COMPARATIVE ANALYSIS OF CHEMICAL COMPOUNDS AND TRACE ELEMENTS IN THE LEAVES OF FEW *OCIMUM* SPECIES PLANTS BY USING DIFFERENT SPECTROSCOPIC METHODS

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*Abstract***.** The objective of present research work was to identify bioactive chemical compounds with their functional groups and trace elements in the leaves of four varieties of *Ocimum* species plants, namely, *Ocimum sanctum, Ocimum kilimandcharicum, Ocimum gratissimum* and *Ocimum tenuiflorum* using gas chromatography-mass spectrometer (GC-MS), Fourier transform infrared spectroscopy (FTIR) and inductively coupled plasma-optical emission spectroscopy (ICP-OES) techniques. The chemical constituents with their functional groups were determined by GC-MS and FTIR. The leaves of *Ocimum* species plants are rich in various bioactive chemical constituents (eugenol, flavone, caryophyllene, palmitic acid, oleic acid, phytol, camphor, artemisinin, limiflavine). FTIR analysis revealed the presence of alcohols, carboxylic acids, alkanes, alkenes, alkyl halides, aliphatic amines and carotenoids. The results of ICP-OES indicated the presence of calcium, potassium, magnesium, sodium, copper, iron and zinc. The results indicated the presence of bioactive chemical constituents and trace elements help to minimize the health disorders. Medicinal formulation of these plants can be designed taking into consideration these elements and chemical compounds profile and provides some scientific basis in the preparation of medicines.

Key words: Chemical compounds, eugenol, functional groups, *Ocimum* species, oleic acid, camphor.

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 [39]. 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

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pharmaceutical industry [2, 8, 32, 34, 41]. 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 [1]. The importance of usage of medicinal plants increases with the advancement of technology.

The plant tulsi is considered for the worship of deity and is known as the queen of plants and mother medicine of nature due to its potential medicinal values [38]. Tulsi means incomparable one or matchless one [11]. It is known as *tulsi* in Hindi, *gujarati* in Bengali and *tulasa* in Marathi. In Tamil it is *thulasi*, *tulasi* in Telugu and *trittavu* in Malayalam. It is named as Holy basil in English. The different plant materials like leaves, stems and seeds are used in the ayurvedic medicine [28]. The species tulsi has many varieties and are grown in many parts of the world. Each species has its own medicinal values. It is also categorised as Vanya and Gramya [20]. The plant is used in the treatment of many disorders like tumour, cancer, diabetics and stroke, etc. [5, 7, 9]. The present work had been carried out on the leaves of *Ocimum sanctum*, *Ocimum kilimandcharicum*, *Ocimum gratissimum* and *Ocimum tenuiflorum* using GC-MS, FTIR and ICP-OES studies.

MATERIALS AND METHODS

COLLECTION AND PREPARATION OF PLANT MATERIALS

The present investigation has been carried out with the leaves of *Ocimum sanctum, Ocimum kilimandcharicum, Ocimum gratissimum* and *Ocimum tenuiflorum* plants. The leaves of the plants (total of four samples) were collected from Central Institute of Medicinal and Aromatic Plants (CIMAP), Hyderabad, Telangana State, India. All the plant samples are collected from without any infection 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 was cut into small pieces and crushed into fine powder by using pestle mortar and stored in a container. Figures 1–4 represent the leaves of four varieties of *Ocimum* species plants.

Fig. 1. *Ocimum sanctum* leaf samples.

Fig. 2. *Ocimum kilimandcharicum* leaf samples.

Fig. 3. *Ocimum gratissimum* leaf samples.

Fig. 4. *Ocimum tenuiflorum* leaf samples.

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 °C and stored at 4 °C until further analysis [17].

Sample preparation for FTIR 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 power by using pestle mortar and stored in a container. 1 g of powdered plant samples were used for analysis.

