CODEN [USA]: IAJPBB ISSN: 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

http://doi.org/10.5281/zenodo.1188396

Available online at: http://www.iajps.com Research Article

COMPARATIVE STUDY OF THE ESSENTIAL OIL, PHENOL, FLAVONOID CONTENTS AND *IN VITRO* ANTIOXIDANT ACTIVITY OF FOUR APIACEAE FRUITS

Ahmed I. Foudah

Department of Pharmacognosy, College of Pharmacy, Prince Sattam bin Abdulaziz University, Al-Kharj, Saudi Arabia.

Abstract:

In Saudi Arabia, the fruits of Apiaceae plants are commonly used in the traditional system of medicine for the treatment of various ailments. The present study aims to determine essential oil, phenol and flavonoid contents as a mean of comparison between four selected fruits. In vitro antioxidant properties of the selected fruits, *Carum carvi (Caraway), Anethum graveolens (Dill), Cuminum cyminum (Cumin) and Foeniculum vulgare (Fennel) was also explored. The present study provides means for chemical differentiation between the four selected fruits and shows their beneficial impact on health expressed by the antioxidant activity.*

Keywords: *Apiaceae fruits; essential oils; GC, total phenol; flavonoid; antioxidant.*

Corresponding Author:

Dr. Ahmed I. Foudah,
Department of Pharmacognosy,
College of Pharmacy, Prince Sattam bin Abdulaziz University,
Al-Kharj, Saudi Arabia.
Phone: +966506448820
Office: +96615886022
Fax: +96615886001
E mail: a.foudah@psau.edu.sa

QR code

Please cite this article in press as Ahmed I. Foudah., **Comparative Study of the Essential Oil, Phenol, Flavonoid Contents and In Vitro Antioxidant Activity of Four Apiaceae Fruits** *, Indo Am. J. P. Sci, 2018; 05(02).*

INTRODUTION:

The family Apiaceae is a large family comprising many aromatic plants of more than 3,700 species and over 434 Genera [1]. Members of the family had several medicinal properties and used in traditional medicine in Arab countries [2]. Recent ethno-medicinal study in Makka region, Saudi Arabia showed that the decoction or infusion of fruits of *C. carvi* is used for digestion, gynecological, general and unspecified respiratory disorders and family planning. *A. graveolens* is used as digestive aid. *C. cyminum* recommended for digestive, gynecological, endocrine and nutritional problems, general and unspecified respiratory diseases. While the fruits of *F. vulgare* are used for digestive, urological, gynecological, general and unspecified neurological conditions, and as blood and immune system enhancer [3]. In the traditional rehabilitations, *C. carvi*, *A. graveolens*, *C. cyminum* and *F. vulgare* are used for digestive, urological, gynecological conditions. They are also carminative, antispasmodic, astringent and generally used for the treatment of digestive ailments, diarrhea, dyspepsia, flatulence, morning sickness and colic, cough remedy, analgesic, rheumatism, toothache, diuretics, diarrhea, antidiabetic, hypertension and epilepsy [4, 5]. Spices have been widely explored in different countries because of their high antioxidant action and beneficial effects in several disease conditions [6]. Spices containing bioactive compounds such as tannins, alkaloids, saponins, diterpenes, vitamins, phenols and flavonoids have antioxidant properties that can protect lipids and oils in food against oxidative degradation, control unpleasant taste, keep food quality, and prolong the shelf-life of foodstuffs [7]. In the present work, a comparative study of the essential oil, phenolic and flavonoids contents, and antioxidant activity was conducted for four selected plants: *C. carvi* (Caraway, Karawia), *A. graveolens* (Dill, Shabat), *C. cyminum* (Cumin, Kamoun) and *F. vulgare* (Fennel, Shamar).

MATERIALS AND METHODS:

Preparation of the essential oils

The dry powder of the fruits of *C. carvi*, *A. graveolens*, *C. cyminum* and *F. vulgare* (100 gm each) were subjected to hydrodistillation for 8 hr. The condensates were separately extracted with ether. The ether extracts were dried over anhydrous sodium sulfate and evaporated.

