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## Estimation of total phenolic content in selected varieties of *Ocimum* species grown in different environmental condition

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**Abstract**

Plants produce specialized metabolites as part of their defence mechanism and the most prominent metabolites are alkaloids, tannins, saponins, steroids, cardiac glycosides, terpenoids, flavonoids and phenolic compounds which have significant medicinal properties that cure several human diseases. The plants of genus *Ocimum* commonly called as Basil belonging to family Labiatae have remarkable therapeutic potentials. Traditionally leaves of basil used in treating cold, coughs, bronchitis, asthma, influenza, eye diseases, mosquito repellent, dysentery and diarrhoea. Essential oil of basil has antibacterial, antifungal, antifertility and antiviral properties. The objective of this research was to estimate the total phenolic content present in methanolic, ethanolic and aqueous extract of *Ocimum sanctum* L, *Ocimum gratissimum* L and *Ocimum basilicum* L when grown in shade and open field. Methanolic extract of *Ocimum gratissimum* L is presented best total phenolic content 0.165 mg GAE/g and 0.196 mg GAE/g when grown in shade and open field respectively.

**Keywords:** *Ocimum* species, metabolites, total phenolic content

**Introduction**

In ethno- botanical literature of India, medicinal plants are richest resources of natural drugs in the traditional and modern systems of medicine. In the recent era, majority of population depend on herbal medicine to cure vital human diseases. The plants of genus *Ocimum* commonly called as Basil belonging to family Labiatae or Lamiaceae have remarkable therapeutic potentials [1]. Traditionally leaves of basil are used in treating cold, coughs, bronchitis, asthma, influenza, skin diseases, eye diseases, as mosquito repellent, dysentery and diarrhoea [2]. Essential oil of basil has antibacterial, antifungal, antifertility and antiviral properties. In many ayurvedic cough syrups *Ocimum sanctum* L is an important component and the fresh juice of leaves, flowers and roots of Tulsi are very good antidote for snake and scorpion bite [2]. Traditionally the leaves of *Ocimum basilicum* are used as antispasmodic, carminative, digestive, tonic and also having antioxidant properties [3]. The leaves of *Ocimum gratissimum* L contain an essential oils which has mosquito repellent, insecticidal properties [4]. Phenol (C<sub>6</sub>H<sub>5</sub>OH) is considered as the simple group of natural compounds found in plants [5]. Phenylalanine ammonia-lyase (PAL) plays a crucial role in phenolic synthesis and many reports highlight on the correlation between increase in PAL gene activity and also increase in the phenolic compounds in response to different stimuli [6].

**Materials and Methods**

Plant materials were collected from two different environmental condition which was shade growing plants under green house and other is naturally growing plants in open field. Seedling of different varieties of Basil were purchased from nursery and allowed it to grow in shade condition at the botanical garden of department of Botany, Gujarat University having 43- 92 % relative humidity and 105 Lux light intensity while open field plant materials collected from Gandhinagar district, Gujarat where plants grown in natural condition having 43-70% relative humidity and 9565 Lux light intensity.

**Identification of plant materials**

The plant were identify by taxonomist of Department of Botany and also from the herbarium of the Department of Botany, Gujarat University.

**Collection of plant materials**

Fresh and mature leaves of *Ocimum sanctum* L, *Ocimum gratissimum* L and *Ocimum basilicum* L were collected and washed under running tap water to remove dust particles and

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shade-dried for three weeks. The dried leaf grind to fine powder and stored in airtight bottles.

### Extraction of plant materials

The dried leaves powder of different variety of *Ocimum* species were weighed accurately to 5 g and the same was filled in a thimble and placed in the central assembly of the soxhlet apparatus. Accurately measured 50 ml different solvents such as aqueous, methanol, ethanol was added to a 500 ml round bottom flask. The extraction was done in this apparatus at 100 °C, 64 °C, 78 °C for 6 h respectively. After the completion of extraction, the obtained liquid extract allowed it to dry at room temperature for 24 hours to evaporated and stored it in refrigerator at 4 °C.

