Research Article

# Comparative Study of Antioxidant Potential of Two Indian Medicinal Plants - *Foeniculum vulgare* and *Eugenia caryophylata*

**Juilee Patwardhan*, Nancy Pandita, Purvi Bhatt**
School of Science, NMIMS (Deemed-to-be University), Vile Parle (West), Mumbai, Maharashtra, India.
***Corresponding author's E-mail:** juilee.pat@gmail.com

**Accepted on:** 30-05-2013; **Finalized on:** 31-07-2013.

**ABSTRACT**

Free radicals are produced in the body as by products of the metabolism and also due to some external agents. Due to increased amount of free radicals, the endogenous antioxidant levels deplete and lead to various diseases due to increased oxidative stress. As synthetic antioxidants are harmful, antioxidant levels can be maintained at the normal level by the use of plant derived products. In this study, the flavonoid content and antioxidant activities of *Foeniculum vulgare* and *Eugenia caryophylata* were evaluated. The flavonoid content was observed to be higher in the alcoholic extract of *Eugenia caryophylata*. The antioxidant activities were evaluated by DPPH, FRAP and ABTS assays. *Eugenia caryophylata* showed better antioxidant potential than *Foeniculum vulgare* as the $EC_{50}$ values were observed to be lower. Hence, the results suggest that *Eugenia caryophylata* has a better antioxidant potential than *Foeniculum vulgare* and can be used for the treatment of diseases caused by oxidative stress.

**Keywords:** Antioxidants, *Eugenia caryophylata*, Flavonoids, *Foeniculum vulgare,* Free radicals.

## INTRODUCTION

Free radicals are molecules that contain an unpaired electron and can hence react with various biomolecules. Free radicals can be generated endogenously as well as exogenously. Endogenous free radicals are produced as by products of normal metabolism. Exogenous free radicals are generated by tobacco smoke, UV radiations, X-rays, gamma radiations, pollutants, hyperoxic environment, pesticides, etc. Some of the medicines are metabolised to form free radical intermediates which further act in increasing oxidative stress.[1] Reactive oxygen species (ROS) is an important free radical which plays a dual role - deleterious and beneficial. Beneficial effects occur at low concentrations which involve defence against invading microorganisms and in cell signalling pathways. The deleterious effect of ROS is to develop oxidative stress which disturbs the balance in the prooxidant/antioxidant status of the cell. Increase in ROS level leads to damage of biomolecules like lipids, proteins, DNA, etc. Therefore, free radicals and oxidative stress have been implicated in a number of diseases like ageing, cancer, neurological disorders, cardiovascular diseases, etc.[2]

Antioxidants are molecules that neutralise free radicals by accepting or donating electrons to eliminate the unpaired status of the free radical. Endogenous antioxidants (superoxide dismutase, catalase, glutathione, etc.) are present in the body to scavenge the free radicals. But, due to increased oxidative stress, endogenous antioxidants get depleted and the redox homeostasis is further altered. Hence, exogenous antioxidants are required to nullify the harmful effects of free radicals. Synthetic antioxidants are not preferable as they are toxic when used at high concentrations.[3] Hence, the use of plant molecules as antioxidants has increased. The various phytoconstituents like phenolics, flavonoids, carotenoids, tannins, sterols, essential oils etc are known to have antioxidant activities.

*Eugenia caryophylata* (clove) belongs to the family Myrtaceae. Clove buds are brown in colour and have a pungent odour and is commonly used in the Indian households as a spice. In Ayurveda, it is used as an antibacterial, antifungal, antiseptic, anti-inflammatory, anaesthetic, expectorant, antiemetic and also used to relieve bronchitis, asthma, cough, etc. In Chinese medicine, clove is used as a kidney tonic. The dried clove bud contains essential oils, phenolic compounds, tannins, flavonoids, resins, proteins, carbohydrates, etc. The antioxidant activity of clove is mainly due to the essential oils and the polyphenolic content in the bud.[4,5]

*Foeniculum vulgare* (fennel seeds) belong to the family Apiaceae. It is also used as a spice and condiment in Indian food. It has various medicinal uses due to its carminative, antimicrobial, diuretic, anti-flatulence properties. It is used in Ayurveda extensively for the treatment of gastrointestinal disorders.[6] Fennel is known to contain essential oils, phenols, flavonoids, tannins, etc. It also has anticancer and hepatoprotective activities.[4]

The current study aims at evaluating the flavonoid content and the antioxidant potential of extracts of *Foeniculum vulgare* and *Eugenia caryophylata* which can further provide insights in the treatment of various diseases caused mainly due to oxidative stress.

