IOSR Journal of Applied Chemistry (IOSR-JAC)
e-ISSN: 2278-5736.Volume 11, Issue 11 Ver. I (November. 2018), PP 45-49
www.iosrjournals.org

Optimization of extraction process and investigation of Antioxidant Activity , DNA Protection Potential and Antimicrobial activities of Trachyspermum ammi seed extract

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Abstract: *The free radicals are produced by various metabolic processes in our body. In excess these free radicals can cause damage to important cellular organs. Antioxidants are substances which can prevent this damage by quenching free radicals. Antioxidants of plant origin are in demand due to less side effects. The plant Trachyspermum ammi (Ajwain) is used to cure many diseases and possess antioxidant and antimicrobial activities. The present research is an attempt to optimize extraction process to enhance antioxidant and antimicrobial properties of seed extract of Ajwain. The methanolic extracts of seeds were prepared by cold , hot and Soxhalate method. Folin-Ciocalteu spectrophotometric method was used to estimate TPC. The antioxidant activity was evaluated by DPPH method. The DNA damage was done using super coiled pBR322 plasmid and antimicrobial activity was assessed by well diffusion method. The results showed all extracts possess antioxidant activity and have ability to prevent DNA damage. The positive antimicrobial property was shown by extract obtained from cold extraction method. The cold extraction method was found to be more effective in enhancing medicinal property of extract.*

Keywords: *Antioxidants, DNA Damage , DPPH, TPC*

Date of Submission: 18-11-2018 Date of acceptance: 04-12-2018

I. Introduction

The spices, herbs and parts of trees are source of medicinal preparation since ancient times. Therefore in Ayurveda, the Indian System of Medicine and Unani System of Medicine extensive research is done to study the medicinal properties of plants. The plant Trachyspermum ammi belongs to family Apiaceae and is commonly known as Ajwain in Hindi, Bishop's weed in English, Yamini in Sanskrit, Ajma in Gujrati, Kath in Kashmiri and Omam in Tamil [1]. The seed extract of Ajwain is a traditional medicine, used for treatments of abdominal discomfort, diarrhea, cough and stomach troubles [2].

The research has reported seeds of Ajwain possess medicinal activities such as antioxidant [2], antimicrobial[3], hypolipidemic[4], antihypertensive[5], antispasmodic[5], antilithiasis and diuretic[6], Antitussive[7]. The free radicals are produced in human body due to various metabolic processes. These free radicals in excess have ability to damage important cellular components of body. Antioxidants are substance which can prevent cellular by quenching free radicals. Therefore diet rich in antioxidant plays an important role in preventing disease. The natural source of antioxidants are in demand because of high cost and side effects of synthetic antioxidants. The study of ajwain plant has revealed that it possess antimicrobial and antioxidant properties. The past research has also reported that the presence of phenolic compound is responsible for antioxidant activity of plants[8-10] .

The aim of present research was to optimize extraction process to prepare antioxidant rich Ajwain seed extract and investigate total phenolic content, antioxidant activity , DNA protection potential and antimicrobial activities.

II. Material And Methods

Plant material: The seeds of Ajwain were purchased from local market from Mumbai, Maharashtra, India.

0.25% Bromophenol blue, Xylene Cyanol FF 0.25%, Glycerol 30% , 7.5% sodium carbonate, Tris base, Glacial acetic acid, EDTA , Methanol . All reagents and chemical used were of Analytical grade.

Chemicals : All reagents and chemical used were of Analytical grade.
DPPH Assay : DPPH extra pure procured from SRL Pvt. Ltd. and methanol
TPC : Folin-Ciocalteu reagent , 7.5% sodium carbonate, Gallic acid

DNA Damage Assay : Agarose from Sigma chemicals and Ethidium bromide (EtBr) from HiMedia. Dipotassium hydrogen phosphate, potassium dihydrogen phosphate and hydrogen peroxide from SD Fine Chemicals Ltd. pBR322 was procured from Thermo Fisher. 0.25% Bromophenol blue, Xylene Cyanol FF 0.25%, Glycerol 30% , Tris base, , 7.5% sodium carbonate, Glacial acetic acid, EDTA , Methanol,
Antimicrobial study : The bacterial and fungal cultures Staphylococcus aureus, Escherichia coli, Bacillus cereus , Pseudomonas aeruginosa, Aspergillus spp., Candida albicans were procured from Department of Microbiology , Royal College, Mira Road. Thane, Maharashtra, India. GYEA (Glucose Yeast Extract Agar) from Prerana enterprises

Preparation of Extracts :
The seeds of Ajwain were dried at room temperature. The methanolic extracts were prepared by three methods :

Soxhlet Extraction: The dried seeds (15g) which were soaked overnight in methanol were loaded in Soxhalate extractor using apx 100ml methanol. The extraction was carried out for 6 hours. To prevent evaporation of solvent ice cold water was circulated through condenser. The extract collected was dried and weighed . The brownish residue obtained was dissolved in known quantity of methanol.

