RESEARCH ARTICLE

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GC-FID based metabolite profiling of leave extracts from naturally growing *Ocimum basilicum* L. *Ocimum gratissimum* L. and *Ocimum tenuiflorum* L.

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ABSTRACT
Ocimum spp., also known as "tulsi" member of Lamiaceae, are widely studied herbal plants. Due to their diverse biological activities, *Ocimum* basils are the most popular traditional herbal remedies against various diseases. The aim of the present study is comparative chemical composition profiling of bioactive compounds from three species viz: *Ocimum tenuiflorum* (Black tulsi), *Ocimum gratissimum* (Jungli tulsi), and *Ocimum basilicum* (Green tulsi). Leaves extracts from dried powder were prepared in acetone/water mixture solvent. GC-FID was used to study the comparative chemical profile of cultivars. The results revealed the presence of various bioactive constituents in acetone extracts but in a species-dependent manner. The main bioactive constituents in *O. tenuiflorum* were: β-bisabolene and tetracontane, in *O. gratissimum* were: 4-methyl - 2 -pentyl acetate and tetracontane, in *O. basilicum* were: β-bisabolene and tetracontane. The results indicated that bioactive compounds from tulsi species can be extracted and used as therapeutic agents having potential biological activities.

Key words: *Ocimum spp*. chemicals, acetone extracts

Introduction

Since the dawn of the Vedic period (3500-16 BC), as described in Ayurvedic and Unani literature, medicinal plants formed the basis of all medical sciences developments (Pattanayak et al., 2010). In alternative and complementary medical practice, aromatic and medicinal plant parts like shoots, leaves, roots, seeds, etc., are major sources of therapeutic agents (Jiang et al., 2015). The antioxidant potential of these plants has been attributed to the richness of bioactive compounds also known as secondary metabolites like alkaloids, flavonoids, phenols, saponins, etc., which are a chemically complex and diverse mixture of molecules (Wu et al., 2016). These components are reported to suppress redox reactions of free radicals in the biological system thus preventing the human body from suffering from various ailments (Gao et al., 2012). Other advantages associated with medicinal plants are global availability, cost-effectiveness, and fewer side effects (Mandave et al., 2014).

Among all medicinal plants, aromatic herbal plants are a major source of bioactive agents useful in medicine. Of these *Ocimum* spp, also known as "Tulsi, holy basil, Queen of plants, Mother Medicine of Nature", received a lot of attention due to its diverse medicinal values (Beatović et al., 2013). There are about 160 species identified under the genus *Ocimum* that belong to the family Lamiaceae and are cultivated worldwide in temperate regions (Hussain et al., 2017). In several countries like Australia, East Asia, Europe, and America they have been cultivated for commercial uses and extensively utilized in the food and perfume industries. In India, this herb is worshiped almost in every home and is the most valuable and holistic plant that is used in traditional medicine due to its therapeutic properties (Singh et al., 2010). Traditionally, aqueous extracts of leaves (dry powder or fresh) from this plant are used in herbal tea or mixed with honey to enhance its medical potential (Pattanayak et al., 2010). Tulsi oil extracted from this pant poses diverse biological activities like analgesic, anti-emetics, hypoglycemic, immune-booster, antibacterial, stress reliever, expectorants, etc. (Yamani et al., 2014). It was cited that antioxidant potential and diverse biological activities of *Ocimum* spp. are by virtue of having a diverse range of secondary metabolites such as phenolics and flavonoids (Naquvi et al., 2012). Keeping all this data in the background, the objective of this study was a comparative chemical profile analysis from leaf hydro-acetone extracts from *O. tenuiflorum* (Black tulsi), *O. gratissimum* (Jungli tulsi) and *O. basilicum* (Green tulsi) using GC-FID.

