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Research article

TO STUDY OF ESSENTIAL OIL COMPOSITION AND ANTIOXIDANT ACTIVITY OF PIMPINELLA PLANT

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ABSTRACT: In this research we have investigated essential oil constituents and antioxidant property of Pimpinella plant. aerial part of plant are collected from around sari, mazandaran, provience of mazandaran (North of Iran). The chemical composition of the essential oil plant obtained by hydrodistillation using a Clevenger. Composite was analysed by GC-MS.the extraction was carried out with soxhlet apparatus. For antioxidant activity investigation used of TPC, DPPH and FTC, TBA method .then result by comparison of vitamin c antioxidant activity (natural antioxidant) and BHT (synthetic antioxidant). 20 components were identified in the essential Oil, that 94.5% of total composition. The major components of essential oil from Pimpinella plant were: α-pinene0.8%, couminalchol55.6%, carvacrol0.9%, terpinolene14.9%, limonen7.8%. Absorption read at plant phenolic compound method 0.298, amount of phenolic compound in this plant 37.63mgGAEg^{-1} and according DPPH test amount of antioxidant with IC_{50} is 334µg/ml. α-pinene and limonene observed in composition have Antimicrobial and fungicidal properties. Limonene also used in providing of polymer, paste and perfume. In totally this Plant used that in cure of whooping cough and instigator cough. Percentage of antioxidant were determined for ethanolic extract of plant under study 27% by FTC method , 63% for TBA method .By regards of this plant in Iran natural ,essence of this plant as an natural antioxidant in industrial food and drugs.

Key words: Essentiall oil, Pinpinella, pinene, limonene, hydrodistillation, GC-MS

INTRODUCTION

From the past until now by used of plants for cure of different disease type in worldwide was usual because they contain components of therapeutic value. This was basic formation of medicinal plant [1-5]. That many of those specie have essential oil, so we decided (made a decision) for isolation and investigation of essence and essential oil and antioxidant property of Pimpinella plant .The Pimpinella belongs to family Apiaceae are growing wild in north part of Iran. This plant is herb, annualy and it has often erect stream or Creeping and often has groove. The leaves of this plant has alternate and or divided leaf, more incision and led to pod that put steam to the cohesive section [6-7]. This plant used for cure of whooping cough and instigator cough. Investigation show that ethanolic extract of plant prohibit from acetaminophen destrictive effects in the rat kidney, this is because of antioxidant activity[7].from that flavonoids and other phenolic compound are found in Medicinal plants , and was reported varieties of biologic activity from this compound like antioxidant effect , anti microbial and anti - inflammation [8-14].
Researchers believe this compound is cause of decrease of heart disease and cancer .This observation is lead to special attention to natural sources for find out of antioxidant molecule [15,16].

MATERIALS AND METHODS

Plant material

First aerial part of this plant were collected around sari in the Mazandran state (north of Iran) [6-7].

Analyses and identification of components

The dried powder of aerial part (100gr) was subjected to hydro distillation for approximately 4h using in a Clevenger-Type apparatus. the obtained essential oil Was dried over a hydrous sodium sulphate and used for GC-Mass analyses.GC-98 shimadzu model of GC System was equipped with flam ionization detectors (FID) from 5Dcolumn Germany company to 60 Meter length, 0.25 mm inner diameter and layer stationary phase thick is 0.25 µ varian 3400 GC-Mas network to Mass spectroscopy with 70 ev Ionization energy, the oven temperature was programmed from 40 to 250°c at 4 c/min. The temperature of the Injector port was held at 260°c.Spectrum identification by use retention Index (RI) by Injection of n-alkanes (c_9-c_{28}) under same condition with essence injection by use of a computer library and comparison was done between distributed amount of different compound reference and standard component, mass spectroscopy and confirmation of further information was done by data generation from a series of known standard of alkaniod [17,18].

Extract plant

The dried powder preparation of Pimpinella extract (100g) was Subjected to study of antioxidant activity use of this plant extraction .amount of 100g of dry powder, extraction by soxhelt ,approximately 8 h .after that ,obtain extract of removing solvent by rotary evaporator .

Antioxidant activity

Extract antioxidant activity of plant investigation by use ferrictiocyanate (FTC) and tiobarbituric acid (TBA) methods, Picrylhydrazyn Diphenyl (DPPH) and FTC-TBA method.

C vitamin and butyl hydroxyl toluene (BHT) were consider as standard and a sample utilized as an oxidant [19].

Ferric thiocyanate method (FTC)

Mixture of 4mg of plant extraction at 4 ml pure ethanol (99.99%) 4.1 ml of linoleic acid 2.52% at Pure ethanol, 8ml phosphate buffer 0.05M (PH=7) and 3.9ml distil water in a covered dish and then kept in oven at 40°c and put in darkness during experiment .add to 0.1 ml from this solution, 9.7ml ethanol 75% and 0.1 ml ammonium tiocyanate 30%, after 3min 0.1 ml ferrochlorid and 3.5% HCl add to the reaction mixture. Absorb of the reaction mixture of 500nm during 24h until the absorbance of blank reached to maximum [20].