## MATERIALS AND METHODS

### Collection of plant material

Clove buds and fennel seeds were collected from Thane district, Maharashtra, India. *Foeniculum vulgare* and *Eugenia Caryophylata* were authenticated at the Blatter

herbarium, St. Xavier's College, Mumbai and the submitted specimen matches with the herbarium specimen number GD-1 and 111221 respectively.

### Preparation of plant extracts

Plant material was shade dried and powdered. 50 g of the plant material was successively extracted using n-hexane, chloroform and alcohol by cold extraction technique. The extract was filtered using Whatman filter paper No. 1 and concentrated using rotary evaporator. The percentage yield of the extracts was calculated.

### Phytochemical analysis of the extracts

The extracts were subjected to qualitative phytochemical analysis to confirm the presence of various phytoconstituents like carbohydrates, proteins, flavonoids, alkaloids, tannins and phenolic compounds by the standard protocols.[7,8]

### Thin Layer Chromatography for flavonoids

Thin Layer Chromatography was performed for the alcoholic extracts of *Foeniculum vulgare* and *Eugenia caryophylata*. Alcoholic extract was dissolved in methanol and was spotted on Silica Gel 60 $F_{254}$ pre-coated plates (Merck). Mobile phase for alcoholic extract of *Foeniculum vulgare* was ethyl acetate: formic acid: glacial acetic acid: methanol (7.5: 0.2: 0.15: 0.4) and that for alcoholic extract of *Eugenia caryophylata* was ethyl acetate: formic acid: glacial acetic acid: methanol (7.5: 0.2: 0.15: 0.3). The bands were observed under UV (254 nm and 366 nm). The plates were then derivatized using 2-aminoethyl diphenylborinate (Natural product) reagent (Sigma). Quercetin, kaempferol and gallic acid were used as standards.[9]

### Quantitative determination

### Total Flavonoid Content

Flavonoid content was determined by the Aluminium chloride method described by Chang *et al.*[10] 0.5 ml of the alcoholic plant extract (1mg/ml) in methanol was mixed with 1.5 ml methanol, 0.1 ml 10% aluminium chloride, 0.1 ml of 1M potassium acetate and 2.8 ml of distilled water. The reaction mixture was kept at room temperature for 30 mins and the absorbance was measured at 415 nm using a UV-Vis spectrophotometer. Quercetin was used as a standard in a concentration range of 10-100 µg/ml.

### *In vitro* antioxidant activity

### DPPH free radical scavenging assay

The DPPH radical scavenging ability of the alcoholic extracts was evaluated.[11] Various concentrations of the alcoholic extracts (2 ml) were prepared and 400 µl of 360µg/ml freshly prepared DPPH (Sigma) was added. Tubes were incubated in dark at room temperature for 30 min and the absorbance was measured at 516 nm. A tube with 2 ml methanol and 400 µl DPPH was maintained as a control. Ascorbic acid was taken as a standard. The percentage scavenging activity was calculated using the formula as given below:

$$\text{Percentage scavenging activity} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

### Determination of reducing power by FRAP assay

The reducing power of the alcoholic extracts was determined by the method described by Oyaizu.[12] Various concentrations of the alcoholic extracts (1 ml) were prepared in methanol. 2.5 ml of 0.2M phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide were added and the mixture was incubated at 50°C for 20 mins. After 20 min, 2.5 ml of 10% trichloroacetic acid was added and the mixture was centrifuged at 3000 rpm for 10 min. 2.5 ml of the supernatant was mixed with 2.5 ml distilled water and 0.5 ml of 0.1% freshly prepared ferric chloride. Absorbance was measured at 700 nm. Ascorbic acid was used as a standard.

