EFFECT OF DIFFERENT GROWING CONDITION ON TOTAL FLAVONOID CONTENT IN THREE VARIETY OF BASIL

[1*]Falguni R Patel & [2]Nainesh R Modi
[1*]Ph.D Student, [2]Associate Professor
[1]Department of Botany, Bioinformatics and Climate Change Management, School of Science, Gujarat university, Navrangpura, Ahmedabad-380009

Received: February 23, 2019 Accepted: March 27, 2019

ABSTRACT: : *Medicinal plants are richest sources of drugs in the traditional and modern systems of medicine and also has great importance to the human health. Mostly the bioactive constituents of drug or medicines were originally obtained from plants such as tannins, saponin, steroids, cardiac glycosides, terpenoids, flavonoids and phenolic compounds. The genus Ocimumcommonly called as Basil belonging to family Labiatae, which is used in treating cold, coughs, bronchitis, asthma, influenza, skin diseases, eye diseases, mosquito repellent, dysentery and diarrhoea. Oils of basil has antibacterial, antifungal, antifertility and antiviral properties. The main intent of this research was to estimate the total flavonoid content of different variety of Ocimum species named as Ocimum sanctum L, OcimumgratissimumLand OcimumbasilicumL when grown in different environmental condition. Some species shows good results in shade and some other shows in direct sunlight.*

Key Words: *Flavonoid, Ocimum, Labiatae.*

I. INTRODUCTION

Among the plants known for medicinal value, the genus *Ocimum*is member of Labiatae or Lamiaceae have important therapeutic potentials (Paton *et al.*,1999).The plant species are annual or perennial herbs, distributed in temperate and tropical regions of the world. In India the family expressed by several important genera such as *Mentha, Ocimum, Leucas, Coleus, Pogostemon*etc. There are 200 genera and 3200 species in this family. (Pandey, 2007)

Plants produce a large, diverse array of organic compounds termed as secondary metabolites that appear to have no direct influence on growth & development of plants.They are categorized into three majorclassesknown as(a) Terpenes (b) Nitrogen containing compounds (c) Phenolic compounds(Taiz and Zeiger, 1991). Metabolites are the intermediate products of metabolism which have various functions such as signalling, stimulatory and inhibitory effects on enzyme activities, defence mechanism etc.(Tiwari &Rana, 2015)

II. MATERIAL AND METHODS

Mature leaves of all variety of *Ocimum* species were collected from two different growing condition such as shade grown plants and open field. Shade grown plant material collected from green house of Department of Botany, Gujarat University, Ahmedabad(NL 23°2 23',EL 72° 32 30")where seedling of all variety were allowed to grow under 43-46% relative humidity and 2096 Lux light intensity for 3-4 months. Plant material was also collected from open field of Gandhinagar district (NL 23°9 12',EL 72° 3829")where plants were grown in natural condition having 32-40 % relative humidity and 10,9400 Lux light intensity.

2.1IDENTIFICATION OF PLANT MATERIALS

The plants were identified by taxonomist of Department of Botany and also compared with the specimensfrom the herbarium of the Department of Botany, Gujarat University.

2.2PREPARATION OF POWDER

Fresh and mature leaves of differentvariety of *Ocimum*species were washed with tap water to remove dust particles and shade-dried for 2 to 3 weeks. The dried leaves homogenized into powder and stored in airtight bottles till further use.

2.3EXTRACTION 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 hours respectively. After extraction, the

obtained liquid extract was allowed to dry at room temperature for 24 hours to evaporated and then stored it in refrigerator at 4˚C.

2.4QUALITATIVE ANALYSIS OF LEAVES OF DIFFERENT VARIETIES OF *OCIMUM* SPECIES

The extracts were subjected to qualitative analysis for various phytochemical constituents such as alkaloids, flavonoids, tannins, carbohydrates, proteins and phenolic compounds, cardiac glycosides, saponins and steroids as described by Harborne, 1998, Adebayo &Ishola, 2009.

