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An efficient approach of Laser Induced Breakdown Spectroscopy (LIBS) and ICAP-AES to detect the elemental profile of *Ocimum* L. species

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ABSTRACT

The present study is aimed to investigate the micro- and macro-element distribution in the leaves of four *Ocimum* species viz. *Ocimum basilicum*, *Ocimum sanctum*, *Ocimum gratissimum* and *Ocimum americanum*. For this, Laser Induced Breakdown Spectroscopy (LIBS) and Inductively Coupled Argon Plasma Atomic Emission Spectroscopy (ICAP-AES) were used. LIBS spectra of different *Ocimum* leaves (in the form of pellets) were recorded in the spectral range of 200–900 nm, which shows the atomic lines of potassium (K), sodium (Na), calcium (Ca), magnesium (Mg) and silicon (Si) along with lighter elements like carbon (C), hydrogen (H), oxygen (O) and nitrogen (N). Similarly, ICAP-AES also confirmed the presence of above mentioned elements as well as their patterns (except the lighter elements due to its identification limits). Results of both the techniques clearly revealed that Ca is the most abundant element in all the species followed by K, Mg and Na. Results further suggested that *O. sanctum* is a highly mineral rich species followed by the *O. basilicum*, *O. gratissimum* and *O. americanum*. Principal component analysis (PCA) was also used on data set of LIBS spectra of *Ocimum* species which revealed PC1 (72%) and PC2 (26%) data matrix explaining 100% variance in the data set. The PCA plots clearly classified the cultivated and wild species and separated them in the two clusters. Conclusively, the present work demonstrated the suitability of LIBS technique due to its rapid, nondestructive and eco-friendly approach and gives the comparative account of mineral availability in *Ocimum* spp.

1. Introduction

In India the exploitation of plants as a resource of medicines and human nourishment has been in trend since antiquity (Rai et al., 2009; Tewari et al., 2012). More than 2500 plant species are reported in India that are credited with medicinal values; however, world health organization (WHO) has listed about 20,000 plant species all over the world which are used to develop various medicines (Tewari et al., 2012). Among the galaxy of medicinal plant, *Ocimum* (Basil) is listed as one of the most popular herbs all over the world because of its medicinal value and therapeutic potentials and therefore, widely utilized due to its economic, nutritional, industrial and medicinal properties (Tewari et al., 2012). The genus *Ocimum* belonging to family Lamiaceae comprises about 65 aromatic species, distributed in tropical and subtropical regions worldwide (Paton et al., 1999). However, among all species, *Ocimum sanctum L.* (Tulsi), *Ocimum gratissimum* (Ram Tulsi), *Ocimum canum* (Dulal Tulsi), *Ocimum basilicum* (Ban Tulsi) and *Ocimum americanum* are the major examples of significantly important medicinal species of genus *Ocimum* grown in different parts of the world (Mukherjee et al., 2006; Gbolad, 2009; Tewari et al., 2012). Some species of this genus like *O. sanctum*, *O. canum*, *O. gratissimum* and *O. basilicum* are widely used for treating diabetic, hypoglycemic, upper respiratory tract infection, diarrhea, skin diseases, pneumonia, cold, cough, conjunctivitis, bronchitis, malaria, stomach disorders, inflammation, heart diseases and various forms of poisoning, and at the same time it is also used to flavor foods and as traditional medicines in Africa and Asia (Yusuf et al., 1994; Hiltunen, 1999; Aguiyi et al., 2000; Nyarko et al., 2002;

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Egesie et al., 2006; Mukherjee et al., 2006; Matasyoh et al., 2007; Abo et al., 2008; Gbolad, 2009; Pattanayak et al., 2010; Oguanobi et al., 2012; Domlur et al., 2013). Various investigations on its essential oils and others activities undoubtedly suggested many pharmacological activities of *Ocimum* i.e. antioxidant, anti-inflammatory, anticancerous, anti-stress, anti-diabetic, immune modulatory activity, gastro protective, antimicrobial, hypolipidemic, anthelmintic, wound healing, antifertility and analgesic radio protective (Hiltunen, 1999; Domlur et al., 2013). Further, antimutagenic activity and anti-HIV activity of *Ocimum* have also been well reported and sincerely discussed worldwide in view of future prospective (Obaseiki-Ebor et al., 1993; Ayisi and Nyadedzor, 2003; Prakash et al., 2011). A range of studies have been done on several beneficial effects of *Ocimum*; however, availability of elements in different *Ocimum* species has not been yet properly studied.

In the present scenario of food and nutritional research, study of element's availability and essentiality in plants and animals is a key area of research throughout the world (Tripathi et al., 2012a, 2012b, 2014a, 2014b), and also an increasing curiosity to diagnose importance and requirement of their minimum and maximum levels of dietary minerals in the prevention of several diseases. It has been well recognized that proper intake of elements such as calcium (Ca), magnesium (Mg), manganese (Mn), sodium (Na), potassium (K), copper (Cu), zinc (Zn) and iodine (I) may possibly decrease individual risk factors of various diseases of human and animals (Mertz, 1982; Anke et al., 1984; Sanchez-Castillo et al., 1998; Ozcan and Akgul, 1998). Therefore, a systematic study of mineral availability in frequently used plants, vegetables, fruits, medicinal plants and animals is essential through quick, reliable and eco-friendly analytical technique.