Sample preparation for ICP-OES analysis. Nitric-perchloric acids digestion

One gram of oven dried powdered sample is transferred to a teflon beaker and 10 mL concentrated nitric acid and 2.5 mL concentrated perchloric acid are added. The sample is then brought very slowly to boiling on a hot plate, heated and to dryness. If blackening occurs during the fuming stage, nitric acid is added drop wise. The sample is then cooled, re-dissolved in 10 mL distilled water and 1 mL concentrated hydrochloric acid and brought to a volume of 25 mL in a volumetric flask. The solution is then analyzed against calibration curves established. The prepared plant materials are subjected to ICP-OES analysis [37].

RESULTS

The present study had been carried out for the identification of chemical compounds with their functional groups and trace elements using GC-MS, FTIR and ICP-OES techniques. The prepared plant samples (leaf) were subjected to GC-MS, FTIR and ICP-OES analysis.

GC-MS ANALYSIS

All the leaf sample extracts of *Ocimum* species plants 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 5–8. Figures 5–8 show the chromatograms of leaf extracts of *Ocimum* species plants.

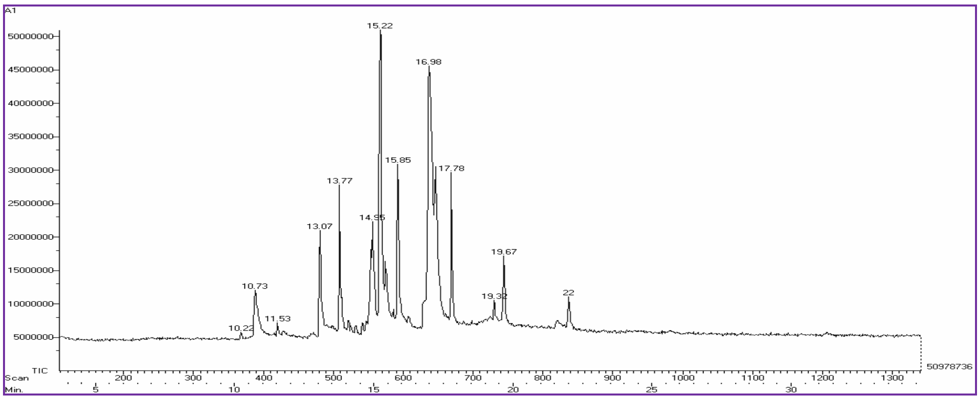

Fig. 5. GC-MS chromatogram of *Ocimum sanctum* leaf sample.

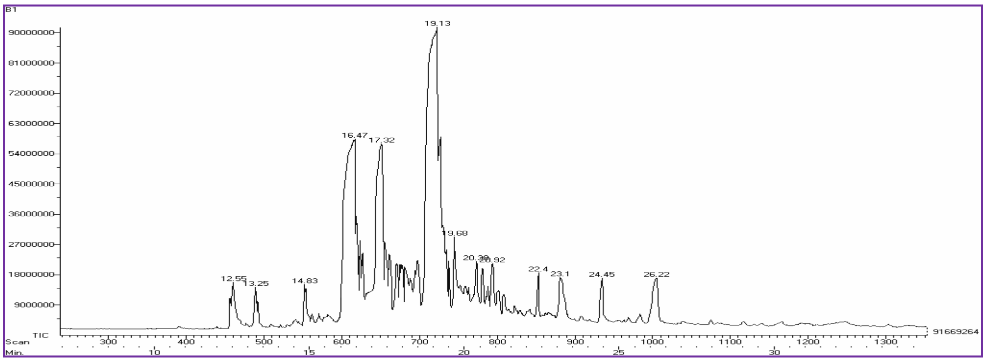

Fig. 6. GC-MS chromatogram of *Ocimum kilimandcharicum* leaf sample.