2.5ESTIMATION OF TOTAL FLAVONOID CONTENT IN LEAF EXTRACT (QUETTIER *et al*, 2000)

500 µl of the extracts of different variety of *Ocimum* species (12.5-300 µg/ml of methanolic, ethanolic and aqueous extracts) was mixed with 1500 µl of 95% of methanol, and then100µl ofAluminium chloride (10%) and Potassium acetate (1M) was added respectively and make volume up to 10ml with distilled water and agitated.Incubation was done for 20-30 minutes at room temperature. The absorbance was assessed at 415 nm against a blank having all the reagents without the sample using spectrophotometer. Measurement was done in triplicates and the total flavonoid quantified by the standard curve of quercetin solution (12.5, 25, 50, 75, 100 and 150 µg/ml, $R^2 = 0.9963$).

III. RESULT & DISCUSSION

3.1 QUALITATIVE ANALYSIS OF DIFFERENT VARIETIES OF *OCIMUM* SPECIES

The result of the preliminary phytochemical screening was carried out with aqueous methanolic and ethanolic extracts. Dried leaf powder of all different varieties of *Ocimum*species were used for qualitative analysis where screening of phytochemicals was more or less similar in both growing conditions. The result disclosed the presence of anextensive range of phytoconstituents including cardiac glycosides, flavonoids, tannins, steroids, phenolic and are illustrated in table 1 and table 2. However, the extract of the plant material was devoid of carbohydrates, saponin, resins and acidic compounds.

Table 1: Qualitative analysis of leaves of different varieties of *Ocimum* species (Grown in open field)

Sr No	Phytochemical constituents	*Ocimum sanctum* L			*Ocimum basilicum*L			*Ocimum gratissimum*L		
1	**Flavonoids**	**AE**	**ME**	**EE**	**AE**	**ME**	**EE**	**AE**	**ME**	**EE**
	Alkaline reagent test	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**+**
	Lead Acetate Test	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**+**
2	**Phenolic/tannin**									
	Ferric Chloride Test	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**+**
	Folinciocalteu reagent	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**+**
3	**Steroids**									
	Libermann Burchard's Test	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**+**
	Salkowski test	**-**	**+**	**-**	**+**	**+**	**+**	**+**	**+**	**+**
4	**Cardiac Glycosides**									
	Keller-Killiani test	**+**	**+**	**+**	**+**	**-**	**-**	**-**	**-**	**-**
	Legal's test	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**+**

Key: AE=Aqueous Extract, ME= Methanolic Extract, EE=Ethanolic Extract, **+:** Present, -: Absent

Table 2: Qualitative analysis of leaves of different varieties of *Ocimum* species (Grown in shade)

Sr No	Phytochemical constituents	*Ocimum sanctum* L			*Ocimum basilicum*L			*Ocimum gratissimum*L		
1	**Flavonoids**	**AE**	**ME**	**EE**	**AE**	**ME**	**EE**	**AE**	**ME**	**EE**
	Alkaline reagent test	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**+**
	Lead Acetate Test	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**+**
2	**Phenolic/tannin**									
	Ferric Chloride Test	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**+**
	Folinciocalteu reagent	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**+**
3	**Steroids**									
	Libermann Burchard's Test	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**+**
	Salkowski test	**-**	**+**	**-**	**+**	**+**	**+**	**+**	**+**	**+**
4	**Cardiac Glycosides**									
	Keller-Killiani test	**+**	**+**	**+**	**+**	**-**	**-**	**-**	**-**	**-**

	Legal's test	-	+	-	+	+	+	-	+	+

Key: AE=Aqueous Extract, ME= Methanolic Extract, EE=Ethanolic Extract, **+:**Present, -: Absent

3.2 DETERMINATION OF TOTAL FLAVONOID CONTENT

Flavonoid content analysing by colorimetric method with quercetin as a standard. The chemical reaction is observed into solution in order to form complexity between flavonoid and aluminum chloridewith colour changing into yellow at wavelength 415 nm. Aluminum chloride forms stable complexes with C-4 keto group, C-3 or C-5 hydroxyl group of flavones and flavonols and also with ortho-dihydroxyl groups in the A or B-ring of flavonoids (Parikh & Kothari, 2016).

