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Quantification of phytochemicals and its association study with leaf oil percentage in different genotypes of *Ocimum tenuiflorum* L.

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Abstract
Six genotypes of holy basil collected across India were analysed for quantification of phytochemicals on leaves and its association with leaf oil percentage were examined and presented in this article. The maximum ascorbic acid and β carotene content in leaf (89.51 & 5.93 mg 100 g-1) was recorded in West Godavari Local genotype followed by Cim-Ayu and Anand Collection 2 (86.37 & 4.66 mg 100 g^{-1}). The highest total phenols in leaf (360.23 mg 100 g^{-1}) was recorded in genotype Anand Collection 2 followed by West Godavari Local (308.60 mg 100 g^{-1}). Anand Collection 2 recorded the highest (3.70%) per cent oil from leaves on dry weight basis which was on par with Cim-Ayu (2.98%) and West Godavari Local (2.97%). Phenols content in leaf (0.844) and aroma score of leaves (0.811) showed significant positive association with per cent oil from leaves on dry weight basis.

Keywords: Sacred basil, phytochemicals, per cent oil, character association

Introduction
Sacred or holy basil or tulsi belongs to family Lamiaceae botanically called *Ocimum tenuiflorum* L. syn. *O. sanctum* L. is worshiping by Indians since from time immemorial also it has its immense contribution in ancient Vedic periods for different ayurvedic preparations from more than 3000 years as it contains diverse health healing biochemical constituents. In Sanskrit Tulsi word means "matchless one" [19]. Holy basil is a highly valued culinary and aromatic medicinal herb. In the Ayurveda system tulsi is often referred to as an "Elixir of Life" for its healing powers and has been known to treat many diverse health issues. In the Indian Materia Medica tulsi leaf extracts are described for treatment of bronchitis, rheumatism, and pyrexia. Other reported therapeutic uses include treatment of hiccups, epilepsy, asthma, cough, skin and hematological diseases, neuralgia, headache, wounds, inflammation, parasitic infections and oral conditions.

The leaves juice has been applied as a drop for earache, while the tea infusion has been used for treatment of hepatic and gastric disorders. The roots and stems were also traditionally used to treat mosquito and snake bites and for malaria [23].

The leaves of this basil on steam distillation yield a bright yellow volatile oil possessing a pleasant odour characteristic of the plant with an appreciable note of cloves. The plant contains mainly phenols, aldehydes, tannins, saponin and fats. The essential oil components are eugenol (about 71%), eugenol methyl ether (20%), nerol, caryophyllene, selinene, α-pinene, β-pinene, camphor, cineole, linalool, carvacrol (3%) etc. A terpeneurobsolic acid possessing anticancer action has also been isolated. The plant is used as a pot herb; leaves are used as condiment in salads and other foods. The leaves, seed and root form medicinally useful part. The leaves also contain ascorbic acid (83 mg 100 g^{-1}) and carotene (2.5 mg 100 g^{-1}) [10].

Although considerable research on the phytochemical analysis of sacred basil oil had been done but leaf phytochemicals analysis is fewer therefore analysis of basil leaf phytochemical composition has been the subject of considerable studies. There is extensive diversity in the constituents of the holy basil leaf and several chemo-types have been established from various nutritional and phytochemicals investigations.

The biochemical composition of sacred basil is highly complex, containing many nutrients and other biological active compounds in their leaves *viz*., ascorbic acid, beta carotene, carbohydrates, proteins, phenols etc., thus nutritional properties of the whole herb in natural form gave the holy basil importance as food and indicates its potential as a source of drugs and also perfumeries as it is rich in aromatic volatile oil. It is well known that the varieties, environmental condition and agricultural practices may significant influence on productivity, oil content, and bio chemical composition of basil leaf.