The compounds n-hexadecanoic acid (15.94 %), flavone (13.36 %) and eugenol (11.9 %) are present in higher amount in the leaves of the *Ocimum sanctum* plant and 5-octen-2-one,5-methyl-isopropyl palmitate (4.31 %) is present in less amount. Leaves of the *Ocimum kilimandcharicum* plant are having compounds camphor (16.91 %), thuiopsene-[12] (13.53 %) and eugenol (27.53 %) in higher amount. The chemical compounds identified in leaves of the *Ocimum gratissimum* plant were: eugenol (30.57 %), clohexene-1-methanol4-[1-

methylethenyl]-acetate (14.67 %), caryophyllene (14.93 %), artemisin (11.84 %). The leaves of the *Ocimum tenuiflorum* plant are having eugenol (11.49 %), benzeneacetic acid (11.68 %), caryophyllene (11.98 %), limiflavine (13.16 %), 5,7,3',4'-tetrahydroxy flavones (10.62 %), phytol (10.41 %).

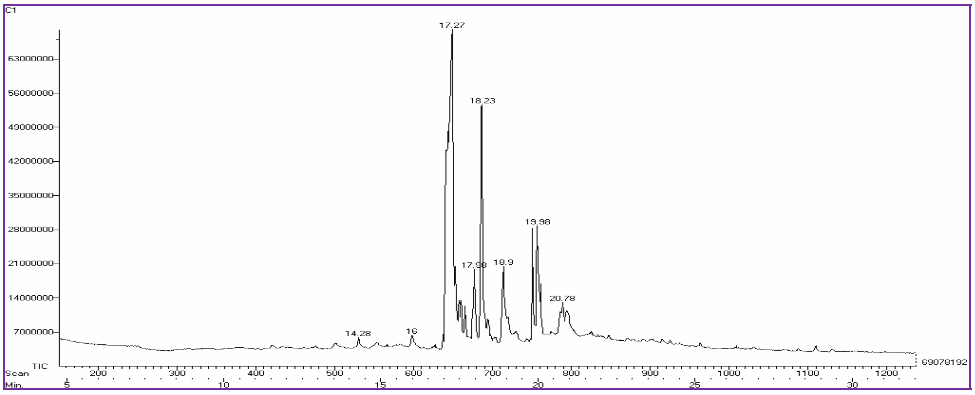

Fig. 7. GC-MS chromatogram of *Ocimum gratissimum* leaf sample.

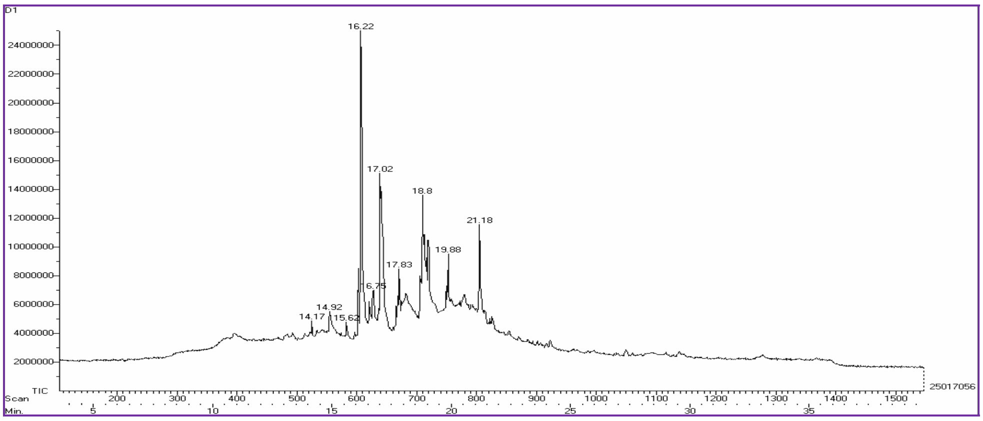

Fig. 8. GC-MS chromatogram of *Ocimum tenuiflorum* leaf sample [36].