Materials and Methods

Plant material and Preparation of plant extracts

Three varieties of tulsi viz: *O. tenuiflorum* (Black tulsi), *O. gratissimum* (Jungli tulsi), and *O. basilicum* (Green tulsi) were collected in summer season (July, 2020) growing under the natural conditions of Jalandhar, India, located at 30º33 north longitude and 71º31 east latitude. Plants were authenticated by Dr. Upma and a voucher (BT-13) was submitted to the department. The city has a humid subtropical climate with cool winters and long, hot summers. The climate is dry on the whole. The mean, max/min temperatures was 40/24°C and day length was 12h50min. Fresh leaves from all *Ocimum* spp. at the flowering stage were harvested, washed with distilled water, and shade dried. The dried leaves were milled to powder with the help of a coffee blender. Plant extracts were obtained by a magnetic stirring of 3.0 g of dried powder with 30 ml of acetone /water (8:2 v/v), for 30 min at room temperature (~37°C). The extracts were kept in dark for 24 h at 4°C.

Phytochemical analysis

Preliminary screening of the presence of various phytochemicals like Saponins, Flavonoids, Terpenoids, Carbohydrates, Amino acids, Protein, Phenol, and Glycosides in Ocimum spp. leave extracts was done following Sharma et al., (2021).

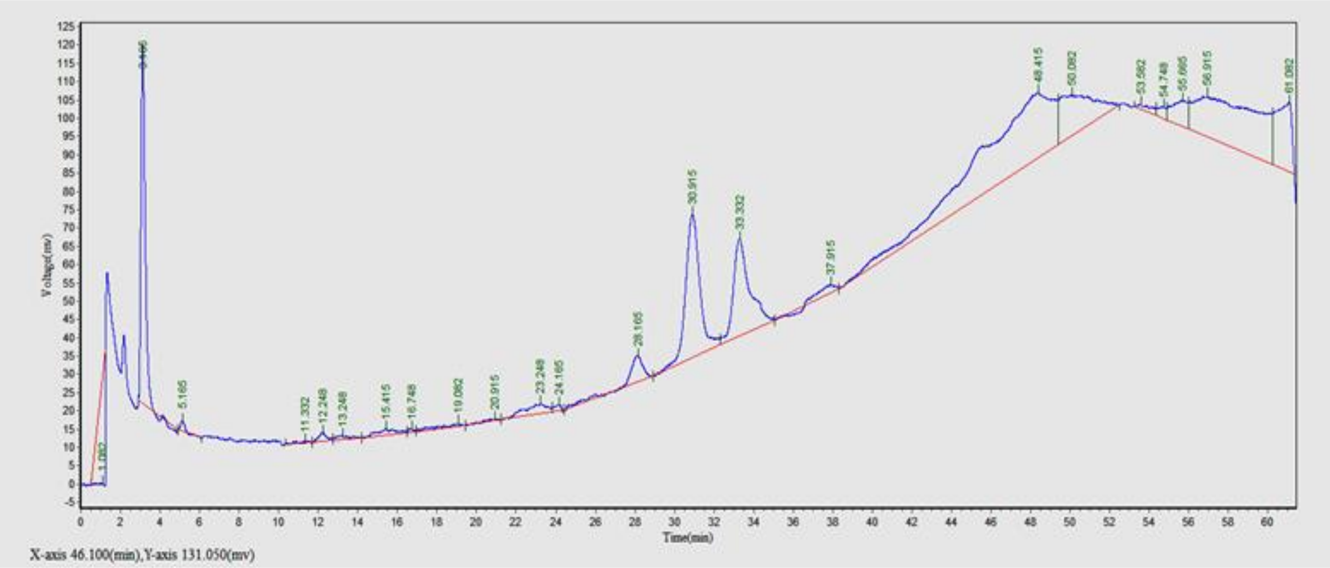
Gas Chromatography Analysis

Gas chromatography (GC-FID) analysis was performed following Sharma et al (2021) using a Chemtron 2045 gas chromatograph coupled with FID (flame ionization detector). A 2 m long column of stainless steel filed with 10% OV-17 on 80-100% mesh Chromosorb W (HP) was used. The carrier gas was nitrogen at a flow rate of 30 ml/min. The detector and injector temperatures were kept at 210 °C and 260 °C, and a 0.1 µl sample was injected. Ramping conditions for the oven were: 110°C (initially maintained) ramped to 200 °C at 2 °C/min. Bioactive compounds from hydro-acetone extracts were identified by comparison of relative retention times of GC-FID spectra with either those of known standards or with published data in the literature.