GC/MS Analyses of the essential oils

One µl of sample oil was injected into the system with the split mode (split ratio 1:20). For the Gas Chromatography-Mass Spectroscopy analysis "Perkin Elmer GC-MS coupled with Clarus 600 T mass Spectrometer (USA)" system was used. The samples were separated on Elite 5 MS (30 m × 0.25 mm i.d., 0.25 µm film thickness) capillary GC column (Perkin Elmer, USA). Analyses were performed with the helium as a carrier gas at a constant pressure mode. The oven temperature was maintained at 40 °C for 2 min, ramped to 150 °C at 8 °C/ min for 2 min and again increased at a rate of 8 °C/ min to 280 °C, ramped with the grade 8 °C /min and held for 2 min. The total run time was 36 min. The injector, ion source and interface temperature were set at 280 °C, 240 °C and 220 °C, respectively. The electron energy was set at 70 eV. The unknown components were identified using "National Institute of Standard and Technology (NIST, 2005) Library" and "WILEY, 2006, Library". GC/EI−MS full-scan chromatograms (m/z 50−600) were recorded from 4.0 to 36.0 min for peak identification.

Table 1: Five most prominent components in the essential oils of *C. carvi*, *A. graveolens*, *C. cyminum* and *F. vulgare* (% and RT in parenthesis).

No.	*C. carvi*	*A. graveolens*	*C. cyminum*	*F. vulgare*
1	Carvone (68.940, 14.27)	Carvone (45.99, 14.12)	α-Thujenal (31.28, 14.94)	*t*-Anethole (42.71, 14.88)
2	Limonene (25.89, 10.01)	Limonene (26.04, 9.87)	*p*-Cymene (17.26, 9.76)	Fenchone (23.89, 11.05)
3	Thymol (0.77, 14.66)	Dillapiole (16.28, 21.33)	2-Methyl-3-phenyl-propanal (15.43, 14.08)	Limonene (12.34, 9.82)
4	E-Limonene-1,2-epoxide (0.69, 11.88)	Dihydrocarvone (3.31, 13.23)	γ-Terpinene (11.06, 10.48)	Estragol (11.32, 13.12)
5	γ-Terpinene (0.56, 10.37)	Thymol (1.83, 14.36)	α-pinene (8.71, 8.84)	Benzaldehyde (2.81, 14.43)

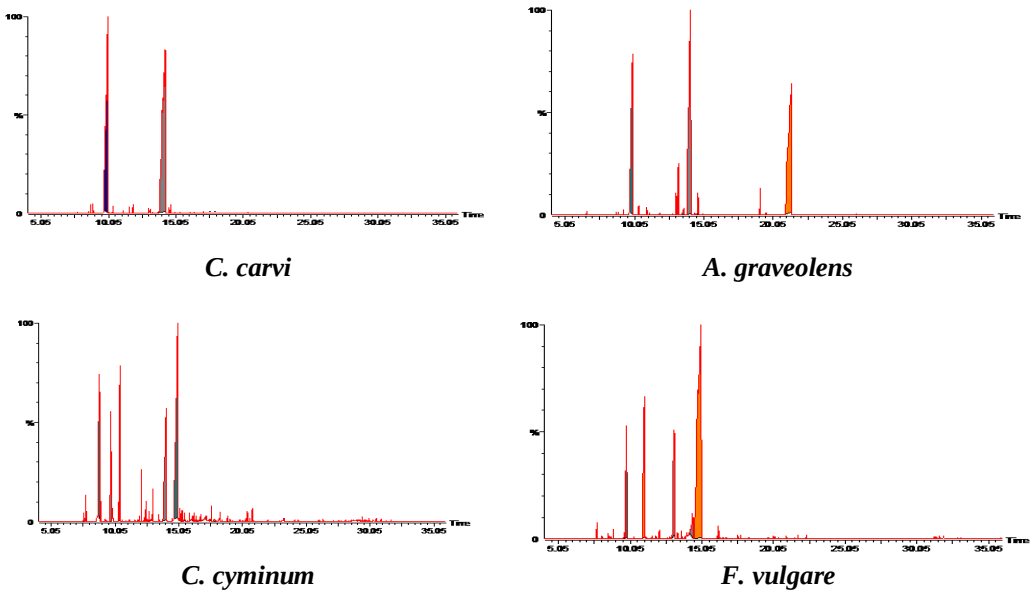


Fig. 1: GC Chromatogram of *C. carvi*, *A. graveolens*, *C. cyminum* and *F. vulgare*.

