as methanol, ethanol, ethyl acetate and water were used to extract the optimum yield of phenolic compounds in all spices. The results showed that the methanol gave good yield for phenolic compounds and followed by the Aniseed *(*20.7±5.0%*)*, Poppy seed (14.3±3.0%), cloves (92.2±10.0%), Fenugreek seeds *(*51.1±4.5%*)* and Ajwain *(*78.0±5.0%), The total phenolic content results coincide with the findings of [15]. For ethanol that showed results were Aniseed *(*18.3±3.0%*)*, Poppy seed (12.1±2.4%), cloves (86.4±4.5%), Fenugreek seeds *(*44.5±2.0%*)* and Ajwain *(*60.30±8.5%), was given in Table 1.

Phytochemical screening

The Phytochemical screening of the spices studied showed that the presence of Flavonoids, terpenoids, Phytosterols and alkaloids in Aniseed (*Pimpinella anisum*), Poppy seed (*Papaver somniferum*), cloves (*Syzygium aromaticum*), Fenugreek seeds *(Trigonella*

Table. 1. Optimization of extraction solvent for Phytochemicals

Spices	Recovery percentage of Phytochemicals			
	Hexane	Methanol	Ethanol	Ethyl acetate
Aniseed	15.3±6.5	20.7±5.0	18.3±3.0	5.69±10.0
Poppy Seed	3.6±1.8	14.3±3.0	12.1±2.4	4.8±1.2
Cloves	29.7±3.0	92.2±10.0	86.4±4.5	49.8±3.0
Fenugreek	17.8±6.0	51.1±4.5	44.5±2.0	14.75±1.0
Ajwain	33.5±4.0	78.0±5.0	60.30±8.5	44.3±2.0

foenum-graecum) and Ajwain *(Trachyspermum ammi*), (Table 2). The extracts of Aniseed, Poppy seed and Fenugreek have revealed the absence of tannins and saponins

Total phenolic content

With regards to the total phenolic content they varied from (312 ± 4.5) to (1540 ± 10.0) (Table2). Cloves had the highest Phenolic content (1540 ± 10.0 mg GA/100g DW) whilst aniseed had the lowest (312 ± 4.5 mg GA/100g DW) as given in table 3.

Table 2. Phytochemical constituent of the five spices

Phytochemicals	Spices				
	Aniseed	Poppy Seed	Cloves	Fenugreek	Ajwain
Phytosterols	+	+	+	+	+
Terpenoids	+	+	+	+	+
Flavanoids	+	+	+	+	+
Tannins	-	-	+	-	+
Saponins	-	-	-	-	-
Alkaloids	+	+	+	+	+
Carbohydrate	+	-	+	-	-
Cardiac Glycosides	+	+	-	-	+

(+) Presence of Phytochemical; and (-) Absence of Phytochemical.

Table 3. Total phenol contents of the five spices

Spices	mg Gallic acid equivalent/100g DW
Aniseed	312 ± 4.5
Poppy Seed	834 ± 2.0
Cloves	1540 ± 10.0
Fenugreek	670 ± 8.0
Ajwain	1240± 15.0

Antioxidant activity

Natural antioxidants that are present in herbs and spices are responsible for inhibiting or preventing the deleterious consequences of oxidative stress. Spices and herbs contain free

Table 4. Free radical scavenging activity (IC5O Values)

Samples	% free radical scavenging activity (IC5O Values)			
	R1	R2	R3	Mean
Aniseed	45.4	46.5	45.8	45.90
Poppy Seed	32.60	33.20	33.4	33.07
Cloves	81.5	83.2	82.7	82.47
Fenugreek	58.2	58.6	57.9	58.23
Ajwain	72.8	71.5	70.2	71.50

Table 5. Antimicrobial activity zone of Inhibition (mm)

