J. Biol. Chem. Chron. **2019**, **5***(1)*, 38-42
ISSN (Print): 2454 – 7468
ISSN (Online): 2454 - 7476
www.eresearchco.com/jbcc/

Phytochemical Analysis of *Ocimum americanum* Linn. with Special Reference to the Impact of *In vitro* Flowering on the Production of Aromatic Compounds

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DOI: http://dx.doi.org/10.33980/jbcc.2019.v05i01.007

(Received 27 Jan, 2018; Accepted 21 Feb, 2019; Published 23 Feb, 2019)

ABSTRACT: Apart from chavicol, methyl chavicol, camphor and linalool another major compound cineole is typically present in a very high amount in the essential oil of *Ocimum americanum* growing in different agro climatic region of West Bengal. Conventionally Cineole (1,8 Cineole) is obtained from sufficiently matured *Eucalyptus* sp. The article deals with the efficiency of the *in vitro* mass propagation of this species as a strategy of alternative cheaper way of commercial production of the aromatic compounds as a constituent of essential oil.

Keywords: Essential oil; cineole; Gas Liquid Chromatography (GLC); *In vitro* flowering and explants.

INTRODUCTION: *Ocimum americanum*, commonly known as lemon basil is an economically important aromatic medicinal herb belongs to the family Lamiaceae. The aromatic nature of the herb is due to the presence of diverse type of aromatic compounds in the essential oil obtained from the leaves and inflorescence. Some unique pharmacological properties and occurrence of bioactive principles of the essential oil has augmented the economic value of the species as a provider of natural compounds to pharmaceutical Industries. The essential oil has anaesthetic activity and the compounds present within it have effects on stress parameters[1]. The analgesic and anti inflammatory activity of the essential oil on laboratory animals has been demonstrated[2]. The species has the potential to control fish pathogens like *Aeromonas hydrophila* and *Gyrodactylus* sp. making its application in aquaculture system[3]. The essential oil constituent of this species varies greatly depending upon the habitat, topography and meteorological parameters[4 & 5].

In view of such outstanding economic values of the germplasm of this species a thorough phytochemical investigation in respect of the major compounds of the essential oil is imperative and need based. The present investigation therefore is aimed to analyse the characteristics of the composition of the essential oil of this species growing in West Bengal, since no such study has been done so far in this regard. Another approach of our investigation is to establish a suitable *in vitro* protocol for mass propagation of this species with the objective to increase herbage yield as an additive and alternative practice to its availability from natural plant materials.

MATERIAL AND METHODS:

Extraction of oil samples: Oil samples were extracted from fresh leaves through steam distillation using Clevenger's apparatus.

GC analysis of essential oil sample: Fresh green vegetative leaf sample of the species was collected from growing populations located at different sites of the state West Bengal during the vegetative phase of growth in order to estimate the constituents in the oil sample. Oil sample was extracted following hydro distillation method using Clevenger's apparatus[6]. The GC analyses of oil sample were made with the help of a CE8000 top model chromatogram using liquid nitrogen as a carrier gas. The oven temperature of the chromatogram was raised from 60-220°C at the rate of 5°C / min with the holding time of the final temperature of the oven for 10 min. The injector and detector temperature was 220°C. The column used for GC analysis was DB-5MS type of capillary column of 30 mt length. The major constituents of essential oil were determined by the following formula:

The conc. (%) of constituent in the test sample = $\frac{M \times A_2 \times 100}{A_1 \times N}$

Where, M = Standard stock concentration (ppm); A1= Area of the standard authentic sample (obtained from chromatogram); A2= Area of the test sample (obtained from chromatogram); N= Stock concentration of the test sample (ppm).

***In vitro* study**: Shoot tip and nodal explant was used for *in vitro* study from matured mother plant. The explants were surface sterilized in 0.1% aqueous $Hgcl_2$ solution for 10 mins. Three different media such as MS, B_5, and White's (WH) with different growth hormone were used for inoculation. The pH of the media was adjusted to 5.7 before sterilization by autoclaving (15 lb/in^2 for 15 mins). The cultures were maintained at 25± 2° C with 80% RH and an exposure to 16 hrs photoperiod.

Statistical analysis: Statistical analysis of data was performed with the help of SPSS software.

RESULTS AND DISCUSSIONS: On the basis of Gas Liquid Chromatographic(GLC) analysis of essential oil sample of *O. americanum* it is revealed that out of fifteen compound four compounds such as chavicol, methyl chavicol, camphor and linalool are major(Fig1.).

