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Essential oil composition and antioxidant activity of trigonella foenum graecum L. plant

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ABSTRACT: Fenugreek plant by scientific name trigonella foenum graecum L is one of the value specis by leguminosae family that this plant are grow up in different domain of iran.at this investigation ,essence and methanolic and ethanolic extracts of fenugreek were studied .aerial part during flowering process around sari city in the mazandran state are collected and then dry at shadow .the chemical composition Of the essence of fenugreek obtained by hydrodistillation and were analyzed by GC/MS .result show fenugreek has 35 components that is 94% of total essence of this plant . main components essence at this plant including:α-bisabolol 2.3% ,γ-cadinene 1.8%,farnesol 0.9%,α-cadinol1.7%,γ-eudesmol o.8%,Trigonellin0.6%,diosgenin0.8%. Aerial part of plant by soxhelt extraction from solvent and fatness. Main component extract at this plant Including:coumarin, fugarsterol,stigmasterol,nicotinic acid . Stigmasterole compound in this plant cause of blood cholesterol decrease and coumarin,trigonelline And nicotinic acid of cause of hypoglycaemic . For antioxidant activity investigation ,used of FRP,DPPH and FTC ,TBA method .then result by comparison of vitamin c antioxidant activity (natural antioxidant) and BHT(synthetic antioxidant). Percentage of antioxidant were determined for ethanolic extract of plant under study %65-27, methanolic extract % 63-32 by FTC method ,%47-38 for ethanolic essence and for methanol essence%40-15 Used 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: trigonella foenum graecum, fenugreek, essence ,extract,antioxidant.

INTRODUCTION

From the past until now by used of plants for cure of different diseag type in worldwide was usual, this was basic formation of medicinal plant . that many of those speice have essential oil, so we decided (made a decision) for isolation and investigation of essence and essential oil and antioxidant property of fenugreek .

Fenugreek plant by scientific name trigonella foenum graecum L is one of the value species of Leguminosae family . this herbal species is papilionaceae (Lee et al. 2007, Kay & Holub 2002).

This plant used for cure of dyabetic and low level cholesterol ,digestive disease ,high fever and blood poressure[9]. from that flavonoids and other phenolic compound are found in Medicinal plants , and was reported variets of biologic activity from this compound like antioxidant effect , anti microbial and anti - inflammation (Bhatt et al. 2006).

Researchers believe that the earn of this compound from diet is cause of decrease of heart disease and canser .this observation is lead to special attention to natural resource for find out of antioxidant molecule (Lee et al. 2007, Kay & Holub 2002, Cha 2007). Oxygen is a big active molecule that by produce of active oxygen piece lead to harm of alive organism (Dostbil 2007,Elango et al 2009).

Active piece of oxygen in cell are cotain of Hypocloride peroxide hydrogen and free radical like Hydroxyl radical and anion superoxide (Guangrong et al 2008).

This oxidants can be harmful for cell, by beginning chemical change reaction such as lipid peroxidation, protein oxidation and DNA that damage to DNA can cause to motation or cancer (Hill 1999, Brisibe et al 2009).

Protein damage is to cause of enzyme limitation and protein decline (Kim et al 2009). Antioxidant ,vitamins ,mineral salts and enzymes are compounds that prevention of free radical activity (Guangrong et al 2008).

MATERIAL AND METHOD

First aerial part of this plant were collected at flowering season .the poder dried aerial part (100g) were Subjected to hydrodisstillation for approximately 4h in a Clevenger –type apparatus .the obtained essence oil was dried over an hudrous sodium sulphate and used for GC/MS analyses .

For study of antioxidant activity use of this plant extraction .amount of 100g of dru powder extraction by soxhelt ,approximately 8 h .after that ,obtain extract of solvent and filteration. Extract antioxidant activity of plant investigation by use ferric tiocyanateFTC and tio barbituric acid TA methods .c vitamin and butyl hydroxyl toluene BHTconsider to a standard and a sample utilized to non oxidant as a blank.

Material

1. Linoleic acid, 2. buthyl hydroxyl toluene(BHT), 3.Ferro chloride, 4. ammonium tio cyanate, 5.phosphate buffer, 6. tio barbituric acid, 7.c vitamin, 8. Ethanol, 9. Methanol,10. picrylhydrazyn (DPPH)
All materials provide from Merk Company.