The present study was aimed to evaluate the phytochemical constituents in sacred basil leaves among different genotypes as preliminary investigation under Godavari zone of Andhra Pradesh, India. Rather, character association study between leaf phytochemical constituents with leaf oil percentage was also constituted with the data recorded to know the significant correlations and presented in this paper which may help the researchers in further investigations.

Material and Methods

An investigation entitled "Performance of sacred basil (*Ocimum tenuiflorum* L.) genotypes in Godavari zone of Andhra Pradesh" was carried out during *kharif* season, 2018-2019 at COH, Venkataramannagudem, West Godavari district. The location falls under Agro-climatic zone-10, humid, East Coast Plain and Hills (Krishna-Godavari zone) with an average annual rainfall of 900 mm at an altitude of 34 m (112 feet) above mean sea level. The geo-graphical situation is 16º 63' 120" N latitude and 81º 27' 568" E longitude. It experiences hot humid summer and mild winter. A total of six sacred basil genotypes *viz*., Cim-Ayu sourced from CIMAP, Hyderabad, Anand Collection 1, Anand Collection 2 sourced from AAU, Gujarat, Mysore Local collected from Mysore, Karnataka, West Godavari Local collected from HRS-Venkataramannagudem, A.P. and IC75030 sourced from NBPGR, New Delhi were taken for study. The experiment was laid out in RBD with four replications. The crop was raised at a plant spacing of 60 cm × 50 cm with plot size 10.8 m^2. The seedlings were transplanted during August, 2018 and herb was harvested during November. A basal fertilizer dose of 25 kg N, 15 kg P2O5 and 10 kg K2O ha^{-1} was given at the time of soil preparation. One month old seedlings were transplanted and need-based plant protection measures were taken up to raise a healthy crop.

Essential oil percentage was recorded by using Clevenger apparatus [6] and expressed on dry weight basis by taking 100 gram fresh sample for analysis. Chlorophyll content of randomly selected 20 leaves from each tagged plant was measured at harvest with the help of a SPAD Meter at full bloom stage and the average chlorophyll content of leaf was calculated. Carbohydrate, total phenol and protein content of leaves were estimated by using Anthrone reagent, Folin-Ciocalteau reagent and Lowry's method respectively as per the methodology adopted by Ranganna [28]. Ascorbic acid and β carotene content of fresh leaf was estimated as suggested by Sadasivam and Manickam [27] and Srivastava [31], respectively. Sensory evaluation was carried out on the fresh leaves using 10-man panel of judges from different departments of Horticulture. Aroma of leaves was rated separately on a scale of 1 to 5. Scores were defined as follows: 1 - dislike extremely, bad; 2 - like only slightly, tolerable; 3 - like, good; 4 – like very much, very good; 5 – like extremely, excellent. Numerical averages were then calculated for a composite test score.

The data obtained in respect to all the characters was subjected to the following statistical analysis. The data were analyzed by the methods outlined by Panse and Sukhatme [22] using the mean values of five random plants in each replication from all genotypes to find out the significance of genotypes effect. Correlations were worked out by using the formula suggested by Karl Pearson [16]. Significance of correlation coefficients was tested by comparing correlation coefficients with the table values [11] at n-2 degrees of freedom at 5% and 1% levels where 'n' denotes the total number of pairs of observations used in the calculation.

Results and Discussion

Leaf Chlorophyll (SPAD)

The leaf chlorophyll content varied significantly among the different genotypes (Table 1). Average chlorophyll content recorded by leaves was 45.24. The maximum total chlorophyll (48.79) content was recorded in West Godavari Local which was on par with Anand Collection 2, Cim-Ayu, and IC75030 (47.20, 47.10 and 45.28 respectively), but significantly superior to Anand Collection 1 (44.86) whereas the lowest leaf chlorophyll (38.20) was noticed in Mysore Local.