Extraction

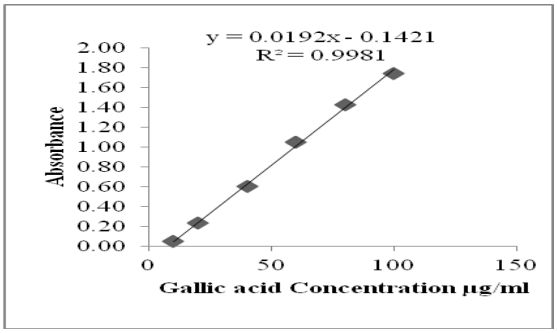
About 100 gm of each fruit powder was extracted with methanol by percolation at room temperature until exhaustion. The combined extract for each plant were evaporated at 40 ^{0}C using rotary vacuum evaporator.

Estimation of Phenols and Flavonoids

The total phenols and flavonoids content were estimated for the four extracts using the reported methods [8, 9] with minor modification. Separately, 1g of methanol extract of each plant was dissolved in 20 ml 50% aqueous methanol, filtered and diluted to 100 ml with 50% aqueous methanol. Gallic acid and quercetin were used as standard of 200 µg/ml in 50% aqueous methanol. From this solution serial dilutions of 5, 10, 20, 40, 80 and 100 µg/ml were prepared.

Total Phenolic contents

The reaction mixtures, 1: 10: 1.5 ml of each dilution, distilled water and $FeCl_3$ were prepared and incubate for 5 min at 25°C. Then 4ml of 20% Na_2CO_3 (w/w) was added, using distilled water, the volume was made up to the 25 ml, stand for 30 min with occasional shaking and at 765 nm, the absorbance was taken using UV/VIS spectrophotometer. The result was expressed as gallic acid in milligram per gram of dry weight of each extract.



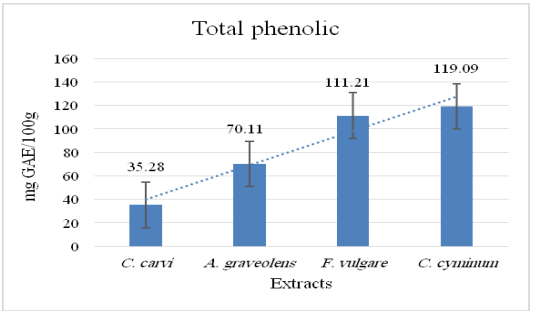


Fig. 2: Standard calibration curve of gallic acid and total phenolic contents in the samples. Each point represents the mean of three experiments.

Flavonoids content

The reaction mixtures composed of 2 : 0.1 : 0.1 : 2.8 ml of each dilution, 10% aluminium chloride hexahydrate, 1 M potassium acetate and distilled water was incubated for 40 min at 25°C. At, 415 nm, the absorption was measured using UV/VIS spectrophotometer and the total flavonoid content was expressed as quercetin in milligram per gram dry weight of each fruit extract.

