e-ISSN:2321-6182
p-ISSN:2347-2332

RESEARCH AND REVIEWS: JOURNAL OF PHARMACOGNOSY AND PHYTOCHEMISTRY

Study of Physicochemical Properties, Antibacterial and GC-MS Analysis of Essential Oil of the Aniseed (Pimpinella anisum Linn.) in Oman.

Sumaiya Mohammed Aslam Albulushi, Hanan Al Saidi, Nirmala Amaresh, and AR Mullaicharam*.

Oman Medical College, Muscat, Sultanate of Oman.

Research Article

Received: 20/08/2014
Revised: 15/09/2014
Accepted: 18/09/2014

*For Correspondence

Oman Medical College, Muscat, Sultanate of Oman.

Keywords: Extraction, antioxidant, antimicrobial test, Gas chromatography, mass spectrometry (GC-MS), Pimpinella anisum Linn.

ABSTRACT

Aniseed plant (*Pimpinella anisum* Linn.) is a flowering medicinal plant and are annual important spices belonging to the family of Apiaceae native to eastern Mediterranean region and Southwest Asia. Aniseed (Pimpinella anisum Linn.) essential oil has been widely used in aromatherapy for breathing difficulties as well as has a good effect on asthma as a natural asthma remedy. Aim The aim of the project is to extract essential oil present in Saunf (aniseed). Objective The main objective of the present study was focused on identification and quantification of chemical constituents present in the essential oil of aniseed by GC-MS methods. Essential oils may be defined as volatile odoriferous oils of vegetables origin. Essential oils are aromatic substances widely used in the Perfume industry, Food and Flavoring, Cosmetics, and Pharmaceutical Products. Aniseed plant (Pimpinella anisum) is an annual belonging to the family Apiaceae . It is a colorless or pale-yellow liquid having the characteristic odor and taste of the fruit. Methods In this study the essential oil of aniseed was extracted by steam distillation. Many chemical constituents were found by gas chromatography and mass spectrometry (GS-MS) analysis from the essential oil of aniseed. It is highly soluble in organic solvent. In addition, the extracted aniseed oil was tested for antimicrobial and antioxidant properties. Result: The essential oil showed resistance properties on E.coli, Staphylococcus aureus, pseudomonas aeruginosa and Klebsiella; however aniseed oil was not particularly inhibitory to bacteria. The antioxidant activity was assessed by the DPPH free radical scavenging method. The total phenolic content was determined with the Folin Ciocalteu Reagent (FCR).Refractive index was calculated (1.4765).

INTRODUCTION

Essential oils contain highly volatile substances that are isolated by a physical method or process from plants of a single botanical species. The oils normally bear the name of the plant species from which they are derived. Essential oils are so termed as they are believed to represent the very essence of odor and flavor. Essential oil plants and culinary herbs include a broad range of plant species that are used for their aromatic value as flavorings in foods and beverages and as fragrances in pharmaceutical and industrial products. Essential oils derive from aromatic plants of many genera distributed worldwide [1].

Aniseed plant (Pimpinella anisum Linn.) is a flowering medicinal plant and is an annual important spice belonging to the family of Apiaceae native to eastern Mediterranean region and Southwest Asia. Anise is believed to be Asian in origin but is currently found in Central and Southern Europe, Egypt, Russia, Cyprus, Syria and North America. Most commercial seed is imported from Turkey, followed by Spain and China [18]. Today, aniseeds are an important raw material which is used for pharmaceutics, perfumery, food and cosmetic industries [2]. Recently, this spice plant has drawn more consideration of consumers due to the antimicrobial, antifungal, insecticidal and anti-oxidative effect of this herb on

e-ISSN:2321-6182
p-ISSN:2347-2332

human health. The world production of aniseed essential oil amounts to 40-50 tons per annum. Because of aniseed plant favors warm climatic conditions throughout the growing season it is cultivated particularly subtropical regions [3,4]. The quantity of aniseed is determined mainly by the essential oil content and its composition.

Aniseed plant is an herbaceous annual plant growing to 3 ft (0.91 m) tall. The leaves at the base of the plant are simple, 0.5-2 in (1.3-5.1 cm) long and shallowly lobed, while leaves higher on the stems are feathery pinnate, divided into numerous leaves. The flowers are white, approximately 3 mm diameter, produced in dense umbels. The fruit is an oblong dry schizocarp; 3-5 mm long [5]. The essential oil is located in the schizogenic oil ducts of anise fruit and shoots [6].