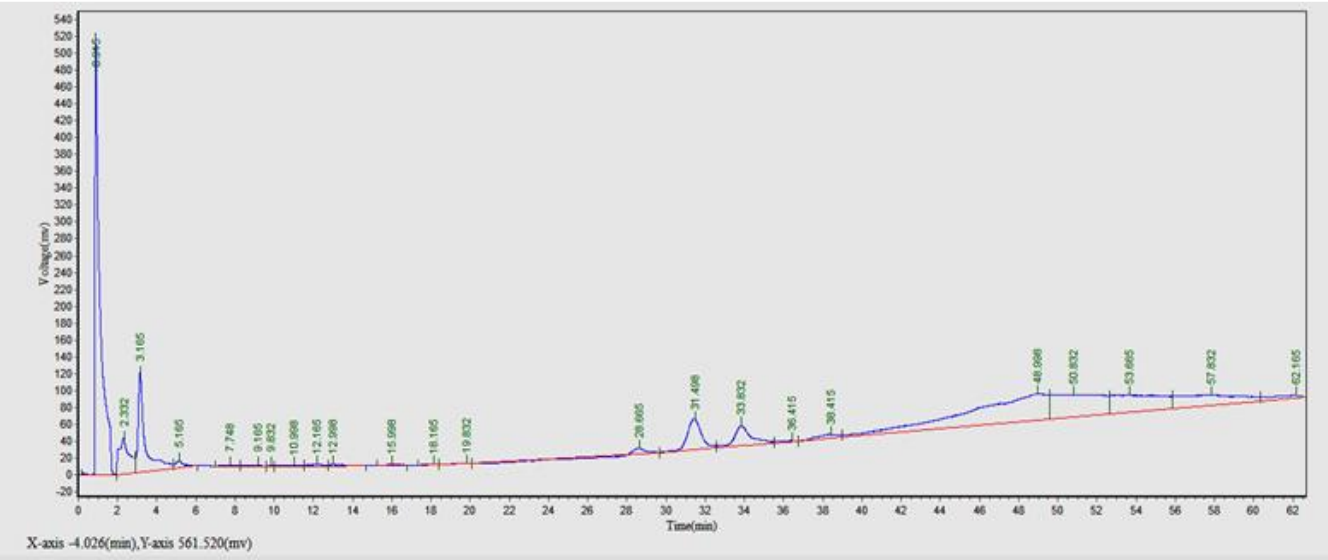
Results and Disscusion

Medicinal plants, especially *Ocimum* spp. are reported to be rich in bioactive compounds, hence immensely used as therapeutic agents in traditional medicine (Naquvi et al., 2012). Results of preliminary studies indicated that *O. basilicum* L. (Green tulsi), *O. gratissimum* L. (Jungli tulsi), and *O. tenuiflorum* (Black tulsi) are rich in polyphenolics (data not shown).