FTIR ANALYSIS

Infrared spectra of all the dried powdered plant samples are recorded using Bruker alpha FTIR Spectrometer with ATR eco ZnSe accessory, atmospheric compensation, interferogram size 15192 points, and FT size 16 K, in the mid IR region, 4000–400 cm^{-1}. Resolution of the screen is given as 512×256 pixels. The functional groups of bioactive chemical compounds are identified, and results are shown in Figures 9–12.

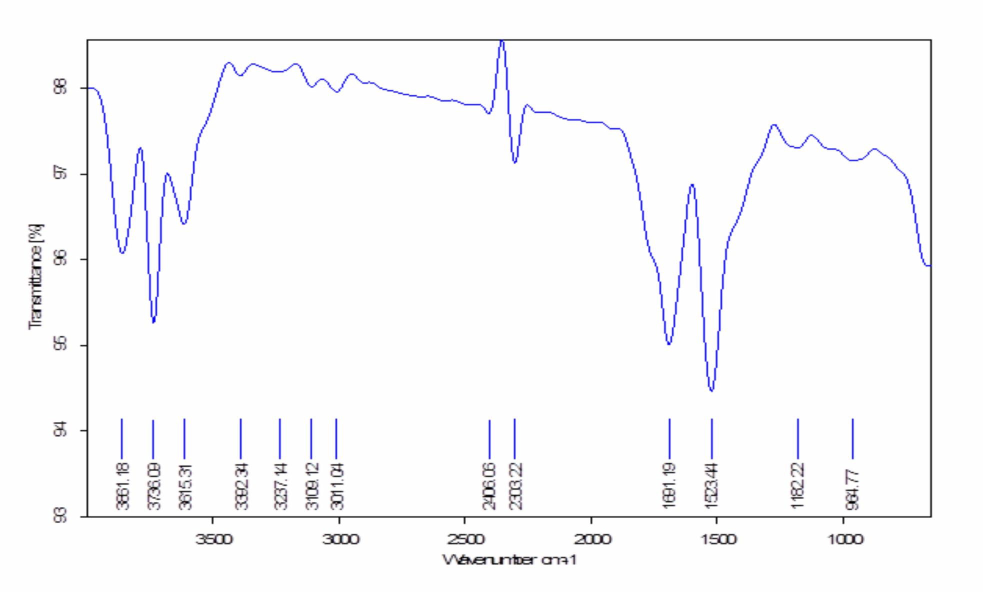

Fig. 9. FTIR spectrum of *Ocimum sanctum* leaf sample.