Tio barbituric acid method (TBA)

To mix 2ml of three chloro acetic acid 20% and 2ml tiobarbituric acid 67% to 1ml of ready solution add to FTC method .Then mix reaction for 15 minute put in banmari after getting cold ,centrifuge for 20 minute with 3000 cycle per minute and maximum sample absorb measure at 532nm wave length [10]. Analysis of absorption at this method to accomplished one day after to get maximum of sample absorption in FTC method. To calculation of percentage of antioxidant activity extraction after read of sample absorption of instance of test in 500nm wavelength for FTC method and 532nm for TBA method.

The percentage of antioxidant activity was calculate by using this formula [21].

%Antioxidant activity = $(A_0 - A)/A_0$ X 100 **(**eq.1**)**

A_0 =blank absorb, A=sample absorb

Picrylhydrazyn Diphenyl method (DPPH)

First ,250 ml of 0.1mM DPPH solution provide in ethanol ,then 250 ml of plant extraction of 550 mg/lit solution, BHT and c vitamin equal 0.1375g of each provide. This made solution ,is mother solution, now different concentration of that (10-20-50-100-150-…550mg/lit) by 1ml from DPPH made in above mixture during 30 minute mixture in darkness, then read the absorption in 517 nm. This work repeat for 3 time and then percentage of antioxidant activity according to the pervious relation [22,23, 24].

TBA –FTC method

Analysis of absorption at this method to accomplished one day after to get maximum of sample absorption in FTC and TBA method.

Total phenolic compounds method (TPC)

0.3 ml of extract was ready in the DPPH method, added 1.2 ml 7.5% Na_2Co_3 and 1.5 ml fulin 10% absorb measure in 765nm wave length.

Total Phenol content concentration in plant is counted according to µg of Gallic acid in mg of extract [20].

Total Phenol =.0005X+0.087

X=amount of Gallic acid according to µg.

RESULTS

In plant compounds were identified at essential oil and table-1 show the main component of essential oil of this plant. Antioxidant activity of ethanolic extracts of this plant by use of FTC, TBA, DPPH, FTC-TBA method are studied. C vitamin (natural antioxidant) and BHT (synthetic antioxidant) consider using a standard and a sample utilized to non oxidant as a blank. Absorption read at plant phenolic compound method 0.298, amount of phenolic compound in this plant 37.63mgGAEg^{-1} and according DPPH test amount of antioxidant with IC_{50} is 334µg/ml. (Table-1 to4and Figures-1 to 4).

Table-1: Main component of the essential oil from Pimpinella

Components	Composition in percent
α-pinene	0.8
couminalchol	55.6
carvacrol	0.9
terpinolene	14.9
limonene	7.8

Table-2 :Absorption earn in FTC method from Pimpinella extract.

Absorption									
Day	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Extract	0.336	0.36	0.421	0.57	0.456	0.493	0.455	0.721	0.931
Control	0.251	0.309	0.421	0.505	0.65	0.809	0.949	0.992	1.182
BHT	0.192	0.2	0.253	0.26	0.276	0.28	0.28	0.288	0.33
C vitamin	0.141	0.176	0.186	0.2	0.234	0.249	0.26	0.274	0.298

Table-3 :Absorption earn in TBA method from Pimpinella extract

Absorption									
Day	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Extract	0.015	0.021	0.032	0.038	0.035	0.032	0.031	0.027	0.034
Control	0.034	0.048	0.049	0.053	0.058	0.061	0.073	0.074	0.081
BHT	0.03	0.033	0.035	0.03	0.03	0.031	0.035	0.037	0.046
C vitamin	0.033	0.038	0.04	0.037	0.035	0.039	0.041	0.044	0.048

Table-4 :Earn result from Pimpinella extract in DPPH method

Absorption											
Concentration	**50**	**100**	**150**	**200**	**250**	**300**	**350**	**400**	**450**	**500**	**550**
Extract	5.82	11.89	20.55	26.78	35.19	40.61	47.41	51.72	56.95	62.94	65.04
BHT	59.1	70.6	74.23	75.07	76.99	76.03	77	71.88	77.95	78	87.22
C vitamin	67.37	80.09	80.42	90.33	89.42	91.23	89.42	91.23	91.84	92.54	92.74