### ABTS free radical scavenging assay

ABTS was dissolved in distilled water to a concentration of 7 mM and the ABTS radical was produced by reacting ABTS stock solution with 2.45 mM potassium persulfate (final concentration). The mixture was kept in dark for 12-16 hrs. ABTS radical was diluted with PBS (pH 7.4) to an absorbance of 0.7 (± 0.02). Various concentrations of the extracts were prepared in methanol. To 10 µl of the sample, 1 ml of the ABTS solution was added and the absorbance was measured at 734 nm after 1 min and up to 6 mins. Ascorbic acid was used as a standard. A tube containing 10 µl methanol and 1 ml of the ABTS solution was maintained as a control.[13] The percentage scavenging activity was calculated using the formula as given below:

$$\text{Percentage scavenging activity} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

## RESULTS AND DISCUSSION

The present study revealed the antioxidant activity of *Foeniculum vulgare* and *Eugenia caryophylata* showing the presence of medicinally active constituents. Table 1 shows the percentage yields of the extracts (n-hexane, chloroform and alcohol) of *Foeniculum vulgare* and *Eugenia caryophylata*. In case of *Foeniculum vulgare*, the n-hexane extract showed the highest yield of 9.15% whereas in case of *Eugenia caryophylata*, the alcoholic extract showed the highest yield of 8.01%.

**Table 1:** Percentage yields of the extracts of *Foeniculum vulgare* and *Eugenia caryophylata*.

| Extracts | *Foeniculum vulgare* | *Eugenia caryophylata* |
|---|---|---|
| n-Hexane | 9.15% | 2.09% |
| Chloroform | 5.81% | 4.35% |
| Alcohol | 4.42% | 8.01% |

Preliminary phytochemical screening of the n-hexane, chloroform and alcoholic extracts of *Foeniculum vulgare* and *Eugenia caryophylata* was carried out. The n-hexane and chloroform extracts showed absence of all the phytoconstituents tested. The alcoholic extract of *Foeniculum vulgare* tested positive for flavonoids and in the case of *Eugenia caryophylata*, flavonoids, tannins and phenolic acids were present. Whereas, carbohydrates, proteins and alkaloids were absent in the alcoholic extract of both the plants.

The alcoholic extracts of *Foeniculum vulgare* and *Eugenia caryophylata* were chosen for the further analysis as they showed the presence of phytoconstituents like flavonoids, tannins and phenolic compounds. Thin layer chromatography (TLC) was performed for the detection of various flavonoids present in the extracts. Figure 1a-b shows the TLC profiles of alcoholic extracts of *Foeniculum vulgare* and *Eugenia caryophylata* respectively. Quercetin, kaempferol and gallic acid were used as standards for TLC as observed in lane 1, lane 2 and lane 3 respectively in both the figures. Alcoholic extract of *Foeniculum vulgare* and *Eugenia caryophylata* showed the presence of 7 and 10 bands respectively. Natural product reagent reacts with the flavonoids and gives different coloured fluorescent bands depending on the functional groups present in the flavonoid. None of the standards used are present in the *Foeniculum vulgare* extract whereas in the *Eugenia caryophylata* extract, quercetin, kaempferol and gallic acid were seen to be present.

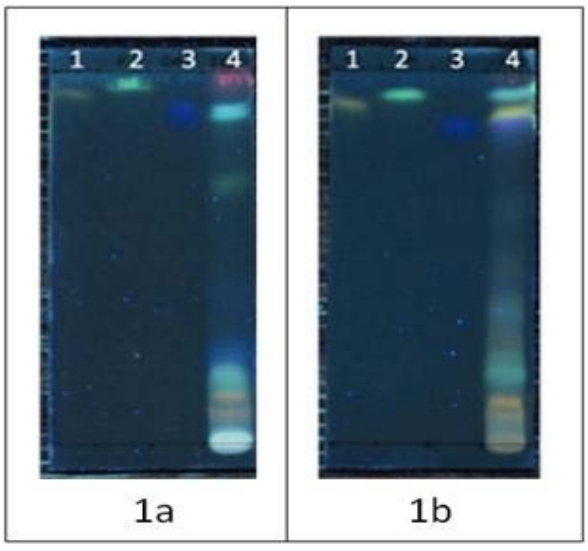


**Figure 1:** TLC profile of the alcoholic extracts after derivatization with Natural product (a) *Foeniculum vulgare* (lane 4) and (b) *Eugenia caryophylata* (lane 4). Lane 1 – Quercetin, Lane 2 – Kaempferol, Lane 3 – Gallic acid.