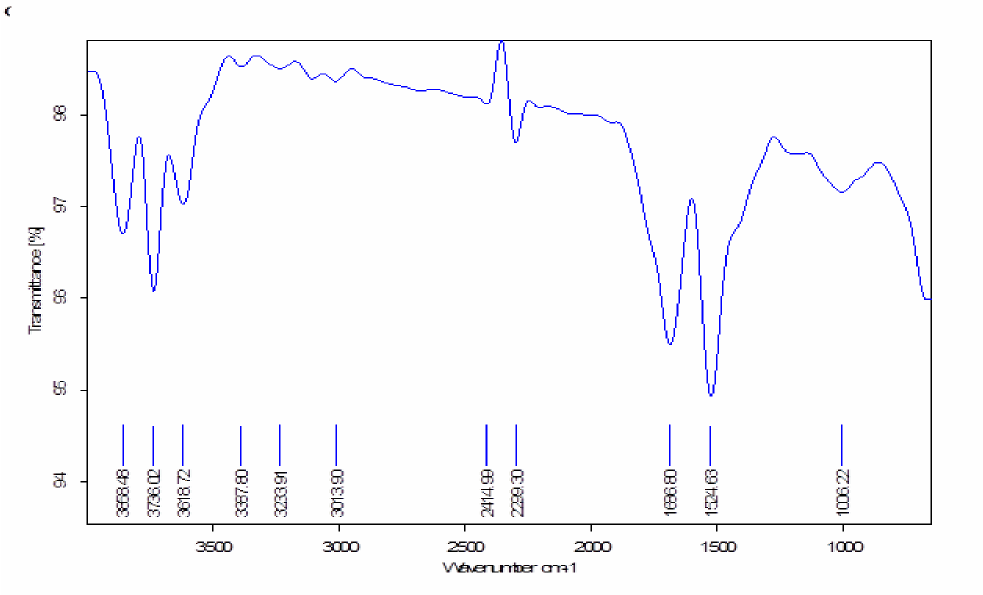

Fig. 10. FTIR spectrum of *Ocimum kilimandcharicum* leaf sample.

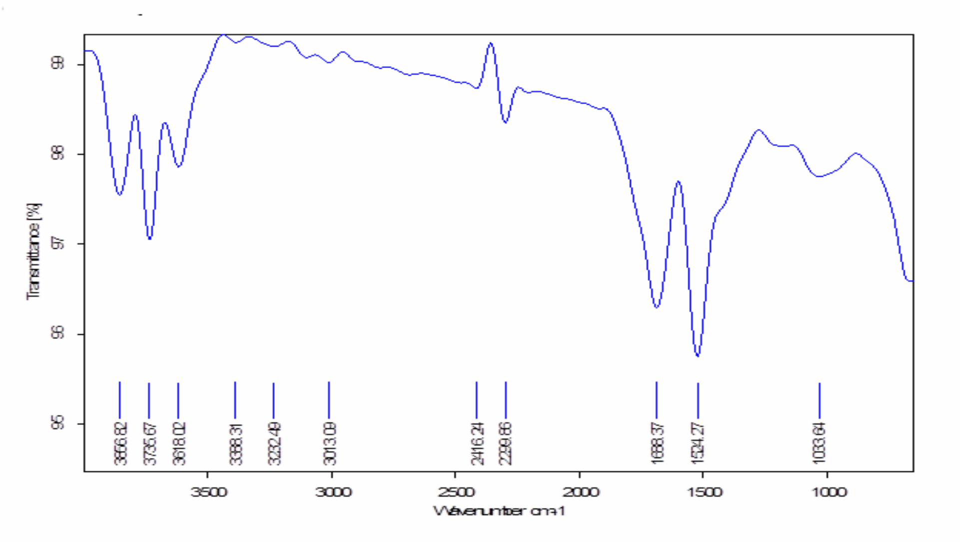

Fig. 11. FTIR spectrum of *Ocimum gratissimum* leaf sample.

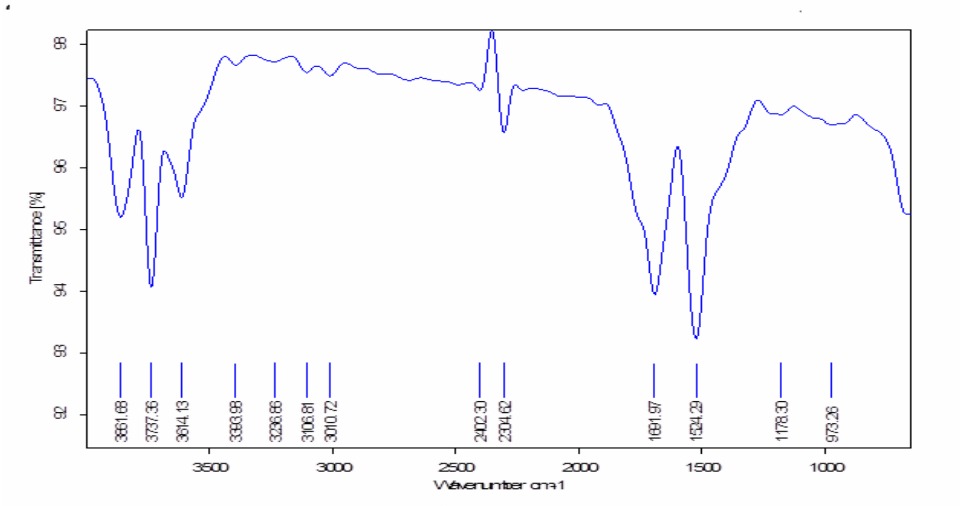

Fig. 12. FTIR spectrum of *Ocimum tenuiflorum* leaf sample.