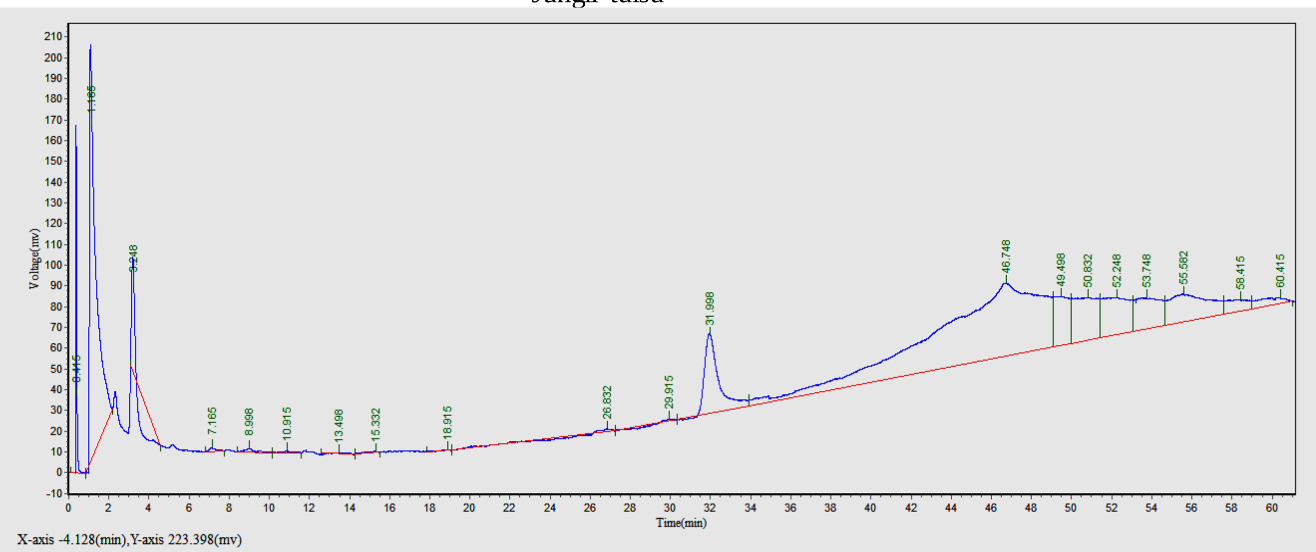
GC-FID profiles of acetone extracts of all tulsi spp. are shown in Figure 1. The superimposed GC-FID profile of all *Ocimum* spp is shown in Figure 2. The bioactive constituents along with their abundance percentage (%) and Retention time (RT) are presented in Table 1. The aroma profile revealed cultivar-dependent occurrence of bioactive compounds. As illustrated in Figure 1 and Table 1, in black and jungle tulsi, a total of 23 peaks were detected which were a combination of both major and minor peaks. In green tulsi, only 20 peaks were detected. In black tulsi, 6 major bioactive compounds were identified followed by 5 bioactive compounds in jungle tulsi and 3 bioactive compounds in green as given in table 1. In black tulsi major identified compounds were: 4 -Methyl - 2 -pentyl acetate, Eugenol, β-bisabolene, Epi—cadinol, Camphene, Tetracontane but with different concentrations. The most common major compound detected was Tetracontane with different concentrations in acetone extracts of all *Ocimum* spp. For instance: black tulsi (31.2%), jungle tulsi (23.6%), and green tulsi (44.5%). This was followed by β-bisabolene with a higher concentration in black tulsi (12.4%), green tulsi (6.3%), and jungle tulsi (4.6%). Jungle tulsi almost depicted the same profile as black tulsi, however, there were quantitative differences. For instance, the abundance of major component tetracontane was 23.6% along with others. In green tulsi, only 3 major bioactive compounds were detected, however, the quantitative differences were apparent. The differences in the chemical composition of the extracts among all *Ocimum* spp. analyzed can be explained as a consequence of differences in growing, environmental factors, soil, and agro-climatic (climatical, seasonal, geographical) conditions of the regions, stage of maturity, genetic differences, and adaptive metabolism of plants. This observation was in consonance with earlier reports highlighting that the chemical constitution of *Ocimum* extracts is highly influenced by various factors like; environmental conditions, edaphic factors, and types of cultivars (Hussain et al., 2017). Some common bioactive constituents were also detected in all three *Ocimum* spp, however, quantitative differences were apparent in three different species of *Ocimum* (Figure 2). Such variation in nature and type of bioactive components in various cultivars of *Ocimum* spp. has been reported earlier (Dev et al., 2011, Balasubramanian et al., 2014; Borah & Biswas, 2018, Mousavi et al., 2018; Manogaran & Muthukrishnan, 2019). The study by Balasubramanian et al., (2014), showed that the major components from methanolic extract of the leaves of *O. sanctum* are Benzene, 1, 2-dimethoxy-4-(2-propenyl)-, Isocaryophyllene and Eugenol. On the other hand, the study by other groups revealed the presence of enzene, α - Farnesene and Cyclohexane, 1, 2, 4- triethenyl and Oleic acid, 2-Isopropyl-5- methylcyclohe xyl 3-(1- phenyl-3-oxobutyl)- coumarin-4-Y, 1-Hexyl-2- Nitrocyclohexa (Borah & Biswas, 2018; Manogaran & Muthukrishnan, 2019). Mousavi et al., (2018), in n-hexane, chloroform, ethyl