The total flavonoid content varied among the all different species of *Ocimum*with different solvents and ranged from 0.002mg/g to 0.068mg/g of dry weight. Ethanolic extract of *Ocimumgratissimum*L showed highest flavonoids (0.068mg/g) than the other species of *Ocimum* when grown in shade (Figure 1). Aqueous extract of *Ocimum sanctum*L indicated lowest flavonoids (0.002mg/g) than the other species of *Ocimum* when grown in open field (Figure 2).

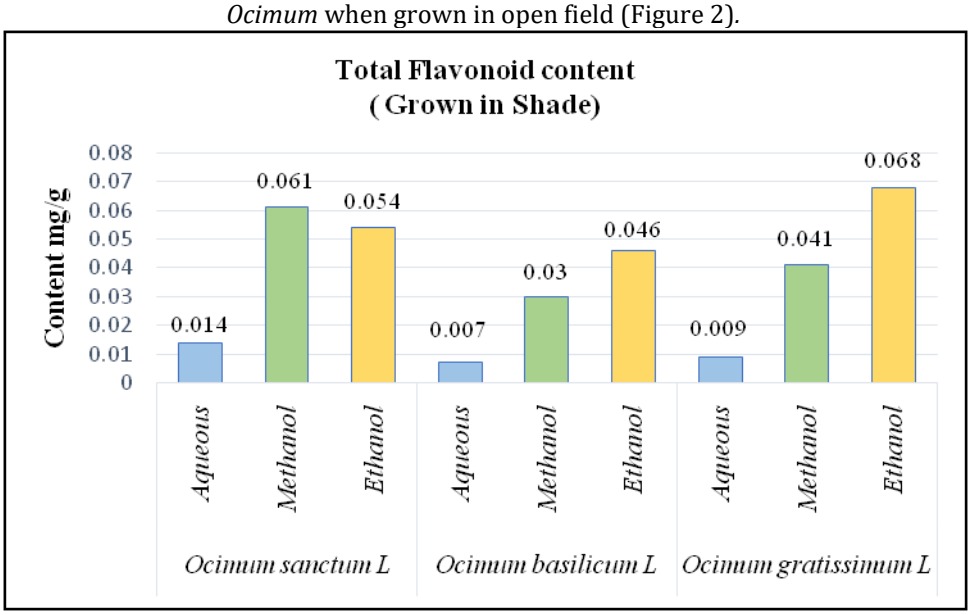

Figure 1: Total Flavonoid content when grown in shade.