In the current technological development, for the detection of mineral profile of different biomaterials, various spectroscopic techniques are being frequently used such as mass spectroscopy, inductively coupled plasma atomic emission spectrometry (ICP-AES), ion mobility spectrometry (IMS), graphite furnace atomic absorption spectrometry (GFAAS) and X-ray florescence (XRF); however, due to lengthy sample preparation and consumption of heavy liquids, these techniques may cause several health hazards and are also not environmental friendly (Tripathi et al., 2011, 2012, 2015; Singh et al., 2015). Furthermore, these techniques are more expensive, time consuming and not suitable for in-situ and point to point detection analysis (Rai et al., 2014; Wang et al., 2015).

Recently, Laser Induced Breakdown Spectroscopy (LIBS) has been introduced as a valuable tool for in-situ and in-vivo analysis of any type of materials including plants (Chauhan et al., 2011a, 2011b; Tripathi et al., 2011, 2012; Kumar et al., 2013; Maurya et al., 2014; Wang et al., 2015). LIBS is based on collection of the emission from ionic, atomic and molecular species in the plasma formed due to high power laser and material interaction (Rai et al., 2009; Tripathi et al., 2012; Devey et al., 2015). LIBS has several unique features like it requires minimal or no sample preparation, it is a very sensitive, nondestructive, reliable, and rapid analytical technique (Chauhan et al., 2011a, 2011b; Tripathi et al., 2011, 2012a, 2012b; Guerra et al., 2015). It is also capable of simultaneous multi-elemental monitoring, capable of real time analysis of material in any phase, i.e. solid, liquid and gas, and requires minimal sample preparation (Rai et al., 2007; Chauhan et al., 2011a, 2011b; Tripathi et al., 2011, 2012).

Various studies have been conducted to analyze different antioxidants, essential oil activities, functional groups and other beneficial compounds of *Ocimum* species; however, to the best of our knowledge no such comparative study has been yet done to compare the elemental profile of different *Ocimum* species through the LIBS or any other spectroscopic technique. Thus the aim of this study is to evaluate the mineral content of four *Ocimum* spices i.e. *O. sanctum*, *O. americanum* and *O. gratissimum* and *O. basilicum* as well as to introduce the feasibility of Laser Induced Breakdown Spectroscopy (LIBS) technique in food crop and medicinal aspects. Further, the trend of results obtained by LIBS was also authenticated with results obtained by inductively coupled argon plasma atomic emission spectroscopy (ICAP-AES).The use of multivariate analysis PCA on the LIBS data is also presented in this study for classification of different *Ocimum* samples.

2. Materials and methods

2.1. Materials

Leaves of *Ocimum* species, i.e. *O. sanctum, O. americanum, O. gratissimum* and *O. basilicum* were collected from the Roxburgh Botanical Garden of Botany Department University of Allahabad, Allahabad. UP, India. Further identification of *Ocimum* species was made with the help of senior taxonomist on Botanical survey of India and herbarium were prepared (Table 1).

2.2. Analysis of micro- and macro-nutrient content by LIBS

The pellets of *Ocimum* leaves were prepared by drying it in hot air oven at 104 °C for 5 h followed by grinding and finally pelletized by hydraulic pressure machine at 10 t pressure. The laser was focused to record LIBS spectra of these samples. The laser beam from a Q-switched Nd: YAG laser, pulse duration of 4 ns and 10 Hz repetition rate was used to create the plasma on the surface of pellet of *Ocimum* species. Each spectrum is the average of 10 laser shots. The data acquired simultaneously by the spectrometer has been stored in a computer through OOILIBS software for subsequent analysis. The laser is capable of delivering 425 mJ at 532 nm. The laser beam was focused through a 15 cm quartz lens on the pellet of *Ocimum* leaves in open atmosphere. The target was slowly translated to avoid surface damage and to make the laser pulse fall on a fresh target surface of *Ocimum* pellet. A schematic diagram of the experimental setup is shown in Fig. 1. The light from the laser induced plasma was collected using a lens fixed at the tip of a fiber bundle and was transferred into the entrance slit of the LIBS 2000+ spectrometer (Ocean Optics), which has four modules, one for low resolution (0.75 nm) in the wavelength region 200–900 nm and the other three are of high resolution. The emission from laser-induced plasma was recorded by adjusting the tip of the fiber bundle at 45° with respect to the laser beam. Five pellets of each *Ocimum* species were used for LIBS analysis. Further, the background of the spectra has been corrected through the Origin 6.1 software to visualize each spectral signature of the elements.

2.3. Estimation of micro- and macro-nutrient content by ICAP-AES

For the determination of nutrient content, dried samples (50 mg) of different *Ocimum* leaves were digested in mixed acid (HNO_3:$HClO_4$; 85:15, v/v) until transparent solution was obtained. The volume of digested sample was maintained up to 30 ml with

Table 1
Detail of *Ocimum* species used in the study.

General name	Local name	Botanical name	Family	Used parts
Shyma Tulsi	Black Tulsi	*Ocimum basilicum*	Lamiaceae	Leaves
Ram tulsi green	Green Tulsi	*Ocimum sanctum*	Lamiaceae	Leaves
Krishna Tulsi	Deona	*Ocimum gratissimum*	Lamiaceae	Leaves
Ban Tulsi	Mamari	*Ocimum americanum*	Lamiaceae	Leaves