Figure 1: Pimpinella anisum flowers

Figure 2: Pimpinella anisum fruits

Essential oil of the genus Pimpinella is a complex mixture of various components that contain sesqquterpens, phenolic compounds (C6-C3) and alkenes. The essential oil is located in the schizogenic oil ducts of fruits, shoots and roots. According to European Pharmacopoeia aniseed as drugs must have an essential oil concentration higher than 2% [7].

It is also clear that the concentration of essential oil can significantly vary among aniseed from different origins [8-10]. Aniseed as well as aniseed essential oil has medicinal values. The aniseed tea is used for children's flatulence, upper respiratory tract problems and bronchial asthmatic attacks [11]. Trans –anethole (4-methoxyphenyl-1-propane), the major component of aniseed oil, is precursor that can produce 2,5-dimethoxybenzaldehyde which is used in the synthesis of psychedelic drugs such as DOB (2,5 dimethoxy-4-bromoamphetamine) [12]. Aniseed is useful in destroying body lice [13], head lice and itching insects11 and the oil can be used by itself [14], which makes it helpful for prediculosis, the skin conditions caused by lice [15]. It can also be used for scabies [16], where it may be applied is an externally in an ointment base [14].

Essential oil of aniseed is used in perfumery, soaps and other toilet-articles and for flavoring culinary preparations, confectionery, beverages and liquor anisette. It is used in perfuming sachets, dental preparations and mouth washes; it is also used in the manufacture of liquors. It has medicinal values also. Oil of aniseed is also reported to be used as an aromatic carminative to relieve flatulence and as an ingredient of cough-lozenges in combination with liquorice. It is a mild expectorant and is used as an antiseptic, and for the treatment of cholera. It may be used in the preparation of gripe water [17].

Essential oils have been shown to possess antibacterial, antifungal, antiviral, insecticidal and antioxidant properties [19,20]. Some oils have been used in cancer treatment [21]. Some other oils have been used in food preservation [22], aromatherapy [23] and fragrance industries [24]. Essential oils are a rich source of biologically active compounds. There has been an increased interest in looking at antimicrobial properties of extracts from aromatic plants of essential oils, in our project we particularly focus on Aniseed Oil [25]. Therefore, it is reasonable to expect a variety of plant compounds in these oils with specific as well as general antimicrobial activity and antibiotic potential [26].

Steam distillation is used in the extraction of Essential Oil from the plant material. It is a special type of distillation or a separation process for temperature sensitive materials like oils, resins, hydrocarbons, etc. which are insoluble in water and may decompose at their boiling point.

The fundamental nature of steam distillation is that it enables a compound or mixture of compounds to be distilled at a temperature substantially below that of the boiling point(s) of the individual constituent(s). Essential Oil contains components with boiling points up to 200°C or higher temperatures. In the presence of steam or boiling water, however, these substances are volatilized at a temperature close to 100°C, at atmospheric pressure [27].

e-ISSN:2321-6182
p-ISSN:2347-2332

Analysis of Essential Oil is done by using Gas Chromatography. The qualitative and quantitative analysis is done to know the constituents in the oil and the percentage of components present in the oil respectively, by doing so we can know the purity of that particular oil [28].

Aim and Objectives

• To validate that the extracted essential oil has the following properties: anti-bacterial activity, anti-oxidant activity.

Objectives

• To extract essential oil from aniseed by stem distillation.
• To perform physicochemical properties for Aniseed Oil.
• To estimate total phenolic content of Aniseed Oil.
• To determine in-vitro anti-oxidant activity by DPPH.
• To screen the Aniseed Oil for its anti-bacterial activity.

MATERIALS AND METHODS

Plant Material

The fresh aniseed is available in the markets from different companies, AlShahi and Al. The collected samples were ground by mechanical grinder, for 15 minutes to obtain powder and conserved for extraction.

Extraction of Essential Oil

There are a number of methods employed for the extraction of essential oil from the plant. In the present study steam distillation method was used. In the process, 50g of sample were taken in a 500mL round-bottom flask. Then 250mL of distilled water and boiling chips were added and mixed with a stirring rod.