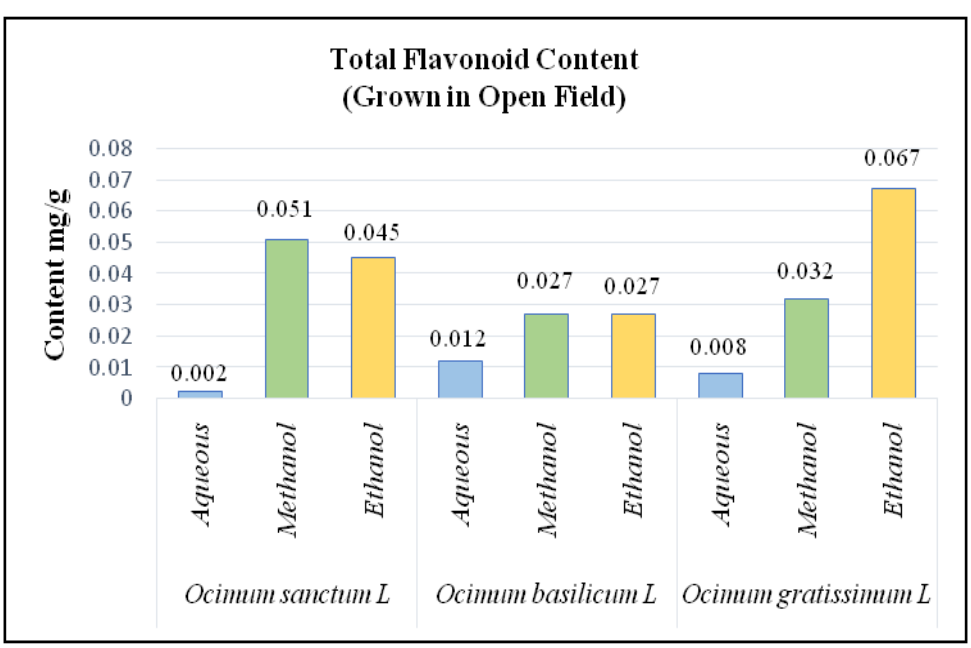

Figure 2: Total Flavonoid content when grown in open field.

3.3 COMPARATIVE STUDY OF TOTAL FLAVONOID CONTENT OF DIFFERENT VARIETIES OF OCIMUM SPECIES WHEN GROWN IN OPEN FIELD AND SHADE.

*Ocimum sanctum*L disclosed maximum flavonoid content (0.014 mg /g) in aqueous extract than the other species of *Ocimum* when grown in shade while *Ocimum sanctum* L is confirmed least flavonoid content (0.002 mg /g) when grown in open field. (Figure 1& 2)

Methanolic extract of all varieties of Basilrevealedhigher concentration of flavonoid content when grown in shade in contrast to grown in open field (Figure 1 & 2). The results concealed that the methanolic extract of Ocimum *sanctum* L showed highest quantity of flavonoid content (0.061 mg /g) while same species of Basil (*Ocimum sanctum* L) represented lower most flavonoid content which is 0.051 mg /g when grown in open field.

All different species of Basil shows greater amount of total flavonoid content in ethanolic extract when grown in shade. Ethanolic extract of *Ocimumgratissimum* L is achieved to contain highest amount of total flavonoid content (0.068 mg/g) followed by*Ocimum sanctum* L (0.054 mg/g) while the ethanolic extract of *Ocimumbasilicum* L is lowest (0.046 mg /g) when grown in shade.

The growth and development of all species of basil also showed variation when grown in different condition. All three varieties of Basil succeeded to complete both the phases of life that is vegetative phase and reproductive phase when grown in natural condition, but plants remained in vegetative phase for longer period of time when grown in shade. Thus, different growing condition might be affecting the production of flavonoids.

The phytochemical screening of the leaf samples studied in the present study exhibit a presence of bioactivecompounds at higher concentration than those reported by Ameh (2010) which showed the presence of glycosides, phenols and tannins appeared in adequate amounts while saponins, flavonoids and steroids were present in low concentrations.

The phytochemicals are naturalcompounds found in plants which fulfil the requirements for the protection of human disease (Alexander, 2016). These phytoconstituents having antibiotic properties which are generallyperformed the function of defensive mechanisms against the plant pathogens. Presence of flavonoids function as anti-inflammatory effects on both severe and chronic inflammation (Boham and Kocipai-Abyazan,, 1974).

3.4 TOTAL FLAVONOID CONTENT

The yield of total flavonoid content in differently grown condition in *Ocimumgratissimum* L revealed fluctuations. Ethanolic extract of this species yielded highest flavonoid content when grown in shade. But in methanolic and aqueous extract of the same species disclosed moderate andlow flavonoid content when grown in shade.