Black tulsu



Jungli tulsu



Green tulsu

Figure 1. *GC-FID profile of all Ocimum spp.*

Table 1. *Chemical profile analysis of acetone extracts of all Ocimum spp.*

Black tulsi Total Number of peaks: [23]			**Jungli tulsi Total Number of peaks: [23]**			**Green tulsi Total Number of peaks: [20]**			**Functional group**
RT	Bioactive components	CONC. (%)	RT	Bioactive components	CONC. (%)	RT	Bioactive components	CONC. (%)	
-	-	-	2.3	unknown	3.9	-	-	-	
3.1	4 -Methyl - 2 - pentyl acetate	9.7	3.1	4 -Methyl - 2 - pentyl acetate	7.3	3.2	4 -Methyl - 2 - pentyl acetate	2.4	Benzaldehydes
28.1	Eugenol	2.3	28.4	Eugenol	0.5	-	-	-	Allylbenzene
31.0	β-bisabolene	12.4	31.0	β-bisabolene	4.6	31.3	β-bisabolene	6.3	Sesquiterpenes
33.3	Epi--cadinol	9.5	33.8	Epi--cadinol	3.6				Sesquiterpenes
37.9	Camphene	0.5	-	-	-	-	-	-	Monoterpene
48.4	Tetracontane	31.2	48.9	Tetracontane	23.6	46.7	Tetracontane	44.5	Alkane C40