Then, distillation was began with a gentle flow of water through the condenser and continued until approximately 100mL of distillate had been collected and cooled to room temperature. The essential oil was lighter than water and so could be separated out. The steam distilled essential oil layer which was collected over water, was extracted and washed with analytical grade chloroform in a 125mL separatory funnel. The chloroform extract of the oil was dried over anhydrous Na2S04 and then filtered. It was collected in vial and kept in the dark, until the moment of analysis. The chloroform was removed in vacuum condition. Thus the essential oil of fresh aniseed was collected.

GC-MS Analysis

The essential oil of Pimpinella anisum Linn. (Aniseed) of two varieties were analyzed by Electron Impact Ionization (EI) method on GC-17A gas chromatograph, coupled to a GC-MS 2010 plus mass spectrometer; fused silica capillary column temperature of 40˚ C (was held 2 min) was maintained with carrier gas helium at a constant pressure of 90kPa. Samples were injected by splitting with the split ratio 10. Essential oil sample was dissolved in chloroform. The operating condition were as follows: name of column- RTS- 5MS, diameter 30 cm, length 0.25mm, temperature of the column- initial temperature 40˚C (was held 2 min) , injector temperature- 220 ˚C, holding time 5 min, column packing was done with 10% diethylene glycol succinate on 100-120 mesh diatomic CAW, splittingsamples were injected by splitting with the spilt ratio 10, carrier gas- helium gas at constant pressure 90 kPa, sample dissolved- in chloroform, range of linear temperature increase- 10˚C per min.

Preparation of Essential Oil Samples for GC-MS Analysis

Essential oil was diluted to 7% by chloroform. An inert gas (i.e. nitrogen) was introduced, from a large gas cylinder through the injection part, the column and the detector. The flow rate of the carrier gas was adjusted to ensure reproducible retention time and to minimize detector dirt. The sample was then injected by a micro syringe through a heated injection part when it was vaporized and carried into the column. The long tube of the column was tightly packed with solid particles. The solid support was uniformly covered with a thin film of a high boiling liquid (the stationary phase). The mobile and stationary

phases were then partitioned by the samples and it was separated into the individual components. The carrier gas and sample component was then emerging from the column and passed through a detector. The amount of each component as concentration by the device and generates a signal which was registered electrically. The signal passed to a detector.

Physicochemical Properties of Anise Oil

There are many physicochemical properties that can be performed on the essential oils, in this study the selected ones are performed due to lack of needed chemicals and glasswares in our college. The oil obtained from aniseed was tested for the following: organoleptic properties, refractive index, and solubility in different solvents, boiling point, and acid value.

Organoleptic qualities: detected by as sight, taste, smell and touch.

Refractive index: the prism assembly of the refractometer was opened and cleaned with ethanol (no need to rub just gentle blotting with soft tissue). Then, 2-3 drops of water/sample was placed in the middle of fixed prism by dropper (never touch the prism with dropper). After that the prism assembly was closed, the lamp switched on and moved toward the prism to illuminate the visual field, the handweel rotated until two distinct fields (light and dark) were visible, and line was centered on cross hair. The refractive index was recorded.

Solubility: A known amount of the solvent was put in a test tube. Then the substance whose solubility is to be determined (oil) was added, a clear layer was observed indicated insolubility.

Acid Value: 5gm of oil and 25mL of neutral alcohol were transferred into a 100mL conical flask, and then heated on a steam bath for 10-15 minutes till the oil got dissolve. After cooling, 4 drops of phenolphthalein were added. Titration of the flask contents began against 0.1N NaOH solution from the burette and pink color was observed. The acid value was calculated.

Determination of Total Phenols by Folin Ciocalteu Reagent (FCR)

Chemicals Required

Sodium carbonate solution,Standard Gallic acid solution, FCR reagent, Ethanol or methanol

Preparation of 7.5% sodium carbonate solution: 7.5g of Sodium carbonate (Na2CO3) was weighted and dissolved in a small amount of distilled water. The final volume was adjusted to 100mL in a volumetric flask.

FCR reagent: was diluted with water (1:10)

Preparation of standard Gallic Acid solution: 10mg of Gallic acid was weighted and dissolved in 10mL ethanol. The concentration of this is 1mg/ml or 1000µg/ml of Gallic acid. This solution was labeled as stock solution. Then the working standards were prepared from this stock solution:

Preparation of plant extract: 0.01g of plant extract was taken and dissolved in 10mL ethanol. The concentration of this solution is 10mg/ml or 1000 µg/ml of plant extract.

