Research Article

Study on Different Parts of *Ocimum tenuiflorum* Plant using GC-MS, XRD and UV-Visible Methods

R. Selvaraju[1], P. Sakuntala*[2], Kaleem Ahmed Jaleeli[3]

1. Physics section, FEAT, Annamalai University, Chidambaram, Tamil Nadu, India.

*2. Department of Physics, RBVRR Women's college, Narayanaguda, Hyderabad, T.S., India.

3. Department of Physics, Nizam College, O.U., Hyderabad, T.S., India.

***Corresponding author's E-mail:** sakuntalap71@yahoo.com

Received: 08-04-2020; **Revised:** 17-06-2020; **Accepted:** 28-06-2020.

ABSTRACT

The present work is aimed to overcome mineral deficiencies, which cause many diseases in our human body. Gas Chromatography–Mass Spectrometry (GC-MS) is a hyphenated method to identify chemical constituents in the plant samples. X-ray diffraction (XRD) is a dominant and non-destructive technique used to characterize minerals present in the medicinal plant. UV–Visible spectroscopy is very valuable method to analyze photosynthetic pigments of samples. In the present study, chemical compounds, minerals and photosynthetic pigments of the leaves, stems and seeds of *Ocimum tenuiflorum* plant are studied. The results confirm the presence of chemical compounds such as Eugenol, Caryophyllene, Limiflavine, Phytol, α-Copaene and 2H-1-Benzopyran-4,7-diol, 3,4-dihydro-2-phenyl-. From XRD studies oxides of calcium, potassium, magnesium, sodium and iron are identified. Phytochemicals such as chlorophylls, phenols and carotenoids are identified from UV-Visible studies. The identified chemical compounds and minerals are associated with the medicinal properties to overcome mineral deficiencies with the reported bioactivities of *Ocimum tenuiflorum* plant.

Keywords: Hyphenated method; *Ocimum tenuiflorum*; Phytochemicals; chlorophylls; carotenoids; bioactivities.

INTRODUCTION

Plant is an important source of medicine and plays an essential role in the world health. Indian tradition has a high impact on the medicinal plant for the drug. Bio-chemical compounds present in the plants are effective, have no side effect and have a low cost. Medicinal plants are being used in our daily life in the preparation of valuable medicines in developing countries like India, Brazil and China etc. These plants are sources of phytochemicals, valuable elements, vitamins and minerals, which can be used in the preparation of drugs in the pharmaceutical industry. Knowledge about medicinal plants has been transmitted gradually from generation to generation. Now a days, modern drugs are synthesized by using medicinal plants. Human knowledge is gradually developed with the formation of civilization with the provision of more facilities. The importance of usage of medicinal plants increases with the advancement of technology. Minerals are special kinds of nutrients that human body requires in order to function properly. Our human body uses minerals to perform various functions from building strong bones to transmitting nerve impulses. Some minerals are even used to make hormones or maintain a normal heartbeat. Mineral deficiencies can lead to a variety of health problems such as weak bones, fatigue, decreased immune system and anemia.[1-4]

The plant *Ocimum tenuiflorum* belongs to a family *Lamiaceae* and is worshipped by Hindus. The different plant materials like leaves, stems and seeds are used in the ayurvedic medicine. The species *ocimum* have many varieties and are grown in many parts of the world. Each species has its own medicinal values. It is also categorized as Vanya and Gramya. The plant is used in the treatment of many disorders like tumour, cancer, diabetics and stroke etc. The present work had been carried out on the three parts of *Ocimum tenuiflorum* plant using GC-MS, XRD and UV-Visible studies. The classification of *Ocimum tenuiflorum* plant is given below.[5-10]

Ocimum tenuiflorum

Kingdom	:	Plantae
Division	:	Magnoliophyta
Genus	:	*Ocimum*
Species	:	*tenuiflorum* L.
Binomial name	:	*Ocimum tenuiflorum* L.
Common name	:	Krishna Tulsi

MATERIALS AND METHODS

Collection and preparation of plant materials

The present investigation has been carried out with the three parts of plant materials - leaves, stems and seeds in *Ocimum tenuiflorum* plant. The leaves, stems and seeds of the plant (total of three samples) were collected from Central Institute of Medicinal and Aromatic Plants (CIMAP), Hyderabad, Telangana State, India. All the plant samples collected from without any infection (leaves, stems and seeds) as per the standard procedure. The

collected plant materials were first washed in water, then washed with distilled water, after that, it is air dried in room temperature for one week. The plant material were cut into small pieces and crushed into fine powder by using pestle mortar and stored in a container.