From FTIR spectral data, the high intense peaks present at 3861 cm^{-1}, 3866 cm^{-1}, 3868 cm^{-1}, 3736 cm^{-1}, 3735 cm^{-1}, and 3737 cm^{-1} are assigned to the O–H stretching vibration of hydroxyl group. The peaks occur at 3515 cm^{-1} 3618 cm^{-1} and 3614 cm^{-1} are attributed to O–H stretching vibration of alcohol group. The weak peaks at 3392 cm^{-1}, 3337 cm^{-1}, 3388 cm^{-1} and 3391 cm^{-1} are mainly attributed to the N–H stretching vibration of amide group. The peaks appearing in the range of 3106–3237 cm^{-1} are attributed to O–H stretching vibration of carboxylic acid group. The peaks at 3010 cm^{-1}, 3011 cm^{-1} and 3013 cm^{-1} are due to the C–H stretching vibration of alkenes. The peaks at 2929 cm^{-1} and 2923 cm^{-1} are assigned to C–H stretching of alkanes. The medium intense peaks at 2406 cm^{-1}, 2414 cm^{-1}, 2416 cm^{-1} and 2402 cm^{-1} are related to the C–H stretching of alkanes. The peaks 2303 cm^{-1}, 2299 cm^{-1} and 2304 cm^{-1} are assigned to C≡N stretching of nitriles. The sharp intense peak at 1691 cm^{-1}, 1666 cm^{-1} and 1668 cm^{-1} are assigned to the C=C stretching of alkenes. The strong peaks at 1523 cm^{-1} and 1524 cm^{-1} arise due to C=C of aromatic ring. The weak peaks at 1182 cm^{-1} and 1178 cm^{-1} are attributed to C–H, wagging of alkyl halides. The peak at 1033 cm^{-1} is identified as C–N stretching vibration of aliphatic amines. The peaks at 964 cm^{-1} and 973 cm^{-1} are attributed to the C–H bending vibration of carotenoids.

ICP-OES ANALYSIS

The prepared plant samples were subjected to ICP-OES analysis using Optima 5300 DV inductively coupled plasma optical emission spectrometer and the results are displayed in the following Table 1. The presence of elements like Ca, K, Mg, Na, Fe, Cu and Zn in the leaves of *Ocimum* species plants is quantitatively estimated and their variations in plants are discussed.

Table 1

Elemental profile of the *Ocimum* species plants (mg/L)

Elements	**Plant name**			
	Ocimum sanctum	***Ocimum kilimandcharicum***	***Ocimum gratissimum***	***Ocimum tenuiflorum***
Ca	3.018	3.085	4.363	6.152
K	1.706	3.473	5.967	2.522
Mg	1.205	1.584	1.636	1.268
Na	4.454	1.110	1.023	5.799
Cu	0.094	0.103	0.110	0.098
Fe	0.216	0.139	0.129	0.260
Zn	0.014	0.012	0.010	0.045

The leaves of *Ocimum tenuiflorum* are having calcium, sodium, iron and zinc in higher amounts. The elements potassium and magnesium are abundant in the leaves of *Ocimum gratissimum*. The higher amount of copper is present in the leaves of *Ocimum kilimandcharicum*.

Figure 13 shows the bar diagram of relative distribution of elements for *Ocimum* species plants. The elements Ca and K are present in higher amounts, where as Mg and Na are in moderate amount. The elements Fe, Cu and Zn are present in trace levels.