Microorganisms	Antimicrobial activity zone of Inhibition (mm)				
	Aniseeds	Poppy seeds	Cloves	fenugreek	Ajwain
Bacillus cereus (MTCC 430)	0.10±0.03	0.14±0.05	1.80±0.54	0.12±0.05	1.35±0.13
Staphylococcous aureus (MTCC 3160)	2.00±0.31	2.00±0.14	3.94±0.40	0.56±0.25	3.11±1.02
Eschericia .coli (MTCC 40)	0.10±0.01	0.10±0.04	5.14±0.17	2.50±0.25	4.60±0.71
Salmonella typhi (MTCC 3224)	0.10±0.01	0.13±0.02	0.24±0.07	0.31±0.25	1.18±0.34
Aspergillus niger (MTCC 281)	2.0 ±0.47	3.00±0.15	0.70±0.15	0.30±0.16	1.20±0.07
Aspergillus oryzae (MTCC 624)	1.00±0.25	3.00±0.65	1.10±0.28	0.80±0.25	2.50±0.06
Aspergillus Flavus (MTCC 227)	2.00±0.04	1.50±0.04	0.40±0.20	0.40±0.25	2.10±0.21
Peniciliium chrusogenum (MTCC 6795)	1.00±0.03	2.00±0.78	0.20±0.25	0.50±0.07	1.00±0.02

radical scavengers like polyphenols and phenolic compounds. In the present paper, we have evaluated the free radical scavenger activity of methanolic extract of Aniseed, Poppy seed, Ajwain, Fenugreek seeds and cloves. Among the five extracts and standard tested for the in vitro antioxidant activity using the DPPH method, the crude methanolic extracts showed antioxidant activity, with IC50 values of Ajwain 71.50, Cloves 82.47 , Aniseed, 45.90, Fenugreek 58.23 and Poppy Seed 33.07 µg/ml, respectively (Table4).

The Phytochemical tests indicated the presence of alkaloids, glycosides and tannins in the crude methanolic extract. Several of such compounds are known to possess potent antioxidant activity[16]. Some of these constituents have already been isolated from this plant. Hence, the observed antioxidant activity may be due to the presence of any of these constituents. The plant exhibited strong anticancer, hepato-protective, antiviral and several other activities. These properties may be due to its antioxidant activity.

Antimicrobial activity

The antimicrobial activities of the spices' extracts results were given in table 5. The methanol extracts of the cloves showed the antibacterial activity zone of inhibition, B. cereus (1.80±0.54 mm), S. aureus (3.94±0.40mm), E.coli (5.14±0.17mm), S typhi (0.24±0.07 mm) and antifungal activity zone of inhibition A. niger (0.70±0.15 mm), A. oryzae (1.10±0.28 mm), A Flavus (0.40±0.20 mm) and P. chrusogenum (0.20±0.25).

The results of the present study suggested that several Phytochemicals as well as antioxidant properties are present in all the six spices extracts. The presence of the Phytochemicals can be correlated with the fact that solvent extracts showed antimicrobial activity against the bacterial and fungal strains. Phytochemicals give plants their colors, flavour, smell and are part of a plant‘s natural defense system and protect them against herbivorous insects and vertebrates, fungi, pathogens, and parasites[17].

CONCLUSION

In conclusion, the results of the present study further demonstrated that in Aniseed (*Pimpinella anisum*), Poppy seed (*Papaver somniferum*), cloves (*Syzygium aromaticum*), Fenugreek seeds *(Trigonella foenum-graecum)* and Ajwain *(Trachyspermum ammi*) possess varying degree of antimicrobial and antioxidant activity. These spices act through their natural inhibitory mechanisms by either inhibiting or killing the pathogens completely. With the increasing awareness of people towards natural food and natural therapies, spices might act as the most obvious alternative. For centuries, Indian spices have made a significant contribution both in the health care system and the food industry. Ancient Asian literature is a treasure of information related to the problems of health care and other environmental aspects. Indian spices have been used since ages in different traditional forms of medicine like Ayurveda, Unani and Sino Tibetian systems.

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