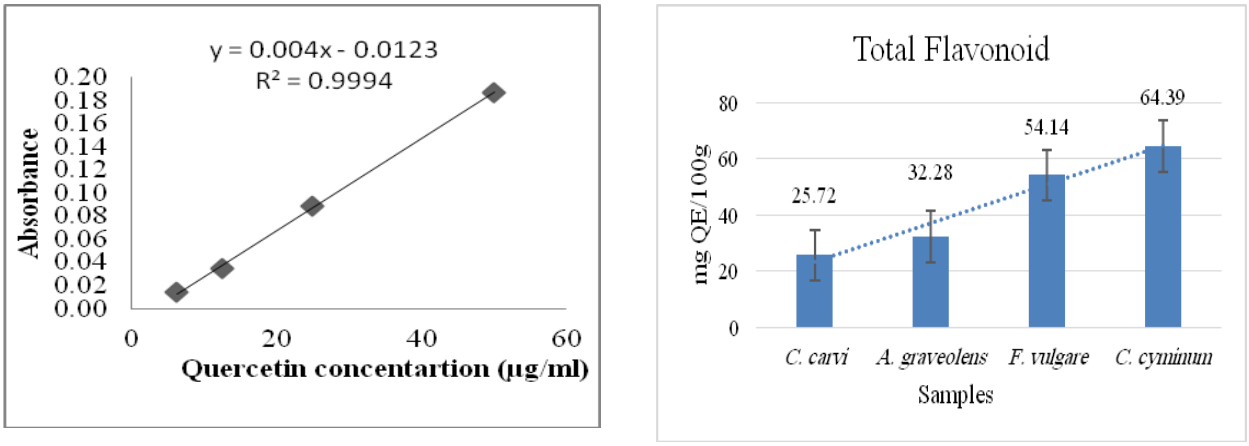


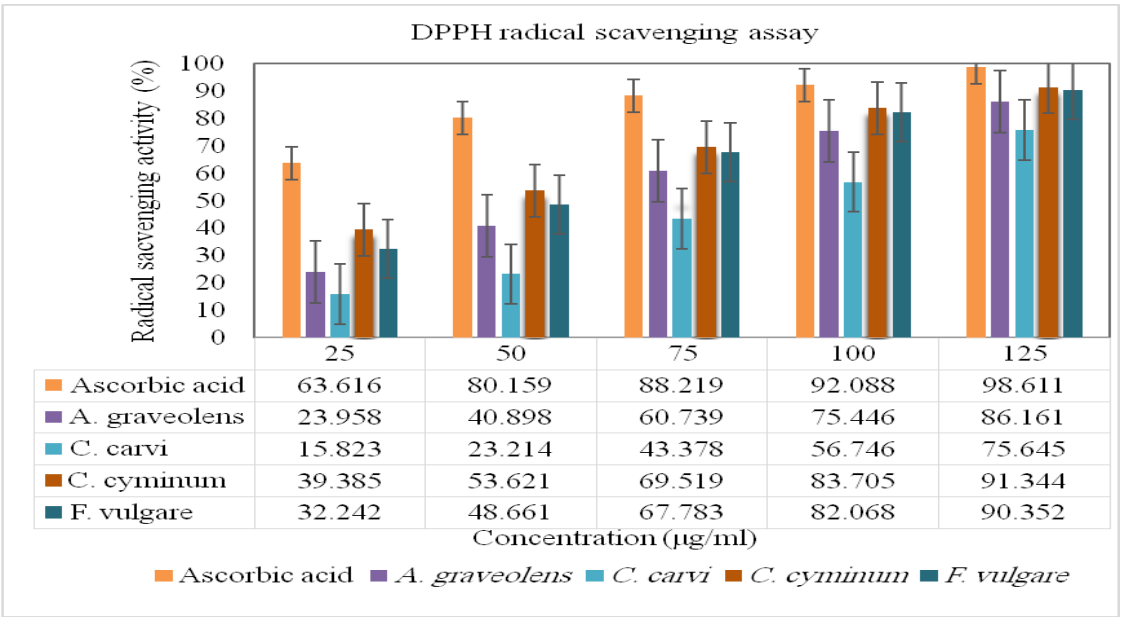
Fig. 3: Standard calibration curve of quercetin and total Flavonoids contents in the samples. Each point represents the mean of three experiments.

***In vitro* Antioxidant activity**

The DPPH (diphenyl-p-picrylhydrazyl) radical scavenging and reducing power assays of the four methanol extracts were performed using the reported method [10].