Experimental procedure

- 1ml of plant extract or standard of different concentration was taken in a test tube.
- 2ml of FCR (diluted 10 fold) was added into the test tube.
- 4ml of sodium carbonate solution was added into the test tube.
- The test tubes were incubated for 30 minutes at 20oC to complete the reaction (for standard). The test tubes were incubated for 1 hour at 20oC to complete the reaction (for plant extract).
- Then the absorbance of the solution was measured at 765nm using a spectrophotometer against blank.
- A typical blank solution contained ethanol.

e-ISSN:2321-6182
p-ISSN:2347-2332

DPPH (2,2-diphenylpicrylhydrazyl) Radical Scavenging assay

Preparation of DPPH solution: 4mg of DPPH was dissolved in 100ml of ethanol (0.004% or 40 µg/ml).

Preparation of Standard Ascorbic Acid solution: Ascorbic acid standard solution was prepared in distilled water by dissolving 100mg in 100ml (1000 µg/mg or 1mg/ml) from the stock solution various serial dilutions were prepared 10, 50, 100, 200, and 500 µg/ml in distilled water.

Preparation of plant extracts: 0.01g of plant extract was taken and dissolved in 10ml of ethanol. The concentration of this solution is 1mg/ml or 1000µg/ml of plant extract. From this stock solution various dilutions in 20, 40, 80, 200, and 500µg/ml in ethanol were prepared.

Experimental procedure

- 1ml of water and 2ml of DPPH solution served as control.
- To 1ml of various concentrations of standard solution or plant extract, 2ml of DPPH solution was added.
- After incubation in dark for 20 minutes, the absorbance of solution was measured at 517nm.

Determination of Anti-bacterial activity by Kirby-Baure procedure

Materials required

Petri plates of sterile Muller-Hinton agar and blood agar, Pure culture suspension,Sterile cotton swab , Antibiotic Discs, Forceps and alcohol.

Test organism

Three strains of gram-negative bacteria *Escherichia coli*, *Klebsiella pneumoniae*, Bacillus subtitils and one strain of gram-positive bacteria *Staphylococcus aureus* were used.

Antibacterial assay

Screening of essential oils for antibacterial activity was done by the disk diffusion method as follows

- The working area was cleaned with ethyl alcohol.
- The steps were carried out under aseptic procedures like Bunsen burner flame.
- A hole was made inside the plate agar by removal of small piece of medium so the oil can be poured within them.
- The broth culture was inoculated on the surface of the medium with help of the sterile cotton swab.
- Plates were labeled appropriately, and the surfaces allowed to dry for 3-5 minutes.
- The oil was poured into the holes by help of meter pipette and incubated for 24 hours at 37oC.
- For the standard or control, the antibiotic disc was deposited on agar surface with help of sterilized forceps.

RESULTS AND DISCUSSION

Oil yield determination and physicochemical properties

The results of physicochemical properties were obtained as follows:

e-ISSN:2321-6182
p-ISSN:2347-2332

Table 1: The physicochemical properties of essential oil of Aniseed

Physical Properties		Aniseed Essential Oil
Oil yield (%)		1.2096%
Taste	Bitter in taste	
Odor		Spicy
Color		Slight yellowish
Appearance at room Temp.		Homogenous, transparent liquid, lighter than water
Refractive index [ηt° C]		1.4765
Solubility		
Hexane		Soluble at any volume
90% alcohol		Clearly soluble at any volume
Chloroform		Soluble at any volume
Distilled water		Not soluble
Chemical Properties		
Acid value		0.3927