Cold Extraction: The dried 15g seeds were soaked overnight in 100ml methanol and then mixed in a shaker at 240 rpm for 6 hours. The seed solution was stored in refrigerator at 4^0C for 15 hour. The cold solution was filtered using Whatmann filter paper. The filtrate was dried at 35^0C in water bath and extract was prepared by dissolving known quantity of residue in methanol.

Hot Extraction : The seeds (15g) soaked in 100 ml methanol for 12 hours and boiled gradually for 20 minutes using heating mentle. The solution was placed in water bath for 6 hours at 50^0C and filtered using Whatmann filter paper. The extract was dried in thermostat at 35^0C and known weight of residue was dissolved in methanol.

Determination of total phenolic content (TPC) :
To evaluate TPC, Folin-Ciocalteu spectrophotometric method described by Singleton and Rossi with some modification was used[11] . To 5ml of Folin-Ciocalte reagent (diluted 10 fold) 1ml of extract and 4ml of 7.5% sodium carbonate was added. The mixture was vortex for 15 sec. The blank / control sample was prepared by adding all the reagents except extract. It was incubated for 30 min at 40^0 C . The absorbance was measured at 765 nm using a spectrophotometer. Total phenols were expressed as Gallic acid equivalents (GAE) per gram dry weight of seeds using calibration curve.

DPPH (1, 1-diphenyl-2- picrylhydrazyl) radical scavenging assay:
The modified method described by Miliauskas et.al [12,13] was used to measure free radical scavenging activity of the extracts. The methanolic DPPH solid was prepared by dissolving 5mg in 100 ml. Then initial absorbance of blank solution was adjusted between 0.9 – 1.0 at 515nm by dilution. The aqueous seed extract of different concentrations (100 -1000µg/ml) was prepared. 3 ml of DPPH solution was mixed with 0.5ml of seed extract and kept in dark for 30 minutes. The control was prepared by mixing 3ml DPPH with 0.5ml methanol. The absorbance at 515nm was measured using Spectrophotometer. The solution of ascorbic acid (5 -100 µg/ml) was used as standard. The experiment was performed in triplicate. The % inhibition was calculated using relation

$$\%\ Inhibition = \frac{\text{Absorbance of control - Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

DNA Damage Inhibition Efficiency
The slightly modified method of Lee et al was used to measure the ability of each extract to prevent DNA damage [14]. To study the protective potential of extract pBR322 plasmid DNA (0.25 µg) in 100 mM potassium phosphate buffer (pH 7.4) was incubated with H_2O_2 (600 mM) .in presence and absence of Ajwain extract (10 µg). The tubes were incubated at 37°C for three hours. pBR 322 plasmid DNA (0.25 µg) in 100 mM potassium phosphate buffer (pH 7.4)along with methanol was kept as a control.

Antimicrobial Assay
The antimicrobial studies were done using Agar well diffusion method Wells were made in nutrient agar plate using bacterial. cultures *Staphyllococcus aureus*, *Escherichia coli*, *Bacillus cereus* and *Pseudomonas aeruginosa* and fungal cultures Aspergillus spp. and Candida albicans. The aqueous extracts were prepared of

concentration 1000 µg/ml and 10,000 µg/ml . 50µl of extracts were added along with control into the wells and allowed to diffuse in refrigerator for 10 minutes. The plates were incubated for 24 hours at 37^0C. The plate of Aspergillus spp was incubated at room temperature for 48 hours. The diameter of inhibition zone (mm) was measured. The analysis was done in duplicate for each extract.