### Determination of yield percentage (%)

The yields of evaporated dried extracts were obtained by gravimetric method. The percentage yield extracts were calculated based on dry weight as:

$$\text{Yield \%} = \frac{W1 \times 100}{W2}$$

Where W1= weight of extract after Solvent evaporation;  
W2 = Weight of the grinded leaf powder

### Determination of total phenolic content [7]

One ml of the extracts of different variety of *Ocimum* species (12.5-300 µg/ml of methanolic, ethanolic and aqueous extracts) was thoroughly mixed with 10 ml of distilled water, added 1.5 ml of Folin-Ciocalteu reagent. After 5 minutes, 4

ml of 20% sodium carbonate (Na<sub>2</sub>CO<sub>3</sub>) was added and adjusted with distilled water up to 25 ml and agitated. Then incubated for 30 minutes at room temperature. The absorbance was measured at 765 nm against a blank having all the reagents excluding the sample using spectrophotometer. This procedure was repeated 3 times for each extract. The total phenols were quantified by the standard curve of gallic acid solution (12.5, 25, 50, 75, 100, 150, 200, 250 and 300 µg/ml) which was prepared using the similar procedure from which the following regression formula was derived.

$$Y = 0.0038X - 0.0026$$

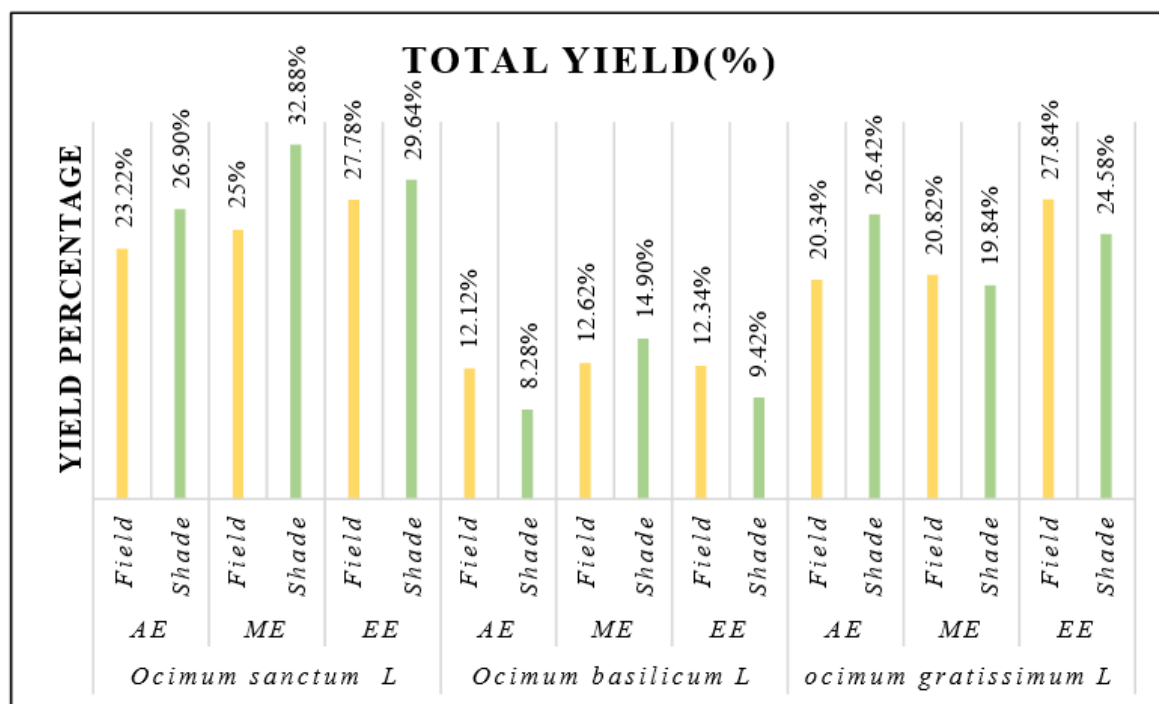
$$(R^2 = 0.9993)$$

### Result

The dried leaves powder of different varieties of *Ocimum* species included *Ocimum sanctum* L, *Ocimum basilicum* L and *Ocimum gratissimum* L were extracted with aqueous, methanolic and ethanolic solvents to examine total phenolic content under two different grown condition, open field and shade.