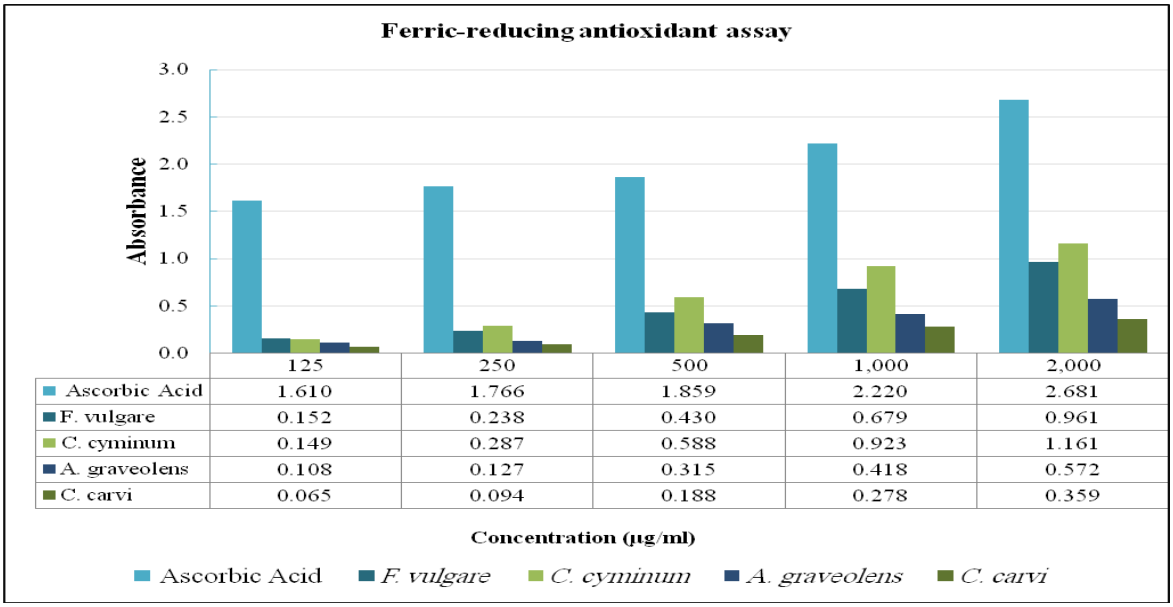
DPPH assay

All the experiments were performed in triplicate, using serial dilution (10-1000 µg/ ml) of both standard and samples and reading was observed at 517 nm using UV/VIS spectrophotometer.



	25	50	75	100	125
Ascorbic acid	63.616	80.159	88.219	92.088	98.611
A. graveolens	23.958	40.898	60.739	75.446	86.161
C. carvi	15.823	23.214	43.378	56.746	75.645
C. cyminum	39.385	53.621	69.519	83.705	91.344
F. vulgare	32.242	48.661	67.783	82.068	90.352

Fig. 4: DPPH radical scavenging assay of methanol extracts (% inhibition vs concentration graph for tested extracts and standard (Ascorbic acid). Values are means of triplicate determinations (*n* = 3).



	125	250	500	1,000	2,000
Ascorbic Acid	1.610	1.766	1.859	2.220	2.681
F. vulgare	0.152	0.238	0.430	0.679	0.961
C. cyminum	0.149	0.287	0.588	0.923	1.161
A. graveolens	0.108	0.127	0.315	0.418	0.572
C. carvi	0.065	0.094	0.188	0.278	0.359

Fig. 5: Ferric Chloride reducing power of methanol extract (absorbance vs concentration graph for tested extract and standard (Ascorbic acid). Values are means of triplicate determinations ($n = 3$).

Reducing power assay

All the experiments were performed in triplicate, using serial dilutions (10, 25, 50, 75 and 100 µg/ml) of both standard and samples and reading was measured at 700 nm using UV/VIS spectrophotometer.

RESULTS:

Preparation of the essential oils

The yields of volatile constituents were 2.51%, 2.95%, 3.91% and 2.10% w/w for *C. carvi*, *A. graveolens*, *C. cyminum* and *F. vulgare*, respectively.

GC/MS Analyses of the essential oils

The results of the GC-MS analyses are presented in Table 1 and Figure 1.

Phenols and Flavonoids in methanol extract

The total phenol contents of the methanol extracts of the selected Apiciacae members *C. carvi*, *A. graveolens*, *C. cyminum* and *F. vulgare* calculated from the calibration curve (Y = 0.0192 X - 0.1421; R^2 = 0.9981) were 35.28, 70.11, 119.09 and 111.21 mg of Gallic acid equivalents/100g (Figure 2). Similarly, the total flavonoid content (Y = 0.004 X - 0.0123; R^2 = 0.9994) were 25.72, 32.28, 64.39 and 54.14 mg of quercetin equivalents/100g, respectively for *C. carvi*, *A. graveolens*, *C. cyminum* and *F. vulgare* (Figures 2, 3).