Apparatus

Ultra violet –visile from jenway company 2- Oven 3- GC-98 shimadzu model of GC System is 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 4- 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 Ingector port was held at 260°c.

Spectrum identification by use retention Index (RI) which Injection of n-alkanes (c_9-c_{28}) under same condition with essence injection and use of a computer library and comparision is done y distriuted compound amount of different reference and by use of standard component ,mass spectroscopy and y information further confirmation was done by data generation from a series of known standard of alkaniod(Adama 1995).

METHODOLOGY

Ferric thio cyanate 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 distilwater inside cover dish and then At oven by 40°c and put in darkness during experiment .add to 0.1 ml fromthis solution ,9.7ml ethanol 75% and 0.1 ml ammonium tio cyanate 30%, after 3min0.1 ml ferro chlorid 0.02 min 3.5% Hcl add To mix reaction .absorb of mix reaction in 500nm measure in any 24h until which absorb of blank reach to maximum .

Tio barbituric acid method (TBA)

To mix 2ml of three chloro acetic acid 20% and 2ml tiobarbituric acid 67% to 1ml from ready solution add to FTC method . then mix reaction for 15 minute put in anmari after getting cold ,centrifyge for 20 minute with 3000 cycle per minute and maximum sample absorb measure in 532nm wave length(Rai et al 2006).Segregation of absorbtion at this method to accomplished one day after to get maximum of sample absorption in FTC method .for calculation ofpercentage of antioxidant activity extraction ,after read of sample asorption of instance of test in 500nm wave length for FTC method and 532nm for TA method.

Percentage of antioxidant activity calculate according to below relation:

$AI\% = (A_0 - A)/A_0 \times 100$

A_0 =blank absorb , A=sample absorb

Picrylhydrazyn Diphenyl method (DPPH)

First ,250 ml of 0.1Mm DPPH solution provider in ethanol then 250 ml of plant extraction of 550 mg/lit solution,BHT and cvitamin 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 3time and then percentage of antioxidant activity calculate according to the pervious relation(Erdemoglu et al 2006).

TBA –FTC method

Segregation of absorbtion at this method to accomplished one day after to get maximum of sample

absorption in FTC and TBA method.

RESULT AND DISCUSSION

In fenugreek plant 40 components were identified at oil essence that contain of 94% of total essence of this plant .

Table 1 is show that the main component of oil essence of fenugreek plant and table 2 show the main component of ethanolic and methanolic extract of this plant .

Table1. Title????

component	Composition in percent
α-bisabolol	2.3%
γ-cadinene	1.8%
farnesol	0.9%
α-cadinol	1.7%
γ-eudesmol	0.8%
trigonellin	0.6%
diosgenine	0.8%
Fatty acid	1.8%
Hydro carbons	20.3%
lactones	1.1%
Phenolic derivatives	8.7%

Sigmasterol component at this plant cause of decrease of blood cholesterol and coumarin component ,trigonelline,nicotinic acid cause of decrease of hypoglycemic .

Antioxidant activity of methalonic and ethanolic extracts of this plant by use of FTC,TBA,DPPH Method are studied .c vitamin (Natural antioxidant) and BHT(synthetic antioxidant) consider to use astandard and a sample utilized to non oxidant as ablank.

Table2. Title????

component	Aerial part oil%(methanolic)	Aerial part oil%(ethanolic)
coumarin	2.42	1.31
phenol	0.21	1.2
Nicotinic acid	-	2.11
fugarsterol	4.3	5.22
stigmasterol	4.61	6.11
sistosterol	3.7	4.12
sotolone	2.48	3.51

At analysis of FTC test result, at this method peroxidation of lipid occurs, which in vicinity of that Fe^{+2} Convert to Fe^{+3} . that present of ammonium tiocyanate formation to red complex of ferric tiocyanate and it absorb in 500 nm .but when antioxidant exist in our sight component ,never we don't have lipid peroxidation in fact here is no ferric which complex with ammonium tio cyanate and occur reduction of absorption . Amount of decrease of absorption show that present of increase of antioxidant (table3) .with attention to diagram (1) of antioxidant activity is below mention :