Sample preparation for GC-MS analysis

10 grams of the plant material powder was soaked in 100 ml of ethanol and it was left for 72 hours. The extract was filtered using Whatman No.1 filter paper and residue was removed. The filtrate was evaporated to dryness at 80^0 C and stored at 4^0 C until further analysis.[11]

Sample preparation for X-Ray Diffraction analysis

The collected plant materials were first washed in water, then washed with distilled water, after that, it is air dried in room temperature for one week. The plant material were cut into small pieces and crushed into fine powder by using pestle mortar and stored in a container. 1 gm of powdered plant samples were used for analysis.

Sample preparation for UV-Visible analysis

10 grams of *Ocimum tenuiflorum* plant parts were soaked separately in 100 ml of double distilled water for 30 minutes. Then, the extract was filtered using Whatman filter paper No.1 and stored in the amber bottle or plastic vials at 4^0 C. The extract is collected and preserved for further analysis. [12]

RESULTS

The present study had been carried out for the identification of chemical compounds, minerals and photosynthetic pigments using GC-Mass, X-Ray Diffraction and UV-Visible techniques. The prepared plant samples (leaf, stem and seed) were subjected to GC-Mass, X-Ray Diffraction and UV-Visible analysis.

GC-MS analysis

All the three sample extracts of *Ocimum tenuiflorum* plant were subjected to detailed GC-MS analysis to determine the chemical constituents using JEOL GCMATE II GC–Mass Spectrometer and the results are displayed in the following figures 1-3. Figures 1-3 show the chromatograms of leaves, stems and seed extracts of *Ocimum tenuiflorum* plant. The leaves of the plant are having Eugenol (11.49%), Benzenacetic acid, 3-ethoxy-4-hydroxy-(11.68%), Caryophyllene (11.98%), Limiflavine (13.16%), 5,7,3′,4′-tetrahydroxy flavones (10.62%), Phytol (10.41%) in higher amount. Whereas 1,4-Cyclohexadiene, 1-methyl-4-[1-methylethyl]-(6.83%), Benzenemethanol,4-hydroxy-3-methoxy(7.45%),Benzene,1,4-bis[1-ethylmethyl]-(6.68%), Flavone (9.75%) were in moderate amount. The compounds identified in the stems in higher amount are: α-Copaene (10.32%), Caryophyllene (11.05%), Flavone (13.99%) and 6-Hydroxyflavone (10.57%). Benzene, [2-methyl-3-butenyl]-(5.28%), gamma-Terpineol (7.35%), Eugenol (9.32%), E-6-Tetradecen-1-ol acetate (8.85%), Heneicosanoic acid, 3,3-dimethyl- (5.63%), Octadecanoic acid, octyl ester(5.73%), 9,12-Octadecadienoic acid[Z,Z]-(7.22%) are present in moderate amount. Whereas the compound 1,4-Cyclohexadiene, 1-methyl-4-[1-methyl ethyl]-(4.69%) is present in trace amount. The compounds Eugenol (16.59%), Caryophyllene (12.29%), 2H-1-Benzopyran-4,7-diol, 3,4-dihydro-2-phenyl-(18.06%) and E-6-Tetradecen-1-ol acetate(11.13%) were identified in higher amount in the seeds and Benzene, [1-methylpropyl]- (6.50%), Cyclohexanol, 1-methyl-4-[1-methylethylidene]- (9.04%), 4-Nonanone,8-methyl-(8.96%), E,E,Z-1,3,12-Nonadecatriene-5,14-diol(9.33%), Phytol(8.10%) are present in moderate amount. The compounds eugenol and caryophyllene are identified in all three parts of the plant and flavone is present in leaves and stems, whereas phytol is present in leaves and seeds of the plant.[13-15]