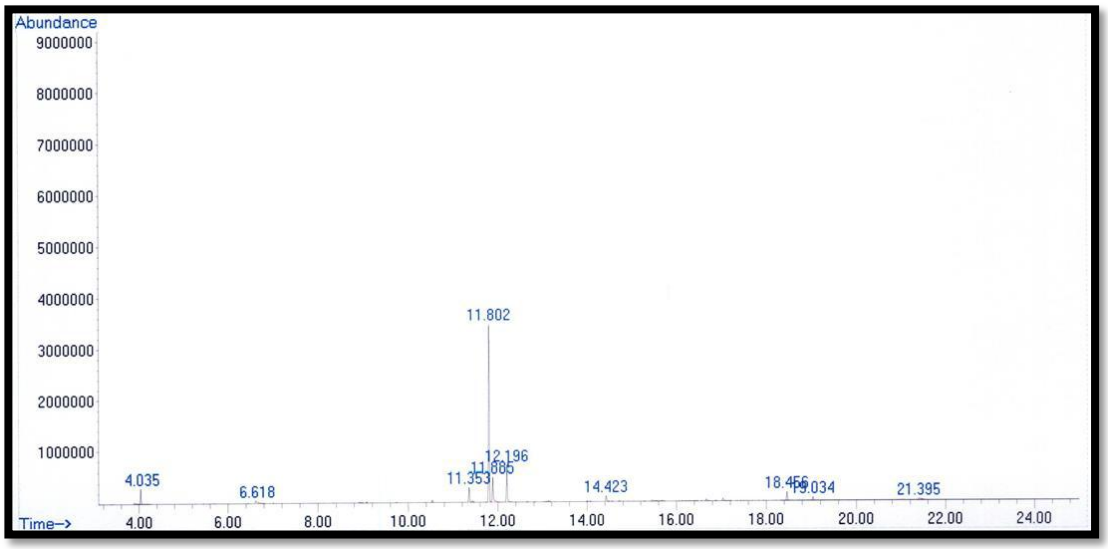
GC-MS Analysis

GC-MS analyzed results which include the active principles with their retention time, and composition of the essential oil of *Pimpinella anisum Linn.* (Aniseed) are presented in Table 2.

Table 2: Chemical constituents of the essential oil of Aniseed

No.	Retention time	Name of the compound	Composition (%) of Aniseed essential oil
1	4.035	Tetrachloroethylene	4.179%
2	6.618	2-heptanal (Z and E)	2.483%
3	11.353	P-anisaldehyde	4.769%
4	11.802	Trans-anethole	55.491%
5	11.881	2,4-decadienal	11.353%
6	14.423	3,5,5,9-tetramethyl (1H) benzocycloheptene	2.028%
7	18.456	2-(1-propenyl)-4-methoxyphenyl	3.098%
8	19.034	4-methoxy-2-(3-methyloxiranyl) phenyl ester	1.545%
9	21.399	1,2-cyclohexanedimethanamine	1.234%

Figure 3: the quantitative and qualitative analysis of Essential Oil extracted from Aniseed



e-ISSN:2321-6182
p-ISSN:2347-2332

Determination of Total Phenols by Folin Ciocalteu Reagent (FCR)

The total phenolic contents were calculated as gallic acid equivalent (GAE) from a calibration curve of gallic acid standard solutions and expressed as mg of gallic acid per 100 µg of essential oil sample, by using one formula of the following:

(y= mx+c) or
C=(cxV) / m,

where:
C= total content of phenolic compounds, mg/g plant extract, in GAE
c= the concentration of gallic acid established from the calibration curve, mg/ml
V= the volume of extract, ml
m= the weight of sample

Table 3: Results of total phenolic content in plant extracts

S. No	Conc. Prepared (µg/ml)	Absorbance	X
1	50	0.1207	5.733
2	100	0.1632	14.688
3	150	0.1923	24.518
4	200	0.2151	34.416
5	250	0.2702	50.6625
6	Aniseed Oil	0.3266	61.237

Table 4: Results of total phenolic content in Aniseed oil

Concentration (ml)	Absorbance	Average	% Inhibition
Aniseed Oil 0.2	0.6849	0.7725	18.68%
Aniseed Oil 0.2		0.8601	
Aniseed Oil 0.4	0.6878	0.7550	20.52%
Aniseed Oil 0.4		0.8223	
Aniseed Oil 0.8	0.9107	0.7507	20.97%
Aniseed Oil 0.8		0.5907	
Aniseed Oil 2	0.9203	0.6957	26.76%
Aniseed Oil 2		0.4712	
Aniseed Oil 5	0.8779	0.7390	22.21%

Antioxidant activity

The prcentage inhibition of DPPH radical of Aniseed oil was calculated according to the following formula:

% Inhibition= [(AB-AA)/ AB] x 100
where: AB absorption of blank sample and AA absorption of tested oil