### Estimation of yield percentage of different varieties of *Ocimum* species

The highest yield of solid residue was achieved using methanol (32.88%) and ethanol (29.64%) as extraction solvents in variety of *Ocimum sanctum* L when grown under shade condition (Figure 1).



**Fig 1:** Total Yield (%) with different extract and different grown condition of different varieties of *Ocimum* species. AE=Aqueous Extract, ME=Methanolic Extract, EE=Ethanolic Extract

### Determination of total phenolic content

The total phenolic (TPC) content of the different extracts of *Ocimum* species was detected using the Folin–Ciocalteu phenol reagent. The reaction is noticed by the colour change from yellow to blue after incubation than measured it using UV-Visible spectrophotometer at 765 nm wavelength. Its colour change occurs due to the reduction which leads to

make complex between phenolic group and the tungsten and molybdenum 8. The value is calculated using linear regression formula  $Y = 0.0038X - 0.0026$ ,  $R^2 = 0.9993$  for methanol solvent,  $Y = 0.0053X + 0.0487$ ,  $R^2 = 0.9966$  for ethanol solvent and  $Y = 0.0051X + 0.0051$ ,  $R^2 = 0.9963$  for aqueous solvent obtained from different concentration of gallic acid which implied good linearity. The phenolic level of

sample is expressed in terms of gallic acid equivalent (mg of GAE/g of dry extract) In the present study, the total phenolic content of three different extracts with three different species of Basil varied from 0.012 mg GAE/g DW to 0.196 mg GAE/g of dry material when grown under different condition such as open field and shade.  
Methanolic extract of *Ocimum gratissimum* L is showed highest total phenolic content (0.196 mg GAE/g) followed by *Ocimum sanctum* L methanolic extract (0.145 mg GAE/g) while the ethanolic extract of *Ocimum basilicum* L is

disclosed lowest phenolic content (0.012 mg GAE/g) when grown in open field (Figure 2).  
Methanolic extract of *Ocimum gratissimum* L is presented best total phenolic content (0.165 mg GAE/g) followed by *Ocimum gratissimum* L aqueous extract (0.126 mg GAE/g) while the ethanolic and aqueous extract of *Ocimum basilicum* L exhibited lowest phenolic content (0.037 mg GAE/g) and (0.028 mg GAE/g) respectively when grown in shade (Figure 3).