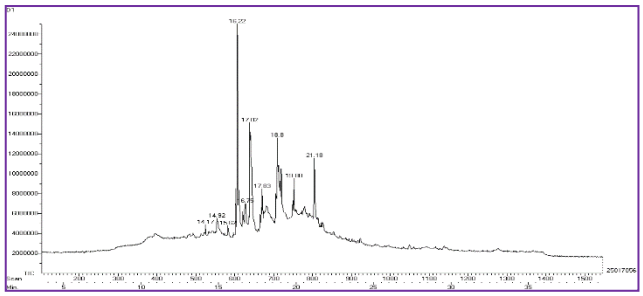

Figure 1: GC-MS chromatogram of *Ocimum tenuiflorum* leaf sample

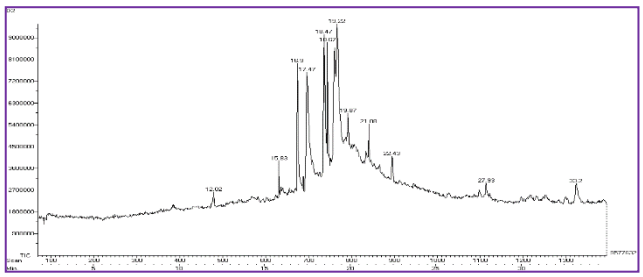

Figure 2: GC-MS chromatogram of *Ocimum tenuiflorum* stem sample

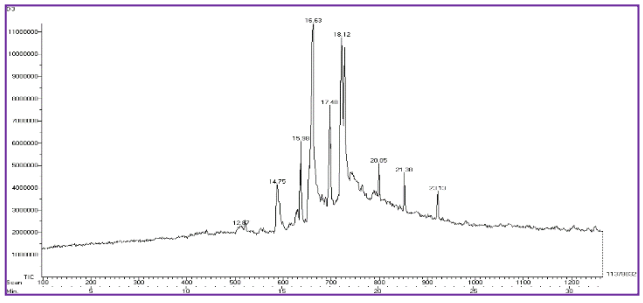

Figure 3: GC-MS chromatogram of *Ocimum tenuiflorum* seed sample

X-Ray Diffraction analysis

XRD spectra of *Ocimum tenuiflorum* plant samples are recorded with Shimadzu X-ray Diffractometer (XRD)–7000 and the spectra are shown in figures 4-6. The data compared with standard values from the Joint Committee on Powder Diffraction data. XRD data confirms the presence of minerals in the samples.

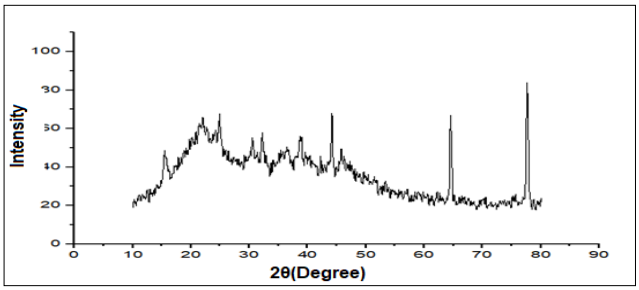

Figure 4: X- ray diffraction spectrum of *Ocimum tenuiflorum* leaf sample

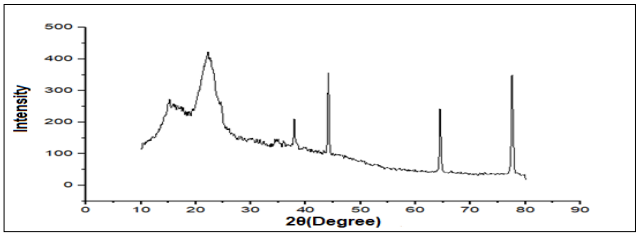

Figure 5: X- ray diffraction spectrum of *Ocimum tenuiflorum* stem sample

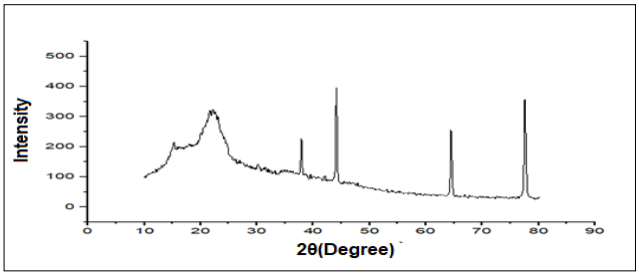

Figure 6: X- ray diffraction spectrum of *Ocimum tenuiflorum* seed sample

The leaves of the plant are having calcium oxide in higher amount, iron oxide in moderate amount and oxides of sodium and magnesium in lower amount. Oxides of sodium, potassium, magnesium and calcium are present in moderate amount in the stems of the plant. The seeds possess oxides of sodium, magnesium, potassium and calcium in moderate amount (JCPDS-2003).