***In vitro* antioxidant study of methanol extracts**

The percentage DPPH radical-scavenging action of the methanol extracts of the selected fruits *C. carvi*, *A. graveolens*, *C. cyminum* and *F. vulgare* are shown in Figure 4. The methanol extract *of C. cyminum* showed the higher % inhibition of DPPH (91.34 %) followed by *F. vulgare* (90.35%), *A. graveolens* (86.16%), and *C. carvi* (75.64%) but lower than, ascorbic acid standard (98.61%) at 125 µg/ml (Figure 4). Similarly, the absorbance in the ferric chloride reducing activity was highest for *C. cyminum* (1.161) and lowest by *C. carvi* (0.359) methanol extracts but the value for *C. cyminum* was lower than the ascorbic acid (2.681) at 2000 µg/ml (Figure 5).

DISCUSSION:

Essential oils & GC/MS Analyses

The yields of volatile constituents for *C. carvi*, *A. graveolens*, *C. cyminum* and *F. vulgare* were 2.51%, 2.95%, 3.91% and 2.1% w/w respectively. *C. cyminum* has the highest essential oil contents, while *F. vulgare* has the least contents. For comparison the five most prominent peaks in the GC/MS analysis of the oils were presented in Table 1. Similarity, qualitative and quantitative differences exist in the oil components allowing an easy identification of each one. Carvone, limonene and thymol are common in *C. carvi* and *A. graveolens* in different proportions. While *C. carvi* is characterized by E-Limonene-1,2-epoxide, γ-Terpinene; *A. graveolens* contain

relatively high concentration of dillapiole and less amounts of dihydrocarvone. *F. vulgare* oil is characterized by the presence of *t*-anithole and fenchone as the most prominent components. The oil of *C. cyminum* contains α-Thujenal, *p*-Cymene, 2-Methyl-3-phenyl-propanal, γ-Terpinene and α-pinene.

Total Phenols and Flavonoids

Polyphenols is a term used to describe simple and complex phenolic compounds. Most naturally occurring phenolic compounds are phenolic acids, flavonoids and tannins [11]. Flavonoids are the major group of the phenolic compounds, and it is highly effective scavengers of most oxidizing molecules and free radicals [12]. The phenolic contents of *C. cyminum* varied in different studies most likely due to environmental factors [13- 15]. The results obtained in the current study indicated that *C. cyminum* is the richest in term of total phenols and flavonoid contents (119.09 and 35.28 mg/100 g, respectively) (Figures 2, 3). *F. vulgare* came in the second place with 111.21 and 54.14 mg/100g of total phenols and flavonoids respectively. The fruits of *C. carvi* has the least amount of total phenols and flavonoids (35.28 and 25.72 mg/ 100 g respectively).

Antioxidant study

The free radical scavenging activity of DPPH is an extensively used method for testing *in-vitro* antioxidant activity of secondary metabolites [16]. In this method, decolourization of the purple colour of DPPH is directly proportional to the potency of the antioxidants. A large decrease in the absorbance of the purple colour indicates significant free radical scavenging activity of the extracts [17]. In $FeCl_3$, reducing assay, the yellow colour of the $FeCl_3$-solution changes to green depending on the antioxidant potential of the test drug. Earlier reports suggested that the reducing activities have been shown to exert antioxidant action by giving of a hydrogen atom to break the chain of free radical and the increasing absorbance is directly proportional to the concentration of the antioxidant compound [18]. Interestingly, the results of the antioxidant study are in complete agreement and support of the total phenolic and flavonoid contents. The fruits showed antioxidant activity in the same order with that of total phenolic and flavonoid contents (Figures 4, 5). Consequently, *C. cyminum* showed the highest antioxidant effect and *F. vulgare* was slightly less in activity. *A. graveolens* fruits were in the third place followed by *C. carvi* with the least total phenol, flavonoid contents and weakest antioxidant activity.

CONCLUSION:

GC/MS can provide an easy tool to identify the oils obtained from the selected fruits and differentiate between them. The carvone is the major components in *C. carvi* and *A. graveolens*. Limonene was common in three fruits with different amounts. *t*-Anethole and fenchone were characteristic for *F. vulgare*. *C. cyminum* has the highest contents of essential oil (3.91%). Antioxidant activity is directly proportional to the phenol and flavonoid contents. *C. cyminum* has the most strong antioxidant activity and highest phenol and flavonoid contents among the selected Apiaceous fruits.