Total flavonoid content of the alcoholic extracts was determined by the Aluminium chloride method. The flavonoid content in the alcoholic extract of *Eugenia caryophylata* was 3.05 mg ± 0.07 which is much higher than that in *Foeniculum vulgare* which was 0.83 mg ± 0.09.

Table 2 shows the $EC_{50}$ values of the alcoholic extract of *Foeniculum vulgare* and *Eugenia caryophylata* observed in the DPPH, FRAP and ABTS assays. The alcoholic extract of *Eugenia caryophylata* showed lower $EC_{50}$ values in all the three assays as compared to that of *Foeniculum vulgare* indicating a better antioxidant potential. The alcoholic extracts of both the plants have an ability to reduce the ferric ions to ferrous form as observed in the FRAP assay which is a very important property of antioxidants. Free radical scavenging ability of the extracts was also observed in the DPPH and ABTS assays.

**Table 2:** $EC_{50}$ values of the alcoholic extracts for DPPH, FRAP and ABTS assays. Results are shown as mean ± SD (n=9).

| | $EC_{50}$ values (µg/ml) | | |
|---|---|---|---|
| | **Ascorbic acid** | ***Foeniculum vulgare*** | ***Eugenia caryophylata*** |
| DPPH assay | 8.70 ± 0.20 | 216.04 ± 4.47 | 16.03 ± 0.84 |
| FRAP assay | 65.08 ± 3.80 | 1267.5 ± 25.13 | 137.80 ± 6.36 |
| ABTS assay | 189.37 ± 6.56 | 3542.12 ± 136.82 | 191.17 ± 3.33 |

Figure 2a-c shows the DPPH radical scavenging ability of ascorbic acid and the alcoholic extracts of *Foeniculum vulgare* and *Eugenia caryophylata*. Figure 3a-c shows the reducing potential of ascorbic acid and the extracts. The reducing potential increased with the increasing concentration of the extracts. Figure 4a-c shows the ABTS radical scavenging abilities of ascorbic acid and the alcoholic extracts.

The higher antioxidant potential of the alcoholic extract of *Eugenia caryophylata* can be attributed to the higher flavonoid content of the extract.

Previous studies on the antioxidant activity of *Eugenia caryophylata* by Wojdylo *et al* [15] showed higher $EC_{50}$ values as compared to the current study. The reported $EC_{50}$ values for DPPH, FRAP and ABTS assays are 884 µg/ml ± 9.04, 2133 µg/ml ± 6.87, 346 µg/ml ± 5.34 respectively.

Anwar *et al* [16] have reported the flavonoid content and the $EC_{50}$ values in the DPPH assay of the alcoholic extract of *Foeniculum vulgare* as 374.88 mg ± 12.89 and 26.75 µg/ml ± 1.06 respectively. The reported $EC_{50}$ value was lower and the flavonoid content was higher showing a better antioxidant activity than that observed in the current study.

## CONCLUSION

In the present study, the flavonoid content and the antioxidant activities of the alcoholic extracts of *Foeniculum vulgare* and *Eugenia caryophylata* were evaluated. *Eugenia caryophylata* showed a higher flavonoid content as compared to *Foeniculum vulgare*.