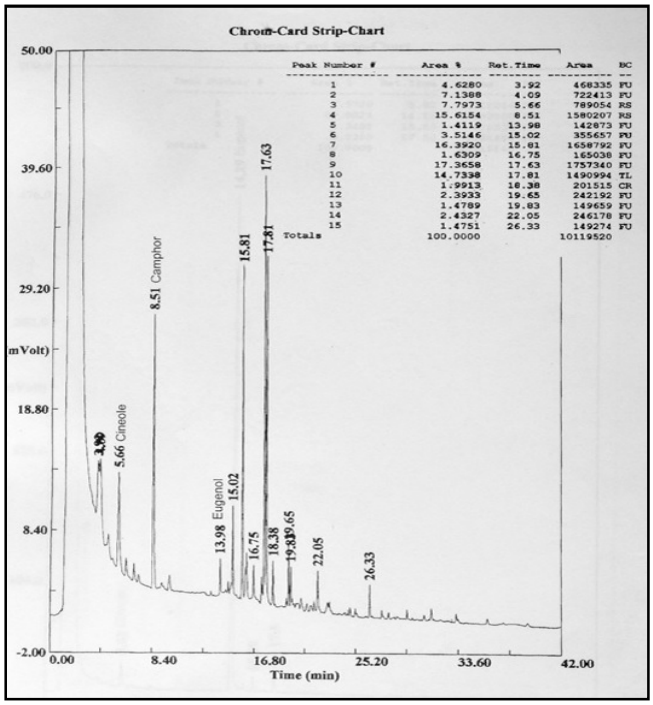

Figure 1: Gas Liquid Chromatogram of the essential oil sample of *Ocimum americanum.*

Cineole has been proved to be the next key compound, the proportion of which is quantitatively estimated as 10.44%. Our study indicates that quantitative occurrences of these major compounds are more or less identical irrespective of the site of collection of essential oil samples from the herbage. Eugenol though presents in the essential oil sample but its quantity has been measured uniformly as low as 3.20%.

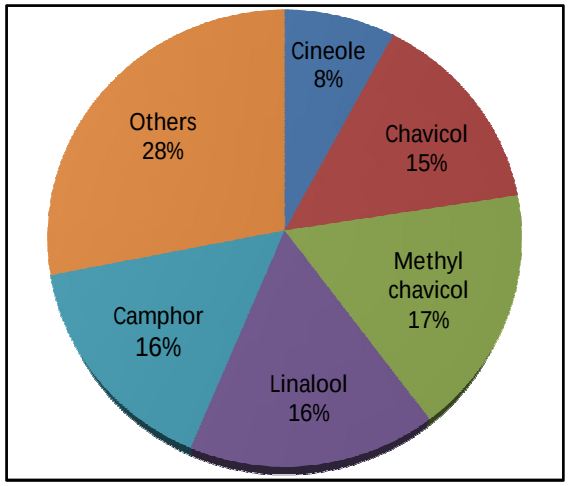

Figure 2: Major constituents of the essential oil sample of *Ocimum americanum* (Quantity shown in terms of relative area percentage).

So in terms of eugenol yield this species would not be a suitable germplasm in comparison to the *O. gratissimum* where eugenol content has been reported as 47.45%[7]. Similarly this species could not be ideal for a substitute of natural camphor (Fig. 2) since its occurrence in maximum quantity has been reported in *O. kilimandscharicum*[8]. Linalool and chavicol both are reportedly high in *O. basilicum* makes the species not up to mark for the commercial yield of those compounds[9]. Cineole is usually obtained from *Eucalyptus* sp as its natural source[10]. The yield of the compound from the plant though high but the plant has to mature sufficiently, which is time consuming. Thus *O. americanum* could be the alternative source of cineole due to its short life span and high herbage yield in nature. Besides, the compound as a constituent of essential oil is present comparatively in greater quantity than other available species of *Ocimum* in respect of the state West Bengal (Fig 3). The species grows luxuriantly in West Bengal during rainy season and flowers during the month of September- October and almost die during the winter. Such short life span may be a good approach for massive cultivation of this species for high herbage yield. Plant regeneration through tissue culture could also be another strategy for augmenting the high herbage yield of this plant in addition to the herbage resource from the nature. Moreover, the herbage could be generated any time through *in vitro* propagation irrespective of the seasonal factors required for the growth of the species. Not only that, once the herbage production from this species through

natural cultivation and *in vitro* approach is commercially established, the *Eucalyptus* sp could be saved in nature from its premature as well as post matured unwanted destruction to grasp green foliage as a major source of the compound.