Disclosure statement

No potential conflict of interest was reported by the author.

REFERENCES:

1. Abou El-Soud NH, Deabes MM, Abou El-Kassem LT, Khalil MY. Antifungal Activity of Family Apiaceae Essential Oils. Journal of Applied Sciences Research, 2012; 10:4964-4973.
2. Saganuwan, Saganuwan A. Some medicinal plants of Arabian Pennisula. Journal of Medicinal Plants Research, 2010; 9: 766-788.
3. Alqethami A, Hawkins JA, Teixidor-Toneu I. Medicinal plants used by women in Mecca: urban, Muslim and gendered knowledge. Journal of Ethnobiology and Ethnomedicine, 2017; 13:62.
4. Al–Yahya M, Collpharm A. Phytochemical studies of plant used in traditional medicine in Saudi Arabia. Fitoterapia. 1986; 57: 179-82.
5. Thakur M, Asrani RK, Thakur S, Sharma PK, Patil RD, Lal B, Parkash O. Observations on traditional usage of ethnomedicinal plants in humans and animals of Kangra and Chamba districts of Himachal Pradesh in North-Western Himalaya, India. Journal of Ethnopharmacology, 2016; 191: 280-300.
6. Baba SA, Malik SA. Determination of total phenolic and flavonoid content, antimicrobial and antioxidant activity of a root extract of *Arisaema jacquemontii* Blume. Journal of Taibah University for Science, 2015; 15: 449-454.
7. Yashin A, Yashin Y, Xia X, Nemzer B. Antioxidant Activity of Spices and Their Impact on Human Health: A Review. Antioxidants, 2017; 70: 1-18.
8. Lin JY, Tang CY, Determination of total phenolic and flavonoid contents in selected fruits and vegetables, as well as their stimulatory effects on mouse splenocyte proliferation. Food Chem. 2007; 101: 140-147
9. Khan TH, Ganaie MA, Siddiqui NA, Alam A, Ansari MN. Antioxidant potential of *Rumex*

vesicarius L.: *in vitro* approach. Asian Pacific Journal of Tropical Biomedicine. 2014; 7: 538-544.
10. Olugbami JO, Gbadegesin MA, Odunola OA. *In vitro* free radical scavenging and antioxidant properties of ethanol extract of *Terminalia glaucescens*. Pharmacognosy Research. 2015; 1: 49-56.
11. Balasundram N, Sundram K, Samman. Phenolic compounds in plants and agri-industrial by-products Antioxidant activity, occurrence, and potential uses. Food Chemistry, 2006; 1: 191-203.
12. Bravo L. Polyphenols: chemistry, dietary sources, metabolism and nutritional significance. Nutr Reviews. 1998; 56: 317–333.
13. Rebey IB, Zakhama N, Karoui IJ, Marzouk B. Polyphenol composition and antioxidant activity of Cumin (*Cuminum Cyminum* L.) seed extract under drought. J Food Sci. 2012; 77: 734-739.
14. Einafshar S, Poorazrang H, Farhoosh R, Seiedi SM. Antioxidant activity of the essential oil and methanolic extract of cumin seed (*Cuminum cyminum*). Eur. J. Lipid Sci. Technol. 2012; 114: 168-174.
15. Ereifej KI, Feng H, Rababah TM, Tashtoush SH, Al-U'datt MH, Gammoh S, Al-Rabadi, GJ. Effect of Extractant and Temperature on Phenolic Compounds and Antioxidant Activity of Selected Spices. Food and Nutrition Sciences, 2016; 7: 362-370.
16. Alhakmani F, Kumar S, Khan SA. Estimation of total phenolic content, *in-vitro* antioxidant and anti-inflammatory activity of flowers of *Moringa oleifera*. Asian Pacific Journal of Tropical Biomedicine. 2013; 8: 623-627.
17. Krishnaiah D, Sarbatly R, Nithyanandam RR. A review of the antioxidant potential of medicinal plant species. Food Bioprod Process. 2011; 89: 217–233.
18. Saeed N, Khan MR, Shabbir M. Antioxidant activity, total phenolic and total flavonoid contents of whole plant extracts *Torilis leptophylla* L. BMC Complementary and Alternative Medicine. 2012; 12:221.