UV-Visible analysis

The extracts were analyzed for phytochemicals using Shimadzu UV-Visible spectrophotometer (model: UV-1800). The samples are scanned at wavelength ranging from 400 to 800 nm using Schimadzu UV-Visible spectrophotometer and the characteristic peaks are detected. The UV spectra are shown in figures 7–9. [16]

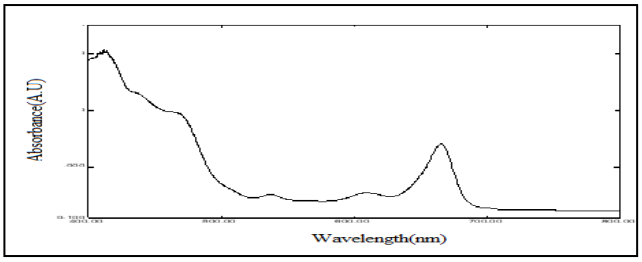

Figure 7: UV spectrum of *Ocimum tenuiflorum* leaf sample

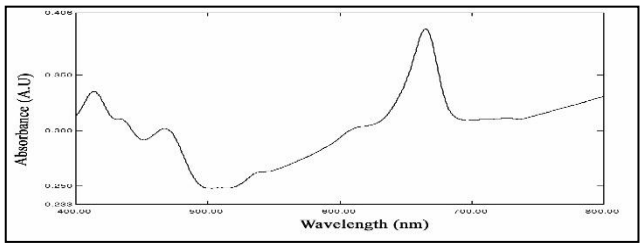

Figure 8: UV spectrum of *Ocimum tenuiflorum* stem sample

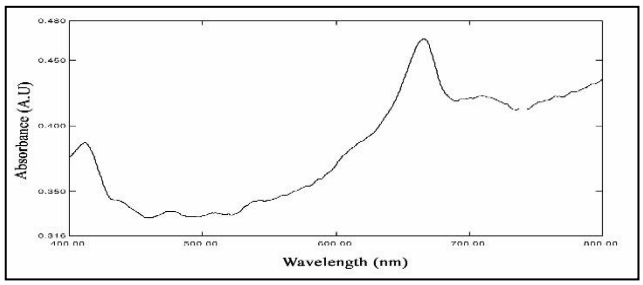

Figure 9: UV spectrum of *Ocimum tenuiflorum* seed sample

The figures 7-9 show UV spectra of leaves, stems and seeds of *Ocimum tenuiflorum* plant. The leaf, stem and seeds show the peaks at 410 nm, 466 nm, 536 nm, 609 nm, 665 nm; 412 nm, 434 nm, 467 nm, 665 nm; and 412 nm, 478 nm, 537 nm, 540 nm, 665 nm, 764 nm respectively. The sharp peaks present at 410 nm (leaf) and 412 nm (stem) are attributed to chromophores, the peaks 466 nm (leaf), 467 nm (stem) and 478 nm (seed) are assigned to carotenoids, the broad peak at 536 nm (leaf) and 537 nm (seed) are due to C=O aromatic compounds. The broad peak at 609 nm (leaf) is assigned to C=O amines and the sharp and most intense peak at 665 nm (leaf, stem) is attributed to chlorophyll. and the broad peak at 764 nm (seed) is assigned to phenols. [17-21]

DISCUSSION

GC-MS analysis indicate that medicinal plant materials (leaves, stems and seeds) of *Ocimum tenuiflorun* contain useful chemical compounds like eugenol, phytol, chlorophyll, γ-Terpineol, α-Copaene, Limiflavine, 2H-1-Benzopyran-4,7-diol, 3,4-dihydro-2-phenyl, flavone and caryophylline etc. The chemical constituents identified are responsible for various medicinal activities. Eugenol is having anti-mycotic, anti-viral, disinfection, anti-parasitic, anti-oxidant, anti-cancer, anti-insect activities and anti-microbial properties help in curing heart and other biochemical disease. Caryophylline is having biological activities like anti-viral, anti-inflammatory, anaesthetic, anti-carcinogenic, anti-microbial, anti-tumour, analgesic, antibacterial, fungicide, cytotoxicity and anti-fungal activities. The constituent of chlorophyll- Phytol can be used in the preparation of synthetic forms of vitamin E and vitamin K. Octadecanoic acid (stearic acid) exhibits antifungal and antialgal effects. Flavones reduce the risk of cancer, heart disease, asthma and stroke. The compound γ-Terpineol possesses antioxidant, anticancer, anticonvulsant, antiulcer, antihypertensive and anti-

nociceptive properties. α-Copaene is having antioxidant and antigenotoxic features. [22-25]