β carotene in leaf (mg 100 g $^{-1}$)

The genotypes varied significantly in terms of leaf β carotene content (Table 1). Mean β carotene in leaves was 3.69 mg 100 g^{-1}. The highest β carotene in leaf (5.93 mg 100 g^{-1}) was recorded by West Godavari Local followed by Anand Collection 2 (4.66 mg 100 g^{-1}). The genotype Mysore Local exhibited minimum β carotene in leaf (0.66 mg 100 g^{-1}).

Carbohydrate content in leaf (%)

Significant variation was recorded for carbohydrate content among the genotypes (Table 1) with a mean 46.92% and the maximum carbohydrate content was observed in West Godavari Local (58.23%) followed by Anand Collection 2 (50.25%) while, the genotype Mysore Local recorded the lowest (39.60%).

The contents of chlorophyll, carotene and carbohydrate revealed that those genotypes with higher levels of chlorophyll and carotene pigments also had a higher quantum of carbohydrates which might be due to the reason that they had larger photosynthetic apparatus in their leaves and *vice versa*. Similar results were obtained by Aluko *et al.* [3], Daly *et al.* [7], Emeka and Chimaobi [9], Idris *et al.* [14], Mlitan *et al.* [20], Pritwani and Mathur [24], Rajeswari *et al.* [25], Tewari *et al.* [32] and Vidhani *et al.* [33].

Total phenols in leaf (mg 100 g $^{-1}$)

Total phenols in leaf differed significantly between the genotypes and the mean recorded was 240.37 mg 100 g^{-1} (Table 1). The highest total phenols in leaf (360.23 mg 100 g^{-1}) was recorded in genotype Anand Collection 2 followed by West Godavari Local (308.60 mg 100 g^{-1}). The minimum total phenols from leaf was recorded by the genotype Mysore Local (118.44 mg 100 g^{-1}).

Ascorbic acid in leaf (mg 100 g $^{-1}$)

There was significant difference in ascorbic acid content of leaf among the different genotypes with mean 56.84 mg 100 g^{-1} (Table 1). The maximum ascorbic acid content (89.51 mg 100 g^{-1}) was recorded in West Godavari Local followed by Cim-Ayu (86.37 mg 100 g^{-1}). Genotype Mysore Local exhibited minimum ascorbic acid content in leaf (24.80 mg 100 g^{-1}).

Aroma score for fresh leaves

The sensory evaluation of aroma in fresh leaves using 5-point hedonic scale revealed that there were significant differences among the various genotypes (Table 1) with a mean of 3.52. Anand Collection 2 had highest (4.78) aroma which is on par with Cim-Ayu (4.56), but significantly superior than West Godavari Local (3.78). Least aroma score was obtained from the genotype Mysore Local (2.00). It is interesting to note from the results of the above parameters that those genotypes showing higher contents of total phenols and ascorbic acid in

the leaves were more aromatic, which might be due to the fact that the aromatic principles are chemically phenolic in nature and the aroma is preserved by the presence of ascorbic acid. The results obtained in the present study are in line with earlier findings of Alakali *et al.* [2], Benabdallah *et al.* [5], Dumbrava *et al.* [8], Emeka and Chimaobi [9], Grzeszczuk and Jadczak [12], Kapoor and Bansal [15], Padmaja and Srinivasulu [21] and Vidhani *et al.* [33].

Protein content in leaf (%)

The results on protein content revealed that there were significant differences among the various genotypes (Table 1) with a mean of 14.60%. Anand Collection 1 recorded maximum protein content in leaf (19.60%) followed by IC75030 (19.00%) whereas, Mysore Local recorded minimum protein content in leaf (8.46%). Amador *et al.*, [4], Emeka and Chimaobi [9], Idris *et al.* [14], Kavitha *et al.* [18], Mlitan *et al.* [20] and Shuaib *et al.* [29] also reported the similar results.