Table 5: Result of antioxidant activity in Aniseed oil

Concentration (ml)	Absorbance	Average	% Inhibition
Control		0.95	
Ascorbic Acid 0.1	0.726	0.745	21.58%
Ascorbic Acid 0.1		0.764	
Ascorbic Acid 0.2	0.091	0.098	85.68%
Ascorbic Acid 0.2		0.105	
Ascorbic Acid 1	0.079	0.087	90.79%
Ascorbic Acid 1		0.096	
Ascorbic Acid 2	0.073	0.073	92.32%
Ascorbic Acid 2		-	
Ascorbic Acid 5	0.055	0.055	94.21%
Ascorbic Acid 5		-	
Aniseed oil 0.1	0.563	0.563	40.73%
Aniseed oil 0.1		-	
Aniseed oil 0.2	0.517	0.517	45.57%
Aniseed oil 0.2		-	
Aniseed oil 1	0.484	0.484	49.05%
Aniseed oil 1		-	
Aniseed oil 2	0.480	0.480	49.47%
Aniseed oil 2		-	
Aniseed oil 5	0.479	0.479	49.57%
Aniseed oil 5			

e-ISSN:2321-6182
p-ISSN:2347-2332

Screening of antibacterial activity

The anti-bacterial activity of Aniseed oil against four bacterial species is summarized in Table 6.

Table 6: Antimicrobial activity of Aniseed oil against E.coli, S. aureus, P.aeruginosa and K. pneumoniae using disc diffusion method

Test organism	Antimicrobial agent	Zone diameter	Susceptibility
E.coli	Ciprofloxacin Anise oil	50mm No inhibition zone	Sensitive Resistance
Staphylococcus aureus	Ciprofloxacin Anise oil	30mm No inhibition zone	Sensitive Resistance
Bacillus Subtilis	Ciprofloxacin Anise oil	30 mm 28mm	Sensitive Sensitive
Klebsiella	Ciprofloxacin Anise oil	20mm No inhibition zone	Sensitive Resistance

DISCUSSION

Natural products are in increasing demand from the manufacturers of foods, cosmetics and pharmaceuticals. Thus the importance of conducting studies on essential oils lies not only in the chemical characterization but also in the possibility of linking the chemical contents with particular bioactive functional properties. The capacity of spices and flavors to minimize disease risk, within the context of culinary use, has not been completely evaluated. There is a strong need to understand the preventive effect of spices and natural flavors for counter acting oxidative damages. Our work suggests that essential oils from spices can be considered as a promising future candidate food supplement.

In the present study, the essential oil of Aniseed fruits was hydro-distillated to evaluate the Aniseed oil yield, physicochemical properties, chemical compositions, antioxidant activity, total phenolic contents and antibacterial activity (Table 4-8). According to our study, the essential oil content on a dry weight basis of Aniseed was 1.2096%.

Our results may have differed compared to previous researches due to several factors such as different experimental conditions, genotype, stage of maturity, cultivation peculiarities, soil composition and climate differences in various geographical locations, which interfere with essential oil content and composition. Moreover, essential oil yield per plant on a dry weight basis will be lower than that on a fresh weight basis for the herbs, and a reduction in essential oil yield may occur when plants are dried before distillation, due to changes that favor the formation of esters and the production of free acids in *Pimpinella anisum*.

The physical and chemical characteristics such as color, appearance, taste, odor, solubility, refractive index, and acid value of the essential oil were determined by conventional methods (Table 4) as described in the methods section. Fluctuation of the oil composition can impart change in the organoleptic properties of the plant belonging to the botanical spices and variety.

GC-MS analyzed results which include the active principles with their retention time, molecular weight and composition of Aniseed essential oil are presented in Table 5. Total 9 chemical constituents of Aniseed essential oil were detected in this study (Trans-anethole, Tetrachloroethylene, 2-heptanal (Z and E), P-anisaldehyde, 2,4-decadienal, 3,5,5,9-tetramethyl (1H) benzocycloheptene, 2-(1-propenyl)-4-methoxyphenyl, 4-methoxy-2-(3-methyloxiranyl) phenyl ester and 1,2-cyclohexanedimethanamine) which were consistent with those of previously published studies but at different concentrations of individual components. Variability in the volatile components among Aniseed essential oil from our results appears to be largely due to stage of harvest, seasonal and environmental factors, in addition to the use of different methods of extracting the volatile components. In Aniseed essential oil, the main compound was Trans-anethole, followed by 2,4-decadienal. Similarly, Trans-anethole was previously reported as major component in *Pimpinella anisum*.