III. Results and Discussion

Total Phenolic Content : The calibration curve of Gallic acid (GA) was used for estimation of total phenolic content . Calibration curve was represented by the linear equation

$Y=0.002 X - 0.027$, $R^2 = 0.973$

The TPC of each extract was calculated in terms of Gallic acid equivalent per gram (GAE/g dry extract). All extracts showed significancant amount of total phenolic concentration (Table 1). However extract prepared by Hot and Cold techniques was comparatively rich in total phenolic content. The extract prepared by Soxhalate method showed minimum TPC. The research of herbal extracts from plant and spices have reported that decrease in oxidative stress is due to presence of polyphenolic content[15]

Table 1 : Total Phenolic content of Ajwain Seed Extracts

Technique	mg GAE/g dry extract
Soxhalate Extraction	15.008
Hot Extraction	19.434
Cold Extraction	20.359

Scavenging Effect on DPPH :

The DPPH assay is a quick method commonly used for the evaluation of the antioxidative potential of natural products[16]. The deep violet methanolic solution of 2,2- Diphenyl-1-picrylhydrazyl (DPPH˙) gives a strong absorption band at 517 nm due to presence of an odd electron. This electron gets paired by accepting proton from an antioxidant producing a change in colour of DPPH solution from violet to yellow. The extent of decolourisation is a measure of antioxidant activity. Fig. 1 shows the inhibitory effect of all the extracts of Ajwain seeds in comparison with standard ascorbic acid.

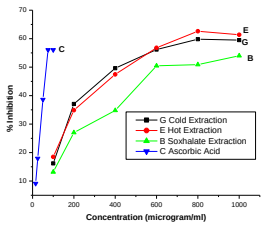

Fig : 1 DPPH Scavenging assay of
Methanolic extract of Ajwain seeds

The experimental data indicates that all extracts have the proton donating ability but it is lesser than ascorbic acid. The extract obtained by hot and cold method displayed similar antioxidant activity. The Soxhalate extract showed lesser antioxidant property at all concentration.

DNA Damage Assay

The protective effect of T. ammi seed extract against H_2O_2 induced DNA damage is presented in Fig. 2.

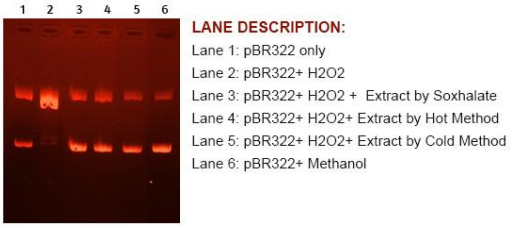

Fig.2. Effect of Ajwain seed extract on DNA damage

Presence of H_2O_2 enhanced DNA strand breakage by converting the super coiled form into circular form in the same manner as that of pBR 322 (Fig. 1; Lane 2). The incubation of DNA with H_2O_2 along with seed extract of Ajwain (10 µg) inhibited the damage caused by strand breakage and the maximum is retained in the super coiled form (Fig. 1; Lane 3- Lane 5). Also results indicate that Ajwain seed extract by hot and cold method showed better protection against DNA damage (lane 4 and 5) as compared to lane 3.

Antimicrobial Activity

The antimicrobial activities of all extracts were studied against four bacterial strains and two fungal strains. Results obtained by agar well diffusion technique, as a qualitative method, are summarized in Table 2.

Table 2 : Antimicrobial Activity of Ajwain extracts by Well diffusion method

Concentration of extract (1000 ppm & 10000ppm)	Zone of Inhibition (mm)					
	Bacterial species				Fungal species	
	S. aureus	*B. cereus*	*Pseudomonas aeruginosa*	*E. coli*	*Aspergillus spp.*	*C. albicans*
Extraction Method						
Soxhalate	-	-	-	-	-	-
Hot	-	-	-	-	-	-
Cold	-	*11.5*	-	*10*	-	-

The past research has reported that the antimicrobial activity of ajwain is due to carvacol and thymol[17]. Depending on concentration, thymol and carvacol, are known to be either bactericidal or bacteriostatic agents[18].

The results of well diffusion method showed that only extract prepared by cold method showed activity against B.cereus and E.coli at both the concentration 1000ppm and 10000ppm.Increase in concentration showed no change in activity. *S.aureus ,Pseudomonas aeruginosa*, *Aspergillus spp.* and *Candida albicans* was found to be resistant to all extracts.

IV. Conclusion

Trachyspermum ammi (Ajwain) is a medicinal plant and is used as traditional medicine. The seed extract prepared by all the three methods showed significant antioxidant activity due to high total phenolic concentration. The results of both DPPH scavenging activity and DNA damage assay have proved Hot and Cold method of extraction equally effective and better than Soxhalate. From the data of present study it can be concluded that cold extraction enhances the antioxidant and antimicrobial activity.

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Aqeela A. S. Qureshi " Optimization of extraction process and investigation of Antioxidant Activity , DNA Protection Potential and Antimicrobial activities of Trachyspermum ammi seed extract." IOSR Journal of Applied Chemistry (IOSR-JAC) 11.11 (2018): 45-49.