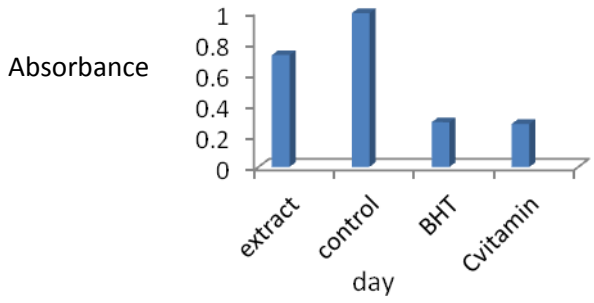

Figure-1: Antioxidant activity by FTC method

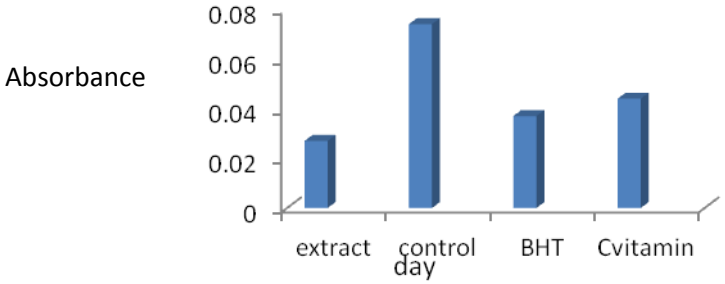

Figure-2: Antioxidant activity by TBA method

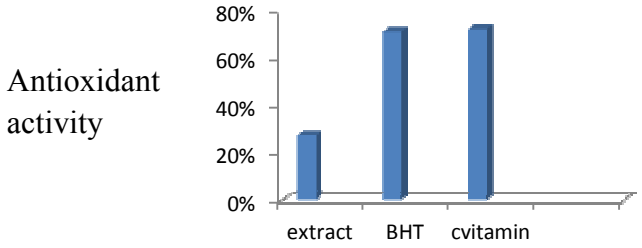

Figure-3: Percentage of antioxidant activity by FTC method

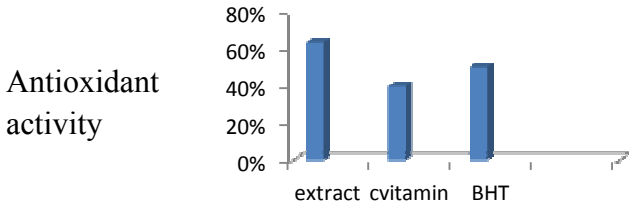

Figure-14: Percentage of antioxidant activity by TBA method

DISCUSSION

α-pinene and limonene observed in composition have Antimicrobial and fungicidal properties. Limonene also used in providing of polymer, paste and perfume [25,26].in totally this Plant used that in cure of whooping cough and instigatorcugh. At analysis of FTC test result, at this method peroxidation of lipid occurs [27] ,which in vicinity of that Fe^{+2} Convert to Fe^{+3} . that present of ammonium thiocyanate formation to red complex of ferric thiocyanate and it absorb in 500 nm .but when antioxidant exist in our sight component , we don't have lipid peroxidation in fact here is no ferric which complex with ammonium thiocyanate and occur reduction of absorption. Amount of decrease of absorption show that present of increase of antioxidant (table2) .with attention to figure-1 antioxidant activity is below mention:

C vitamin≥ BHT□extract.

Then Cvitamin show absorption look like BHT, then have most antioxidant activity and extract plant show most absorption then less antioxidant activity.

At analysis of TBA test result in Pimpinella plant, in this method obtain peroxide from FTC method is destroyed and get Malonaldehid (MA) that 532nm absorb .we have maximum of oxidation that earn most malonaldehid and cause of increase of absorption but when antioxidant present in component we have at least oxidation and less rate of malonaldehid that cause of decrease of absorption in fact when less absorption, we have more antioxidant activity [28].

With attention to figure-2, plant have less absorption, then show most antioxidant activity.

Then antioxidant is below mention:

Extract □BHT □ C-vitamin

Total antioxidant activity for plant extract were determined by per oxidation linoleic acid by use of FTC-TBA method and equation (1) [28]. Percentage of antioxidant activity were determined for ethanolic extract of plant under study 63%, C vitamin 40%, BHT50% by TBA method and27% for extract of plant, C vitamin 72%, BHT 71% for FTC method Percentage of activity in eighth day have been consider to the blank .In this plant by use of TBA method plant show most antioxidant activity which showed important of this plant. By the result of this plant can use as natural antioxidant in industrial food and drug. Absorption read at plant phenolic compound method 0.298, amount of phenolic compound in this plant 37.63mgGAEg^{-1} and according DPPH test amount of antioxidant with IC_{50} is 334 µg/ml. IC_{50} have lowest possible concentration in DPPH method are able to trap of free radical whatever amount of plant phenolic compound is more show increase antioxidant activity. By regards of deep situation and medicinal plant history at different disease cure at herbal medicine , recommendation of this plant because of have high power of antioxidant and as Prevention of different disease and also as help of disease cure more attention beside of new drugs.

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