The total phenolic contents in the examined plant extracts using the Folin-Ciocalteu's reagent is expressed in terms of gallic acid equivalent (the standard curve equation: $y = 0.0007x + 0.087$, $R^2 = 0.9802$). The values obtained for the concentration of total phenols are expressed as mg of GA/g of extract (Table 7). The total phenolic contents in the examined extracts ranged from 18.68 to 26.76 mg GA/g. The total phenolic contents in plant extracts of the species *Pimpinella anisum* depends on the type of extract, i.e. the polarity of solvent used in extraction. High solubility of phenols in polar solvents provides high concentration of these compounds in the extracts obtained using polar solvents for the extraction.

The antioxidant activity of *Pimpinella anisum* was determined using an ethanol solution of DPPH reagent. DPPH is very stable free radical. Unlike in vitro generated free radicals such as the hydroxyl radical and superoxide anion, DPPH has the advantage of being unaffected by certain side reactions, such as metal ion chelation and enzyme inhibition. A freshly prepared DPPH solution exhibits a deep purple colour with an absorption maximum at 517 nm. This purple colour generally fades when antioxidant molecules quench DPPH free radicals (i.e. by providing hydrogen atoms or by electron donation, conceivably via a free-radical attack on the DPPH molecule) and convert them into a colourless-/bleached product (i.e. 2,2-diphenyl-1-hydrazine, or a substituted analogous hydrazine), resulting in a decrease in absorbance at 517 nm.

We tested the antioxidant activity of Aniseed essential oil at five different concentrations of 10, 50, 100, 200, and 500µg/ml using % DPPH inhibition as described in the methods section. It was found to be active at concentration of 500µg/ml. The concentrations that perform the highest antioxidant activity have the highest concentration of phenols. Phenols are very important plant constituents because of their scavenging ability on free radicals due to their hydroxyl groups. Therefore, the phenolic content of plants may contribute directly to their antioxidant action.

In the present study, effectiveness of essential oils was determined by agar disc diffusion method in order to detect growth inhibition zones in presence and absence of each essential oil (Table 9). Based on growth inhibition zone diameters obtained bacterial strains were divided in to three categories i.e. sensitive, resistant (>7 mm), intermediate (>12 mm), and susceptible (>18 mm). The results showed that there was no inhibition zone for tested organisms except Basillus Subtitis.

CONCLUSION

The configuration of the system used in the present work was effective to extract the essential oil of aniseed. Extraction of Essential Oils using Steam Distillation can be used on industrial scale to make various finished products which includes body oils, cosmetic lotions, baths, hair rinses, soaps, perfumes and room sprays.

Analysis using Gas Chromatography-Mass Spectrometer was found to be the best method to identify even the minor components of particular oil along with major components.

Results of our study suggest that *Pimpinella anisum* represents one of the best potential sources of potent natural antioxidant that promote health and lower the risk of cancer, hypertention and heart diseases. Moreover the results showed that their antibacterial activity against bacillus subtitis.

REFERENCES

1. D.pandey and PS Rao Virendra. Extraction of essential oil from Eucalyptus leaves (B-tech project), NIT Rourkela.
2. Ross IA. Medicianl plants of the world: chemical constitutes, traditional and modern medicinal uses, Volume2, Humana press, Totowa, New Jersey, 2001, p. 363-374.
3. Reineccius G. Source book of flavours. 2nd ed. Chapman and Hall, New York, 1994.
4. Hänsel R.,Sticher O., Steinegger E., Pharmakognosiephytopharmaize.6.Auflag. Springer-verlag, Berlin, Heidelberg, 1999, pp.692-695.
5. Chevallier A. The encyclopedia of medicinal plants. Wolfe Publishing Ltd., London, 1996.
6. Figueiredo AC, Barroso JG, Pedro LG, Scheffer JJC. Factors affecting secondary metabolite production in plants: volatile components and essential oils . Flavour Fragr J. 2008;23:213-226.
7. Terapelli CR, Andrade CR, de Cassano AO, de Souza FA, Ambrosio SR, Costa FB, da oliveria AM. Antipasmodic and relaxant effects of the hydroaicoholic extract of Pimpinella anisum (Apiaceae) on rat anovoccygeous smoth muscle. J Enthopharmacol. 2007;110(1):23-29.