C vitamin › BHT› methanolic extract

Cvitamin › BHT› ethanolic extract

Table 3. Absorption earn in FTC method from methanolic extract

day	0	1	2	3	4	5	6	7	8	9
Fenugreek extract	0	.357	.388	.399	.463	.422	.43	.469	.436	.587
control	0	.251	.309	.402	.505	.65	.809	.949	.992	1.182
BHT	0	.192	.2	.253	.26	.276	.28	.28	.288	.33
Vitamin c	0	.141	.176	.186	.2	.234	.249	.26	.274	.298
Fenugreek extract	0	.318	.385	.445	.472	.398	.424	.505	.491	.582

Then both of methanolic and ethanolic extracts c vitamins have less absorption .then show most antioxidant activity and extracts plant show most absorption then less antioxidant activity.

At analysis of TBA test result in fenugreek 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,wehave more antioxidant activity (table 4).

Table 4. Absorption earn in TBA method from methanolic extract

day	0	1	2	3	4	5	6	7	8	9
Fenugreek extract	0	.039	.046	.042	.038	.038	.044	.045	.047	.049
Control	0	.034	.048	.049	.053	.058	.061	.073	.074	.081
BHT	0	.03	.033	.035	.03	.03	.031	.035	.037	.046
Vitamin c	0	.033	.038	.04	.037	.035	.039	.041	.044	.048
Fenugreek extract	0	.013	.029	.037	.034	.039	.044	.037	.04	.056

With attention to data plant show absorption look like cvitamin and both of them show absorption more than BHT.then antioxidant methalonic extract is below mention:

BHT› cvitamin≥methanolic extract

According to the diagram plant show absorption like to the cvitamin and BHTthis represent that antioxidant activity of ethanolic extract is equall to antioxidant activity of cvitamin and BHT.

Total antioxidant activity for plant extract were determined by peroxidation linoleic acid by use of FTC-TBA method .table (5)

Percentage of activity in eighth day have been consider to the blank . by attention to digram most antioxidant activity in FTC method is related to methanolic extract of plant and TBA method is related to ethanolic extract of this plant.at this plant by use of TBA method show activity more than cvitamin and BHTwhich showed important of this plant .By the result of this plant can use as natural antioxidant in industrial food and drug.

Table 5. Percentage of antioxidant activity in FTC and TBA method

TBA	FTC	
%40-15	%63-32	Methanolic extract
%47-38	%65-27	Ethanolic extract

Table 6. Earn result from ethanolic extracr plant in DPPH method

concentration	0	50	100	150	200	250	300	350	400	450	500	550
Fenugreek extract	0	.05	.085	1.02	3.35	6.85	11.07	13.56	18.22	24.49	26.24	27.84
BHT	0	59.1	70.6	74.12	75.07	76.99	76.03	77	71.88	77.95	78	87.22
Vitamin c	0	67.37	80.09	80.42	90.33	89.42	91.23	89.42	91.23	91.84	55.51	92.74
Fenugreek extract	0	7.6	8.69	12.13	13.86	17.77	25.13	26.33	27.65	30.44	33.75	36.07
BHT	0	76.94	85.79	86.73	87.27	87.94	86.86	87.67	85.66	84.85	87.94	85.26
Vitamin c	0	26.41	52.55	70.64	96.11	95.58	95.31	96.11	96.38	95.31	94.77	97.32

Table7. Absorbtion aern in phenolic component method

Fenugreek ethanolic extract	0.298
Fenugreek methanolic extract	0.245

Table 8. Concentration earn

Phenolic component mg/g ethanolic extract	Ic_{50}	Phenolic componentmg/g methanolic extract	Ic_{50}
37.63	212	28.16	317

By attention to the DPPH method (table 6) diagram percentage of acceptance according to the Methanolic and ethanolic extract concentrationof this plant in comparsion by cvitamin and BHT are designed . by attention of data table 7,8 plant ethanolic extract have more absorb from methanolic extract. plant ethanolic extract have most amount phenolic component .this matter show that amount of absorption by phenolic component rate .

IC_{50} have lowest possible concentration in DPPHmethod are able to trap of free radical.

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