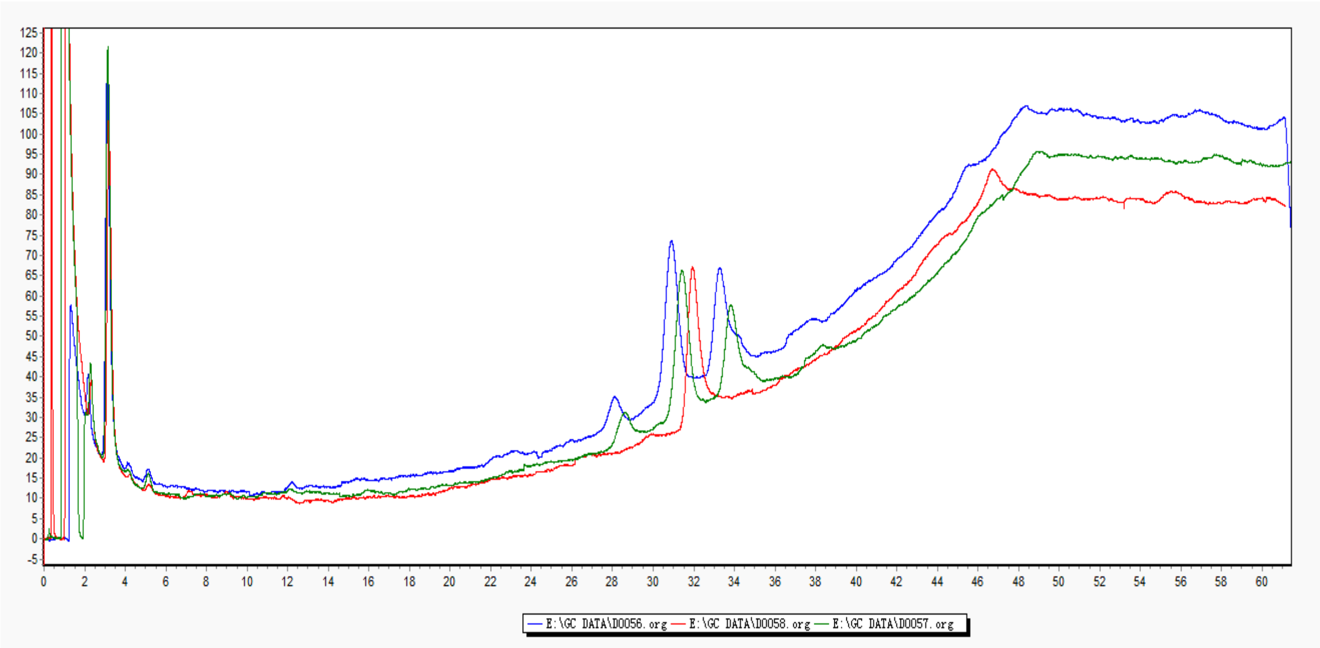


Figure 2. *Superimposed GC-FID profile of all tulsi spp.*

acetate, methanol, water and methanolic leaves extracts from *O. tenuiflorum* reported 3,4-dimethoxycinnamic acid, caffeic acid, permethrin, rosmarinic acid, kaempferol, luteolin (flavonoid), kaempferide (flavonol), chrysoeriol (flavon), xanthomicrol (flavonoid), isosakuranetin (Flavanone) and robinetin trimethyl ether (flavonoids). Dev et al., (2011) in *O. basilicum* hexane extract identified major chemical compounds like 1,2-dimethoxy-4-(2-propenyl) benzene, 2-pentanone, caryophyllene oxide, acetic anhydride, tricyclo, aromadendrene oxide-(2) and 1,3-benzodioxide. The antioxidant potential of plant extracts largely depends on the type of bioactive constituents present in them. Eugenol, which comprised about 0.5-2.3% of bioactives in Ocimum spp. Extracts, has shown to be largely responsible for therapeutic potentials and a number of biological activities like antioxidant, anti- inflammatory, acaricide anticancer, efficient vasodilator, and therapeutic for neurological antispasmodic, antibacterial, antiviral, insecticide (Sailaja et al., 2010). Another study by Wang et al. (2010) reported eugenol from *Ocimum* species to be responsible for the antifungal activity against yeasts and filamentous fungi, such as human-pathogenic, food-borne, phytopathogenic fungi and such as *Alternaria spp.* and *Botryosphaeria rhodina*. The different leaf extracts of Tulsi (*O. sanctum*), showed antimicrobial activity against three human pathogens *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans*. (Balasubramanian et al., 2014). Beta-bisabolene, a sesquiterpene, which comprised of 6-31% of bioactives in *Ocimum* spp. Extracts, has been identified as the abundant volatile compounds present in the essential oils of *O.*

tenuiflorum and *O. gratissimum*, and were shown to possess some antimicrobial activity (Joy et al., 2020). Another study reported beta bisabolene as a natural sweetener as a food additive and poses anticancer properties (Nuutinen, 2018). Epi—cadinol, which constituted 3-33% of total bioactives in *Ocimum* spp, has been reported to be as a phytotoxic agent (Abd-ELGawad et al., 2021). Due to these this rich biodiversity of bioactives *Ocimum* plants various biological properties like antiseptic, anti-inflammatory, anti-stress, analgesic, immunomodulatory, antimicrobial, hypoglycemic, cardioprotective, hypotensive, and antioxidant (Tanwar et al., 2015).

Conclusion

Owing to the presence of a greater number of identified bioactives, it was concluded that black tulsi hydro-acetone leaf extracts were rich in phytocompounds as compared to other tulsi species. The major bioactive compound identified was tetracontane, however, quantitative differences were observed in all *Ocimum* spp. These results indicated that from *Ocimum spp*. by using selective solvent one can extract a sufficient level of bioactive compounds having high biological activities which could be used as a source of antioxidants in food and pharmaceutical-based industries.

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