With this view, our study shows that MS (Murashige & Skoog's) medium[11] supplemented with 2.0mg /lt BA (Benzyl aminopurine) and 0.5 mg/lt GA_3 (Gibberellin) in combination is most effective for high percentage of proliferation. The percentage of proliferation of microshoots is also more or less equally effective in the same medium supplemented with 1.0 mg/ lt BA alone (Table 3).

Among three different type of media used for *in vitro* study it was revealed that the response was significantly greater in MS medium in comparison to others (Table 4).

The use of nodal segment as explant was most suitable for increasing proliferation response, length of microshoots and number of microshoots per culture (Table 5). The microshoots show huge rootlets induction in MS medium supplemented with 1.0 mg/lt IBA (Indole Butyric acid).

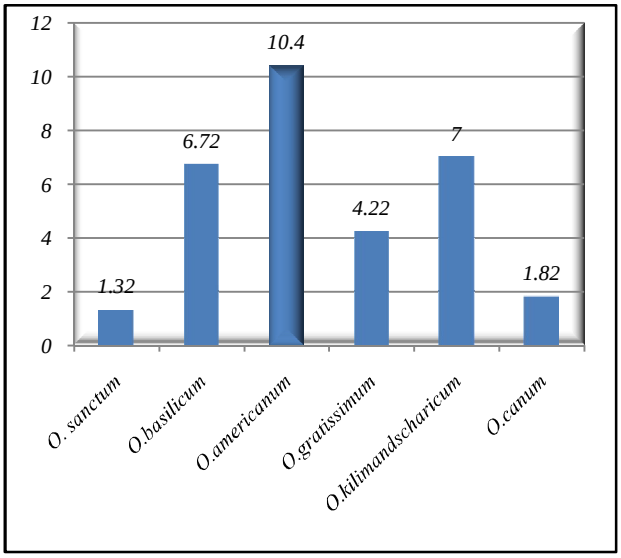

Figure 3 :Comparative study of Cineole content in (w/v) the essential oil sample of the different species of *Ocimum* growing in West Bengal.

Table 3: Mean comparison due to hormone concentrations for % proliferation, number of microshoots per culture and average shoot length in *O. americanum* Linn.

Hormone Concentrations				% of Proliferation	Number of micro-shoots / culture	Average length of micro-shoots
	BA	NAA	GA_3			
1.	0.5	-	-	12.50 (16.192)	1.13	0.90583
2.	1	-	-	23.67 (25.964)	2.07	1.40583
3.	1	1	-	11.67 (18.198)	1.49	1.50333
4.	1	1	0.5	8.33 (13.298)	1.02	1.13417
5.	2	-	-	19.75 (24.045)	0.96	0.76333
6.	2	1	-	11.17 (17.210)	1.06	1.21250
7.	2	-	0.5	24.17 (25.545)	1.46	1.15833
8.	2	2	-	18.33 (22.741)	1.07	0.67417
9.	2	2	0.5	7.50 (13.960)	0.92	0.67500

Table 4: Mean comparisons due to media for percentage proliferation, number of microshoots per culture and average length of microshoots in *O. americanum* Linn.

Type of media	% of proliferation	No. of microshoots per culture	Average length of micro-shoots
1) MS	22.28 (23.624)[a]	1.35	1.03667
2) B_5[12]	14.31 (20.813)[ab]	1.37	1.16556
3) White's media[13]	9.11 (14.615)[b]	1.00	0.94194

Similar alphabet shows homogeneous mean

Table 5: Mean comparison due to explant for percentage of proliferation, number of microshoot per culture and length of microshoots in *O. americanum* Linn.