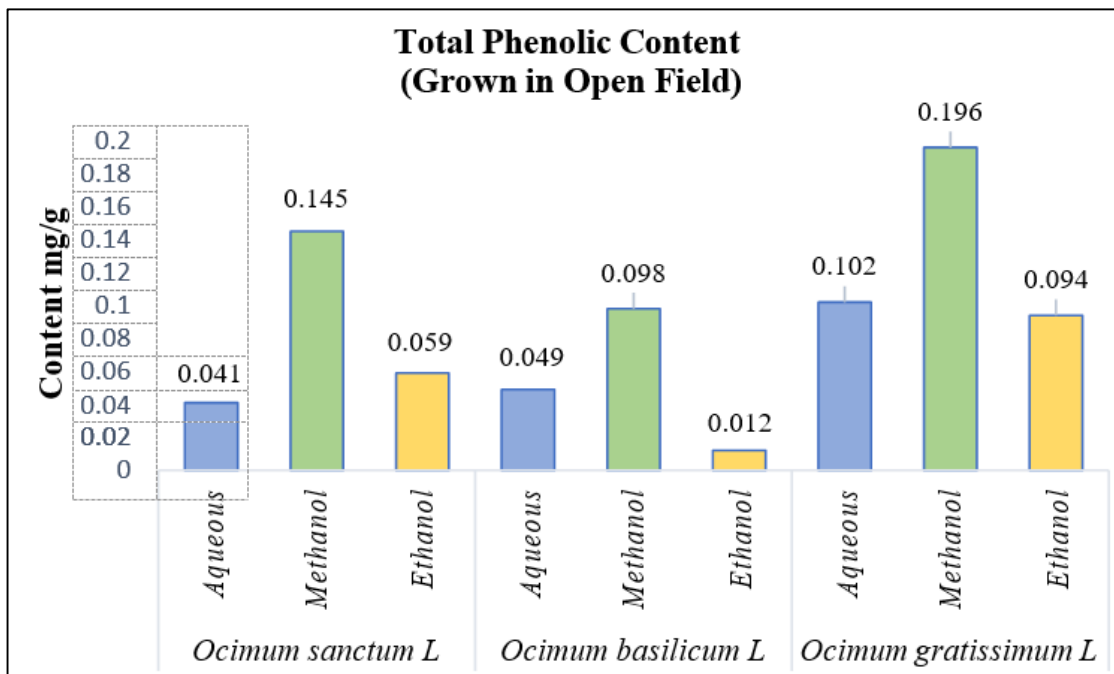


Fig 2: Total phenolic content when grown in open field.

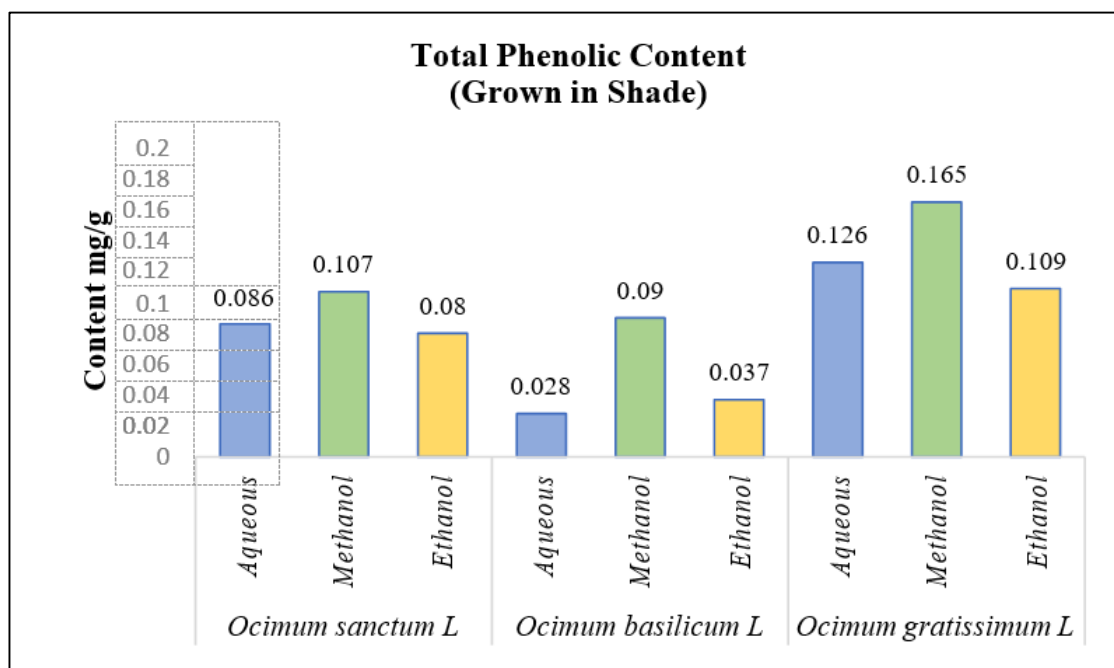


Fig 3: Total phenolic content when grown in shade.

Comparative Study of Total Phenolic Content of Different Varieties of *Ocimum* species when grown in Open Field and Shade.  
In the present study, the total phenolic content of the three different species of Basil with the three different extracts

obtained significantly when grown under different conditions. *Ocimum gratissimum* L shows excessive phenolic content (0.126 mg GAE/g) in aqueous extract than the other species of *Ocimum* when grown in shade. *Ocimum basilicum* L ensured least phenolic content (0.028 mg GAE/g) in contrast

to grown in open field estimated 0.049 mg GAE/g phenol concentration.