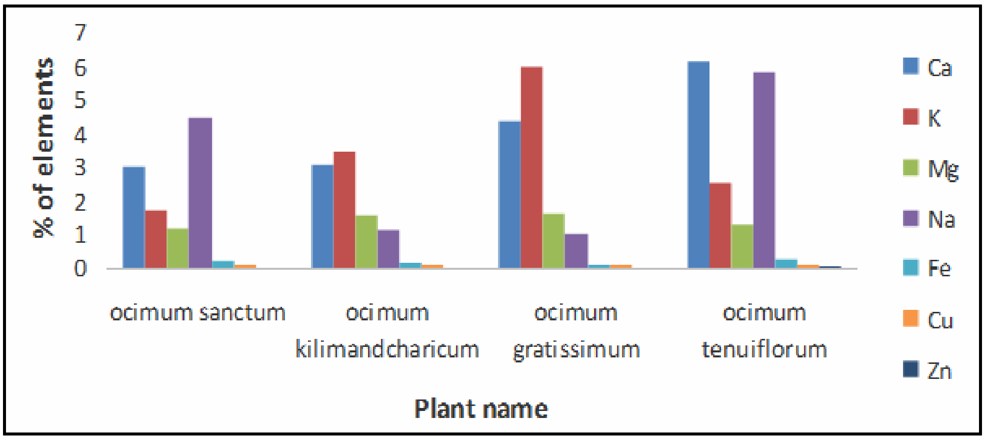

Fig. 13. The relative distribution of elements in leaves of *Ocimum* species samples by ICP-OES method.

DISCUSSION

GC-MS ANALYSIS

GC-MS analysis indicate that medicinal plants contain useful chemical compounds like eugenol, phytol, chlorophyll, limiflavine, camphor, flavone and caryophylline, etc. The chemical constituents identified are responsible for various medicinal activities. Eugenol is having anti-mycotic, anti-viral, desinsection, anti-parasitic, anti-oxidant, anti-cancer, anti-insect activities and anti-microbial properties help in curing heart and other biochemical disease. Caryophyllineis having biological activities like anti-viral, anti-inflammatory, anesthetic, anti-carcinogenic, anti-microbial, anti-tumor, 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 camphor is reported to have anti-candida, anti-inflammatory and anti-diabetic activities. Recent investigations have proved that artemisin is more effective in treating cancer and shows antimalarial activity. The confirmation of the presence of valuable chemical compounds responsible for medicinal activities is done through GC-MS; GC-MS analysis. The component, 5-octen-2-one,5-methyl-isopropyl palmitate reported in the leaves of *Ocimum sanctum* plant is rarely identified. The leaves *Ocimum* species of plants proved to be a reservoir of bioactive constituents which have phytopharmaceutical importance. The study creates a platform to screen many bioactive compounds to treat various disease in human beings and these are confirmed by [4, 12, 13, 14, 15, 16, 25, 28, 29, 32, 36, 38, 40].

FTIR ANALYSIS

FTIR studies were used to identify functional groups of chemical compounds in the plant materials. All the functional groups of chemical constituents were studied in the region 4000–400 cm^{-1}. The functional groups such as C=O, O–H, C–N, C≡N and C=C were identified. The FTIR study of the samples shows the presence of keto, alcohol, amino groups in aromatic and aliphatic components in fingerprint-like spectra. It confirms the results from [34]. This helps to elucidate the chemical structure and effort was taken to understand the significance of functional groups as bio-active constituents for the treatment of various diseases and these are confirmed by the results from [6, 10]. The peaks related to toxic elements are not present in the region 2220–2260 cm^{-1}, confirmed that absence of toxic elements in these plants and can be safely used in the preparation of medicines and it confirms the results from [23].