From X-ray diffraction (XRD) technique, minerals present in the plant materials of *Ocimum tenuiflorum* such as oxides of calcium, potassium, magnesium, sodium and iron are identified. The phytochemicals like carotenes, chlorophylls, phenols, chromophores, amines and aromatic compounds are identified using UV-Visible studies.

CONCLUSION

The present study emphasizes the analysis of leaves, stems and seeds of *Ocimum tenuiflorum* plant belongs to *lamiaceae* family. The samples are analyzed through GC-MS, X-ray diffraction and UV- Visible spectroscopic studies. The minerals present in the plant samples are identified by using X-ray diffraction technique. The results of UV-Visible studies confirm the presence of flavonoids, phenol and its derivatives. The presence of potential bioactive chemical constituents like eugenol, caryophyllene and flavones, having biological activities like anti-viral, anti-inflammatory, anaesthetic, anti-carcinogenic, anti-microbial, anti-tumour, analgesic, anti-bacterial, cytotoxicity and anti-fungal activities are identified with the GC-MS studies. The major chemical constituents, minerals and photosynthetic pigments are discussed along with their medicinal properties. Medicinal formulation of this plant parts can be designed taking into consideration these chemical constituents and minerals and provides some scientific basis in the preparation of medicines. The presence of various bioactive compounds, minerals and photosynthetic pigments, justifies the use of *ocimum teniuflorum* plant for curing various disease caused due to mineral deficiencies.

Acknowledgement: I, P. Sakuntala would like to express my heartfelt thanks to Prof. R. Selvaraju and Dr. Kaleem Ahmed Jaleeli for their constant guidance and support in completing my research work. Also express my thanks to CRL, R.B.V.R.R. Women's College, Hyderabad and SAIF, IIT, Chennai for providing necessary facilities to complete this work successfully.