Explant	% of proliferation	No. of microshoots per culture	Average length of micro-shoots
1. Nodal segment	24.00 (28.304)	1.68	1.37537
2. Shoot tip	6.46 (11.064)	0.80	0.72074

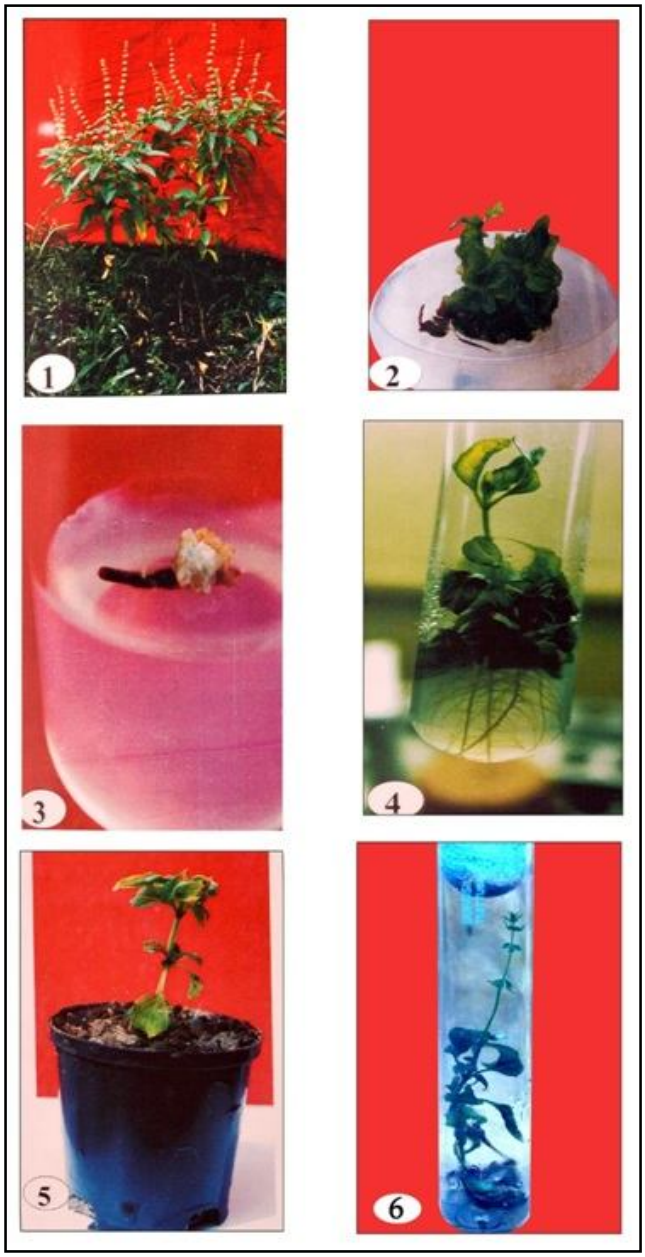

Figure 4: Different stages of *In vitro* culture of (*O. americanum*) 1. Mother Plant (*O. americanum*) 2. Multiple shoot proliferation 3. Introduction of callus from shoot tip explant 4. Complete *In vitro* grown plantlet showing profuse rootlet initiation 5. Plant at the hardening stage 6. *In vitro* flowering of *O. americanum.*

Phytochemical analyses of essential oil of natural and *in vitro* grown plants (Fig. 4) reveal that the constituents are more or less identical substantiating the fact that the regenerated plants are true to type. But the regenerated plants show *in vitro* flowering response in the root induction medium within two weeks of duration which is a remarkable observation. Such *in vitro* flowering is not only responsible for further shortening of life span of the plant but it also might have certain role in the acceleration of the production of aromatic compounds as rate of aroma gene expression is enhanced during the flowering time. Report in this regard is available in apple where over expression of FT homologous gene induces early flowering[14]. The over expression of candidate genes related to aroma formation during flowering could be determined through transcriptomic analysis as it is reported in *Osmanthus fragrans* [15] . Thus a thorough molecular investigation is required to show at what extent over expression of aromatic genes occur during *in vitro* flowering in *O. americanum.* It is also to be worked out at the molecular level whether early *in vitro* flowering response is due to somaclonal variation.

CONCLUSION: In conclusion it could be said that among the major economically important aromatic compounds present in the essential oil of *O. americanum*, cineole is the important ones since it is highest in amount in comparison to other species of *Ocimum* and this compound could be obtained from this species as a chief alternative source**.** Our investigation underlines that *in vitro* propagation of this species could be an alternative and preservative approach for better herbage yield to extract essential oil. *In vitro* flowering of the plant could be a new exploratory field since it has immense potentiality to enhance the production of aroma compounds through the increase of herbage yield by means of limiting life span and over expression of aroma genes as well.

ACKNOWLEDGEMENT: The author is grateful to Prof. P. Ghosh; Dept. of Botany, Kalyani University and Prof. S. K. Mukherjee, Dept. of Microbiology, Kalyani University for their guidance and support.

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