Total flavonoid content of leaf extract of *Ocimum sanctum* L, are lesser in 99.9% methanolic extract than those reported by Kaur & Mondal (2014) concluded total flavonoid contents of *Ocimum sanctum* Lis 20.50 mg / 100 g fresh weight in 98% methanol. Total flavonoid content of leaf extract of *Ocimumbasilicum* L and *Ocimumgratissimum* L in 99.9% methanolic extract found to be graterquantity than those described by Uyoh*et al* (2013) revealed that total flavonoid content of *Ocimumbasilicum* L and *Ocimumgratissimum* L are 0.022 mg RE/100g and 0.0053 mg RE/100g fresh weight in 90% methanol.

IV CONCLUSION

The total flavonoid contents of three medicinal herbs that is *Ocimum sanctum* L., *Ocimumbasilicum* L. and *Ocimumgratissimum* L. in order to realize the health benefits from potential phytochemicals present in them under different growing condition.

The result concluded that, the total amount of flavonoid content observed huge fluctuations when grown in open field and shade. Methanolic, ethanolic and aqueous extract of all varieties of *Ocimum*presented high yield flavonoid content when grown in shade except aqueous extractof *Ocimumbasilicum* L species.

REFERENCES

1. Adebayo EA, Ishola OR**. 2009.** ―Phytochemical and antimicrobial screening of crude extracts from the root, stem bark, and leaves of Terminalia glaucescens."Afr. J.Pharm. Pharmacol; **3(5):** pp.217-221.
2. Alexander, P. (2016). Phytochemical Screening and Mineral Composition of the Leaves of Ocimumgratissimum (Scent Leaf). International Journal of Applied Sciences and Biotechnology, 4(2), 161-165.
3. Ameh, G. I. (2010). Evaluation of the Phytochemical Composition and Antimicrobial Properties of Crude Methanolic Extract of leaves of Ocimumgratissimum L. ASSET: An International Journal (Series B)}, 9(1), 147-152.
4. Boham, B. A., &Kocipai-Abyazan, R. (1974). Flavonoids and condensed tannins from leaves of Hawaiian vaccinium vaticulatum and V. calycinium.Pacific sci, 48, 458-463.

5. Harborne, JB. **1998**. —Phytochemical methods‖. A Guide to modern techniques of plant analysis. 3rd ed., Chapman and Hall Int., New York.
6. Kaur, S., & Mondal, P. (2014). Study of total phenolic and flavonoid content, antioxidant activity and antimicrobial properties of medicinal plants. J Microbiol Exp, 1(1), 00005.
7. Pandey, B. P. (2007). Botany for Degree Students: S. Chand & Company Ltd, New Delhi.
8. Parikh, N. H., & Kothari, C. S. (2016). Phytochemical Analysis and Total Phenolic and Flavonoid Contents Determination of Methanolic Extract of Ocimumbasilicum L seed. International Journal of PharmTech Research, 9(4), 215-219.
9. Paton, A., Harley, R. M., & Harley, M. M. (1999). Ocimum-an overview of relationships and classification. Ocimum Aromatic Plants-Industrial Profiles. Amsterdam: Harwood Academic.
10. Taiz, L., & Zeiger, E. (1991). Plant Physiology The Benjamin. Cummings Redwood City, 565.
11. Tiwari, R., & Rana, C. S. (2015). Plant secondary metabolites: a review. Int J Eng Res Gen Sci, 3(5), 661-670.
12. Uyoh, E. A., Chukwurah, P. N., David, I. A., & Bassey, A. C. (2013). Evaluation of antioxidant capacity of two Ocimum species consumed locally as spices in Nigeria as a justification for increased domestication. American Journal of Plant Sciences, 4(02), 222.
13. QUETTIER, D.C., GRESSIER, B., VASSEUR, J., DINE, T., BRUNET, C., LUYCKX, M.C., CAYIN, J.C., BAILLEUL, F., TROTIN, F. (2000): Phenolic compounds and antioxidant activities of buckwheat (Fagopyrum esculentumMoench) hulls and flour. J. Ethnopharmacol. 72, 35-42.