Methanolic extract of all different species of *Ocimum* are exhibited higher concentration of phenolic content when grown in open field compared to the one grown in shade. The result concealed that the methanolic extract of *Ocimum gratissimum* L accompanied maximum phenolic content (0.196 mg GAE/g) while methanolic extract of *Ocimum basilicum* L shown lowermost phenolic content (0.098 mg GAE/g) than the other species of *Ocimum* when grown in open field. The total phenolic content in methanolic extract of *Ocimum gratissimum* L is 0.165 mg GAE/g, in *Ocimum sanctum* L (0.107 mg GAE/g) and in *Ocimum basilicum* L (0.090 mg GAE/g) when grown in shade.

Ethanol extract of all different species of *Ocimum* are represented remarkable concentration of phenolic compound grow in shade than the open field. The notable concentration of phenolic compound found in ethanolic extract of *Ocimum gratissimum* L is 0.109 mg GAE/g, followed by in *Ocimum sanctum* L (0.080 mg GAE/g) and in *Ocimum basilicum* L (0.037 mg GAE/g) when grown in shade.

### Discussion

In present study, various changes were observed in growth and development of the plants in both growing conditions. All three varieties of Basil are grown in open field in normal condition such as optimum light, nutrition and other environmental factors which leads to growth of plants and are succeeded in two phase of life that is vegetative phase and reproductive phase. But there was vast difference in growth and development of the plants which were grown under shade. Plants grown under shade showed a very little reproductive phase.

The highest yield of solid residue was achieved using methanol, thus methanol proved to be better solvent to extract active compounds. Most of the active components have been generally found to be water insoluble, hence it is expected that low polarity organic solvent yield more active extract. These was already reported in literature, where methanol noticed to be better extracting solvent [9].

### Total phenolic content

The yield of total phenolic content in differently grown condition in *Ocimum gratissimum* L revealed fluctuations. Methanolic extract of this species yielded low amount of phenolic content when grown in shade than open field. But in aqueous and ethanolic extract of the same species disclosed highest phenolic content when grown in shade.

The same way, amount of total phenolic content in differently condition in *Ocimum sanctum* L shown variabilities. Aqueous and ethanolic extract of this species yielded maximum phenolic content while methanolic extract of this Basil species is expressed lowest phenolic content when grown in shade.

The total concentration of phenol in all extract of *Ocimum basilicum* L showed variation when grown in different condition. Ethanolic extract of *O. basilicum* appeared higher yield while aqueous and methanolic extract of the same species expressed lower yield when grown in shade.

The phenolic content of *Ocimum sanctum* L in 95% ethanolic extract is low than those reported by Kaur & Mondal (2014) expressed the phenolic content of *Ocimum sanctum* L in 80% ethanolic extract was 3.65 mg/100 g fresh weight.

The total phenolic content in methanolic extract (99%) of *Ocimum basilicum* L and *Ocimum gratissimum* L are higher than those reported by Uyoh, E. A. *et al.* 2013 determined total

phenolic contents of *Ocimum basilicum* and *Ocimum gratissimum* which was ranged from 0.009 –0.027 mg GAE/10 g DW in 90% methanol. *Ocimum* species showed high phenolic content according to the results by Veeru *et al.* (2009). Total phenolic compounds in basil accessions were greater than the other Lamiaceae plants [13].

### Conclusion

The total amount of phenolic content observed huge variation when grown in open field and shade. Methanolic extract of all varieties of *Ocimum* seems to be present at low concentration of phenolic compounds while ethanolic extract of all species of *Ocimum* are seen to have high yield phenolic compound when grown in shade. Aqueous extract of *Ocimum sanctum* L and *Ocimum gratissimum* L appeared as maximum phenolic content when grown in shade in contrast to open field, while aqueous extract of *Ocimum basilicum* L yielded more in open field. Thus direct light effects the yield positively or negatively in different species of *Ocimum*.

*Ocimum* species contains medicinally important bioactive compounds in both growing condition that have their traditional medicinal applications, that may be successful for future modern therapeutic applications. Therefore they may be cultivated in large quantities in open field or shade which will be helpful to standardize, quality control of precious indigenous drug for pharmaceutical industries.

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