ICP-OES ANALYSIS

The present study emphasizes the importance of medicinal plants, which belong to *Ocimum* species to overcome mineral deficiency and diseases. The concentration of Ca, K, Mg and Na was highest compared to other and that confirms the results from [3, 21].

The present study emphasizes the analysis of leaves of *Ocimum* species plants belonging to *Lamiaceae* family. The samples are analyzed through GC-MS, FTIR and ICP-OES studies. The presence of potential bioactive chemical constituents like eugenol, artemisin, caryophyllene and flavones, having biological activities like anti-viral, anti-inflammatory, anaesthetic, anti-carcinogenic, anti-microbial, anti-tumor, analgesic, anti-bacterial, cytotoxicity and anti-fungal activities are identified with the GC-MS studies and are confirmed by [24, 26, 27]. Using FTIR, functional groups such as such as C=O, O–H, C–N, C≡N and C=C were identified. The elemental determination of *Ocimum* species plants was done using ICP-OES analysis. Quantitative measurement of the elements present Ca, K, Mg, Na, Fe, Cu and Zn has been made. The elemental variation of different *Ocimum* species plants is identified and these are confirmed by [22]. Calcium is important in maintaining strong bone. It also palys vital role in the activities related to pancreas. Research works have proved that the risk of type II diabetes can be reduced with the supplementation of calcium. The present findings have shown that the plants are having higher amount of calcium. It confirms the results from [33, 35]. Potassium is one of the most important elements for human body. It regulates acid base equilibrium and osmotic pressure of body fluids [35]. Magnesium is essential for enzymatic activities, which are responsible for the biochemical metabolism in the body. Magnesium also plays an active role in the transport of calcium and potassium ions across cell membranes [30, 31, 39]. Sodium is an essential electrolyte that helps in balancing water in and around the cells in the body. It takes active part in keeping the body healthy and also involves in the proper functioning of muscles and nerves [33]. Copper is an essential nutrient and involves in the formation of red blood cells. It help us in maintaining healthy bones, prevents heart disease and osteoporosis and also involves in various metabolic processes, transfers electrons to perform catabolic and anabolic reactions. The high level of copper is toxic and leads to chronic liver disease. Concentration of copper was found to be more in seeds of *Ocimum sanctum* and it confirms the results from [39].

Iron plays major role in transporting oxygen to all the parts of the body and involves in the formation of red blood cells. It involves in the transportation of oxygen and electrons in the body and takes active part in the synthesis of hemoglobin and regulates the immune system. Its deficiency causes several disorders like anemia, menstrual disorders and intestinal disease and are confirmed from [18]. Zinc is an essential trace element required to have normal metabolic activities. It controls the immune system and also helps in treating various disorders like common cold, diarrhea and wound healing. It is effective in gastric lesions, which are in initial stages. It is essential for activities of enzymes, especially in the synthesis of DNA and RNA and also involves in metabolic activities related to bones. The deficiency of zinc leads to various disorders like growth hindrance, poor appetite and psychological disturbances and these are confirmed with [19, 42].

CONCLUSIONS

The elemental analysis of these plants shows that these are rich in biologically important elements; it could be utilized as supplement of the elements in the human body. Medicinal formulation of these plants can be designed taking into consideration this elemental profile and provides some scientific basis in the preparation of medicines. The relation between concentrations of element present in the specific plant could be used to assess which plant can be used to cure a particular disease based on the elements. Literature has proved that consumption of elements such as sodium, magnesium, calcium, potassium, iron, copper and zinc helps to minimize the health disorders. These plants are rich in calcium, magnesium, potassium and sodium, possess moderate amount of iron and copper, and zinc in trace amount. The results indicate the presence of elements like Ca, K, Mg, Na, Cu, Fe and Zn that are associated with the medicinal properties with the reported bioactivities of these medicinal plants. Medicinal formulation of these plants can be designed taking into consideration this elemental profile and provides some scientific basis in the preparation of medicines.

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