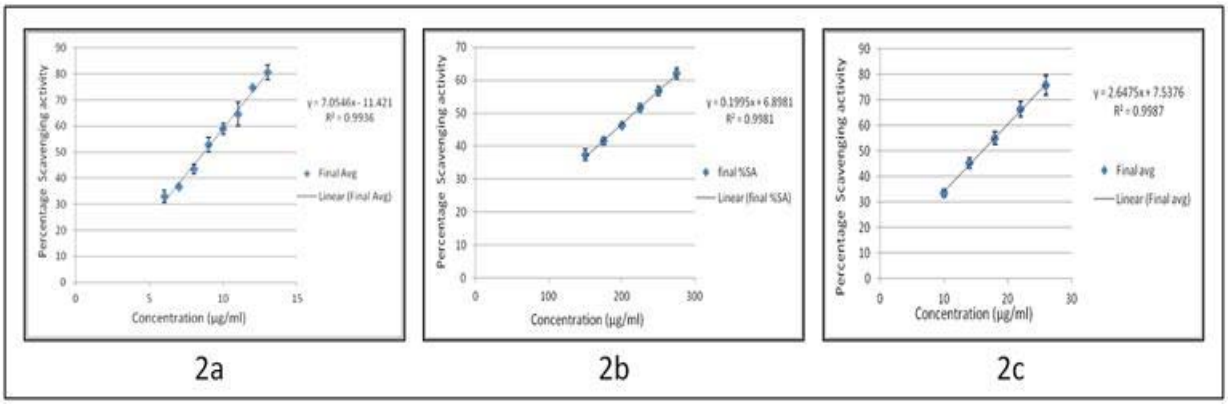


**Figure 2:** DPPH radical scavenging ability of (a) ascorbic acid, (b) alcoholic extract of *Foeniculum vulgare* and (c) alcoholic extract of *Eugenia caryophylata.*

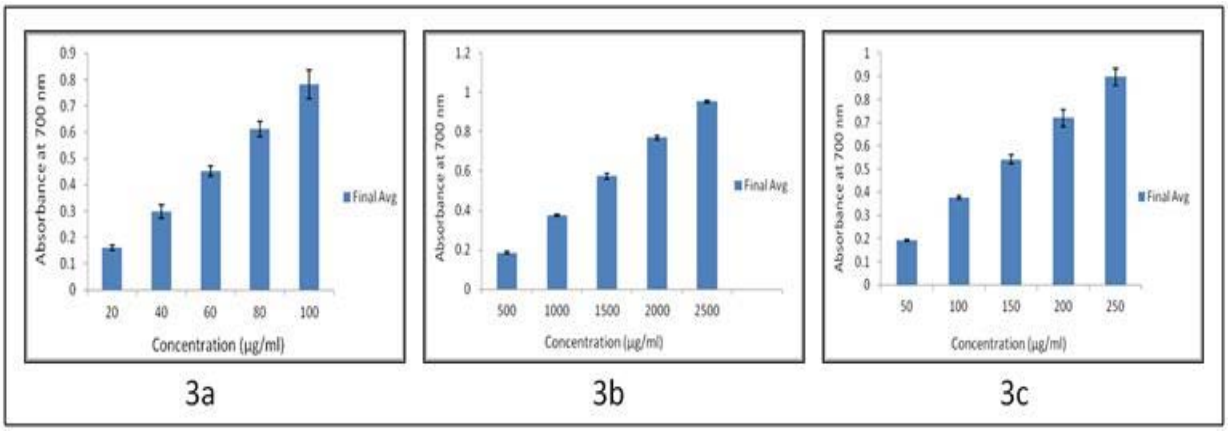


**Figure 3:** FRAP assay of (a) ascorbic acid, (b) alcoholic extract of *Foeniculum vulgare* and (c) alcoholic extract of *Eugenia caryophylata.*

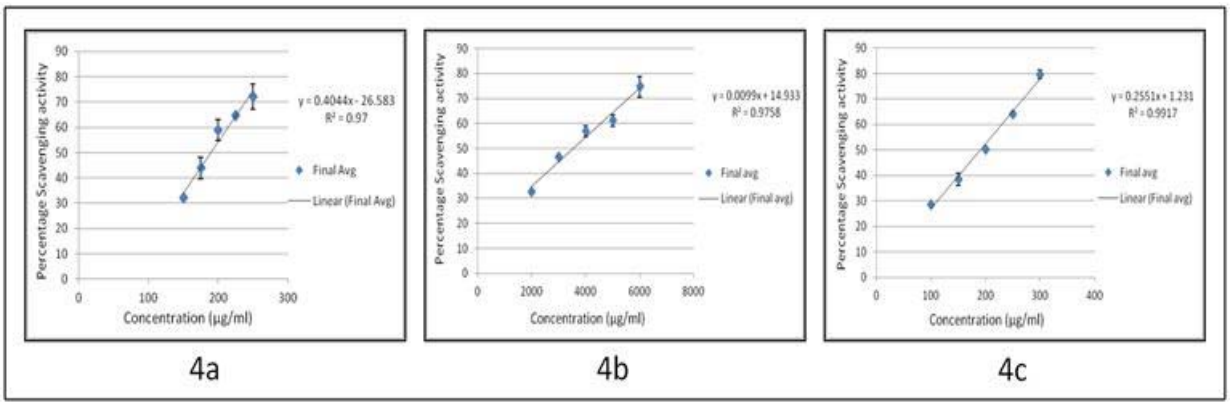


**Figure 4:** ABTS radical scavenging ability of (a) ascorbic acid, (b) alcoholic extract of *Foeniculum vulgare* and (c) alcoholic extract of *Eugenia caryophylata.*