8. Tabanca N, Demirci B, Kirimer N, Baser KHC, Bedir E, Khan IA, Wedge DE. Gas chromatographic-mass spectrometric analysis of essential oil from Pimpinella aurea, Pimpinella corymbosa, Pimpinella perigrina and Pimpinella pubberula gathered from Eastern and Southern Turkey. J Chromatogr A. 2005;1097:192-198.
9. Tabanca N, Demirci B, Kirimer N, Baser KHC, Bedir E, Khan IA, Wedge DE. Gas chromatographic-Mass spectrometric analysis of essential oil from Pimpinella species gathered from Central and Northern Turkey. J Chromatogr A. 2006;1117:194-205.
10. Orav A, Raal A, Arak E. Essential oil composition of Pimpinella anisum L. fruts from various European countries. Natural Prod Res. 2008;22 (3): 227-232.
11. Buchman D. D., Herbal medicine: the natural way to get well and stay well, century Hutchinson, London, 1987.
12. Waumans D., Bruneel N., Tytgat J., Anise oil as a precursor for 2-alkoxy-5-methoxybenzldehydes. DEA Microgram J. 2006;2 (1): Retrieved on 9 December.
13. Spoerke DG. Herbal medications, Woodbridge Press Publ. Co., Santa Barbara CA,1980, pp. 83.
14. Hoffmann D. Thorsons guide to medicinal herbalism: a comprehensive and practical introduction. Thorsons, London, 1991
15. Newall CA, Anderson LA, Phillipson JD. Herbal medicines- A Guide for Health-Care Professionals, The Pharmaceutical press London, 1996.
16. Ody P. Handbook of over-the-counter herbal medicines, Kyle Cathie, London, 1993.
17. Pruthi JS. Spices and condiments; National Book Trust. New Delhi, India, 1976, 27-30.
18. The Herb Society of America — 9019 Kirtland Chardon Road, Kirtland, OH 44094 (440) 256-0514 — http://www.herbsociety.org
19. Burt SA. Essential oils: their antibacterial properties and potential applications in foods: a review. Inter J Food Microbiol. 2004;94:223-253.
20. Kordali S, Kotan R, Mavi A, Cakir A, Ala A, Yildirim A: Determination of the chemical composition and antioxidant activity of the essential oil of Artemisia dracunculus and of the antifungal and antibacterial activities of Turkish Artemisia absinthium, A. dracunculus, Artemisia santonicum, and Artemisia spicigera essential oils. J Agric Food Chem. 2005, 53:9452-9458.
21. Sylvestre M, Pichette A, Longtin A, Nagau F, Legault J: Essential oil analysis and anticancer activity of leaf essential oil of Croton flavens L. from Guadeloupe. J Ethnopharmacol. 2006;103:99-102.
22. Faid M, Bakhy K, Anchad M, Tantaoui-Elaraki A, Alomondpaste : Physicochemical and microbiological characterizations and preservation with sorbic acid and cinnamon. J Food Prod. 1995;58:547-550.
23. Buttner MP, Willeke K, Grinshpun SA: Sampling and analysis of airborne microorganisms. In Manual of Environmental Microbiology Edited by: Hurst CJ, Knudsen GR, McInerney MJ, Stetzenbach LD, Walter MV. ASM Press: Washington, DC; 1996:629-640.
24. Van de Braak SAAJ, Leijten GCJJ: Essential Oils and Oleoresins: A Survey in the Netherlands and other Major Markets in the European Union. CBI, Centre for the Promotion of Imports from Developing Countries, Rotterdam. 1999:116.
25. Milhau G, Valentin A, Benoit F, Mallie M, Bastide J, Pelissier Y, Bessiere J: In vitro antimicrobial activity of eight essential oils. J Essent Oil Res. 1997;9:329-333.
26. Darokar MP, Mathur A, Dwivedi S, Bhalla R, Khanuja SPS, Kumar S: Detection of antibacterial activity in the floral petals of some higher plants. Curr Sci. 1998, 75:187.
27. Essential oil/ http://www.wikipedia.org
28. HJ Williams, Ahmed Mahmoud, Al Scott, JH Reibenspies, and TJ Mabry. New sesquiterpene a-methylene lactones from the Egyptian plant Jasonia candicans. J. Nat. Prod. 1993;56:1276–1280.