REFERENCES

1. Villiathan M. S. "Healing plants", *Current sciences,* 75(11), (1998), p 1122-1127.
2. Davidson Hunt I, "Ecological ethno botany: stumbling toward new practices and paradigms", *MASA J*, 16(1), (2000), p 13.
3. Harborne JB, "Text book of Phytochemical methods", 1st edition, Chapman and Hall, London, (1973).
4. Santhosh Kumar S, Uma C, " Pharmacognostical and phytochemical screening of an Ayurvedic Medicinal Plant 'Karunthakali' (Solanum rubrum Mill)", *International Journal of Ayurvedic Medicine*, 4(4), (2013), p 328-341.
5. Pandey G, "Pharmacological activities of Osimum sanctum (Tulsi): A review", *Int J. pharma Sci Rev. Res.* 5(1), (2010), p 61-66.
6. Buddhadev SG, "A review article on Ocimum sanctum Linn.", *Int. Peer Revd. Ayur. J.*, 2(2), (2014), p 1-6.
7. Kumar PK, "Pharmacological actions of Ocimum sanctum. Review article", *Int. J. Advnc. Pharm. Bio. Chem.,* 1(3), (2012), p 406-414.
8. Joseph B, "Ethano pharmacological and photochemical aspects of Ocimum sanctum Linn. The elixir of life", *Brit. J Pharma. Res*. 3(2), (2013), p 273-292.
9. Gupta SK, Prakash J, Srivastava S, "Validation of claim of Tulsi, Ocimum sanctum Linn. as a medicinal plant", *Indian J. Exp. Biol*. 40(7), (2002), p 765-773.
10. Lethika D Nair, Santosh K Sar And Arun Arora, "Chemical characterization of Ocimum Sanctum in Bhilai- Durg Region of Chhattisgarh", *International Journal of Applied Science-Research and Review,* 3(4), (2016), p 130-137.
11. Lakshmi Kanta Kanthal, Akalanka Dey, Satyavathi K, and Bhojaraju P, "GC-MS analysis of bio-active compounds in methanolic extract of Lactuca runcinata DC", *Pharmacognosy Res,* 6(1), (2014), p 58-61.
12. Geetha Ravi, Thirunavukkarasu Ashokkumar, Selvaraj Tamilselvan, Kasivelu Govindaraju, Mohamed Sadiq, Ganesan Singaravelu,"Green synthesis of gold nanoparticles and their anticancer activity", *Cancer Nano*, 4, (2013), p 91–98.
13. 13. Mani P, Elamparithi D and Moorthy V, "Antimicrobial activity and GC-MS analysis of Ocimum tenuiflorum and Acalypha hispida extract against Streptococcus pyogenes", *Malaya Journal of Biosciences*, 1(4), (2014), p 259-266.
14. Sarma Biswajit and Goswami Bhabesh Ch, "Qualitative elemental analysis of some selected antidiabetic medicinal plants of Assam using X-ray Fluorescence (XRF) technique", *Asian Journal of Plant Science and Research*, 6(3), (2016), p 71-79.
15. Rastogi PR, and Mehrotra BN, "Compendium of Indian Medicinal Plants", 5, p 551, 583, PID, New Delhi, (2004).
16. Delia-Gabriela Dumbrava , Camelia Moldovan, Diana-Nicoleta Raba, Mirela-Viorica Popa, "Vitamin C, chlorophylls, carotenoids and xanthophylls content in some basil (Ocimum basilicum L.) and rosemary (Rosmarinus officinalis L.) leaves extracts", *Journal of Agroalimentary Processes and Technologies,*18 (3), (2012), p 253-258.
17. Malarvili Thekkumalai, Ramya Bashyam, Velavan Sivanandham, "Evaluation of Phytoconstituents of Bryonopsis laciniosa fruit by UV-Visible Spectroscopy and FTIR analysis", *Pharmacognosy Journal,* 7(3), (2015), p 65-170.
18. Rajalakshmi K, Banu N, "Extraction and Estimation of Chlorophyll from Medicinal Plants", *International Journal of Science and Research*, 4(11), (2015), p 209-212.
19. Selvaraju R, Sakuntala P and Kaleem Ahmed Jaleeli, "Extraction, estimation and analysis of chlorophyll from medicinal plants", *International Journal of Research Culture Society (IJRCS)*", Special Issue 2, (2017), p 119-123.
20. Premila Chanu Oinam, Kishan Kshetrimayum , Renuka Devi N, Palinchandra Sharma Aribam, Usharani Longjam, Karuna Devi Longjam , Archana Ng , Santosh Keisam, "Quantitative Estimation of total Chlorophyll and Carotenoid content In

Oreopanax Xalepensis", *International Journal of Pharmacy and Biological Sciences*, 8(4), (2018), p 410-415.

21. Dhivya SM and Kalaichelvi K, "UV-Visible Spectroscopic and FTIR Analysis of *Sarcostemma brevistigma*, Wight. And Arn.", *International Journal of Current Pharmaceutical Research*, 9(3), (2017), p 47-48.
22. Igwe KK, Nwankwo PO, Otuokere IE, Ijioma SN, Amaku FJ, "GCMS analysis of Phytocomponents in the Methanolic Extract of Moringa oleifera Leave", *Journal of Research in Pharmaceutical Science*, 2(11), (2015), p 01-06.
23. Balasubramanian S, Ganesh D, Shridhar Reddy P, Surya Narayana VVS, "GC-MS Analysis of Phytocomponents in the methanolic extract of *Ocimum sanctum* (Tulsi)", *Asian Journal of Pharmaceutical Analysis and Medicinal Chemistry*, 2(2), (2014), p 71-75.
24. Nandave M, Ojha SK, Joshi S, Kumari S and Arya D DS, "Moringa oleifera Leaf Extract Prevents Isoproterenol-Induced Myocardial Damage in Rats: Evidence for an Antioxidant, Antiperoxidative, and Cardioprotective Intervention", *Journal of Medicinal Food,* 12(1), (2009), p 47-55.
25. Waseem Hassan, Shakila Rehman, Hamsa Noreen, Shehnaz Gul, Neelofar, Nauman Ali, "Gas Chromatography Mass Spectrometric (GCMS) Analysis of Essential Oils of Medicinal Plants", *Advances in Animal and Veterinary Sciences,*4(8), (2016), p 420-437.

Source of Support: None declared.

Conflict of Interest: None declared.