DPPH and ABTS assays showed that the alcoholic extracts contain a free radical scavenging property. FRAP assay showed that the extracts contain a reducing potential which is an important property of antioxidants. Alcoholic extract of *Eugenia caryophylata* was found to be a better antioxidant than the *Foeniculum vulgare* extract. The results suggest that *Eugenia caryophylata* can be exploited further for the treatment of various diseases caused due to oxidative stress.

**Acknowledgement:** The authors are thankful to Dr. Krutika Desai and Dr. Brijesh Sukumaran for their constant help and support during this study. Thanks are due to Dr. Brijesh for critical reading of the manuscript. The authors are also grateful to Dr. Aparna Khanna, Dean, School of Science, Mumbai for providing the necessary facilities for carrying out the experimental work.

## REFERENCES

1. Rahman K, Studies on free radicals, antioxidants, and co-factors, Clinical Interventions in Aging, 2(2), 2007, 219-236.
2. Valko M, Leibfritz D, Moncola J, Cronin MTD, Mazur M, Telser J, Free radicals and antioxidants in normal physiological functions and human disease, The

International Journal of Biochemistry and Cell Biology, 39, 2007, 44-84.

3. Lüa JM, Lina PH, Yaoa Q, Chena C, Chemical and molecular mechanisms of antioxidants: Experimental approaches and model systems, Journal of Cellular and Molecular Medicine, 14(4), 2010, 840-860.
4. Parthasarthy VA, Chemapakam B, Zahariah TJ, Chemistry of Spices, CAB International, Oxfordshire, 2008, 146-164, 227-241.
5. WHO monographs on selected medicinal plants, World Health Organization, 2, 1999, 45-54.
6. Kaur GJ, Arora DS, Antibacterial and phytochemical screening of *Anethum graveolens*, *Foeniculum vulgare* and *Trachyspermum ammi,* BioMed Central Complementary and Alternative Medicine, 9, 2009, 30.
7. Khandelwal KR, Practical Pharmacognosy, 19, Nirali Prakashan, Pune, 2008.
8. Soni H, Sharma S, Patel SS, Mishra K, Singhai AK, Preliminary phytochemical study and HPLC analysis of flavonoid from methanolic extract of leaves of *Annona squamosa,* International Research Journal of Pharmacy, 2(5), 2011, 242-246.
9. Wagner H, Bladt H, Plant Drug Analysis-A Thin Layer Chromatography Atlas, 2, Springer-Verlag, Heidelberg, Germany, 1996, 195-244.
10. Ebrahimzadeh MA, Pourmorad F, Bekhradnia AR, Iron chelating activity, phenol and flavonoid content of some medicinal plants from Iran, African Journal of Biotechnology, 7(18), 2008, 3188-3192.
11. Kedare SB, Singh RP, Genesis and development of DPPH method of antioxidant assay, Journal of Food Science and Technology, 48(4), 2011, 412-422.
12. Fakruddin M, Mannan KS, Mazumdar RM, Afroz H, Antibacterial, antifungal and antioxidant activities of the ethanol extract of the stem bark of *Clausena heptaphylla*, BioMed Central Complementary and Alternative Medicine, 12(232), 2012.
13. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C, antioxidant activity applying an improved ABTS radical cation decolorization assay, Free Radical Biology and Medicine, 26, 1999, 1231–1237.
14. Chang CC, Yang MH, Wen HM, Chern JC, Estimation of total flavonoid content in Propolis by two complementary colorimetric methods, Journal of Food and Drug Analysis, 10(3), 2002, 178-182.
15. Wojdyło A, Oszmian´ski J, Czemerys R, Antioxidant activity and phenolic compounds in 32 selected herbs, Food Chemistry, 105, 2007, 940–949.
16. Anwar F, Muhammad A, Abdullah I H, Muhammad S, Antioxidant and antimicrobial activities of essential oil and extracts of fennel (*Foeniculum vulgare* Mill.) seeds from Pakistan, Flavour and Fragrance Journal, 2009.

**Source of Support:** Nil, **Conflict of Interest:** None.