Per cent oil from leaves (Dry weight basis)

The variation was significant for per cent oil from leaves (Table 1). The mean value recorded was 3.00%. Anand Collection 2 recorded the highest (3.70%) per cent oil from leaves on dry weight basis which was on par with Cim-Ayu (2.98%) and West Godavari Local (2.97%), but significantly superior to IC75030 (2.93%) whereas the lowest per cent oil from leaves (2.63%) was noticed in Mysore Local. Aburigal *et al.* (1), Ibrahim *et al.* (13) in basil, Kassahun *et al.* [17], in mint, Ramachandra *et al.* (26) in patchouli and Srivastava *et al.* [30] in mint also confirmed the same.

Table 1: Phytochemicals in leaves of sacred basil (Ocimum tenuiflorum L.) genotypes

Genotype	Leaf chlorophyll (SPAD)	β carotene in leaf (mg 100 g^{-1})	Carbohydrate content in leaf (%)	Total phenols in leaf (mg 100 g^{-1})	Ascorbic acid in leaf (mg 100 g^{-1})	Aroma score of fresh leaves	Protein content in leaf (%)	Leaf oil percent (DWB)
CIM-Ayu	47.10	4.45	47.84	288.40	86.37	4.56	10.67	2.98
Anand collection 1	44.86	2.62	42.32	162.31	35.07	2.67	19.60	2.79
Anand collection 2	47.20	4.66	50.25	360.23	86.37	4.78	15.98	3.70
Mysore Local	38.20	0.66	39.60	118.44	24.80	2.00	8.46	2.63
West Godavari Local	48.79	5.93	58.23	308.60	89.51	3.78	13.86	2.97
IC75030	45.28	3.83	43.31	204.26	58.99	3.33	19.00	2.93
Mean	45.24	3.69	46.92	240.37	56.84	3.52	14.60	3.00
S Em±	1.17	0.06	2.36	11.22	0.45	0.15	0.09	0.23
CD (0.05)	3.54	0.17	7.11	33.83	1.37	0.44	0.27	0.68

Character association among phytochemicals in leaves of sacred basil

Correlation coefficients among different phytochemicals in leaves of holy basil are presented in Table 2.

Leaf chlorophyll (SPAD)

Leaf chlorophyll recorded significant positive association with carbohydrate content (0.817), phenols (0.834), ascorbic acid (0.872) and aroma (0.813); highly significant positive association with β carotene (0.963).

Table 2: Correlation matrix among different phytochemicals in leaves of sacred basil (*Ocimum tenuiflorum* L.) genotypes

	A	B	C	D	E	F	G	H
A	1							
B	0.963**	1						
C	0.817*	0.901*	1					
D	0.834*	0.878*	0.824*	1				
E	0.872*	0.937**	0.856*	0.957**	1			
F	0.813*	0.807	0.655	0.949**	0.934**	1		
G	0.411	0.257	-0.009	0.060	-0.003	0.071	1	
H	0.569	0.572	0.475	0.844*	0.676	0.811*	0.241	1

A	: Leaf chlorophyll content (SPAD)	D	: Total phenols in leaf (mg 100 g $^{-1}$)	G	: Protein content in leaf (%)
B	: β carotene in leaf (mg 100 g $^{-1}$)	E	: Ascorbic acid in leaf (mg 100 g $^{-1}$)	H	: Per cent oil from leaves (DWD)
C	: Carbohydrate content in leaf (%)	F	: Aroma score for fresh leaves		
*	Significant at 5% level of significance;	**	Significant at 1% level of significance.		

β carotene in leaf (mg 100 g $^{-1}$)

Carbohydrate content (0.901) and phenols (0.878) recorded significant positive association with β carotene in leaf. Highly significant positive association of leaf β carotene content was found with leaf chlorophyll (0.963) and ascorbic acid (0.937).

Carbohydrate content in leaf (%)

This character exhibited significant and positive correlation with leaf chlorophyll (0.817), β carotene (0.901), phenols (0.824) and ascorbic acid (0.856).

Total phenols in leaf (mg 100 g $^{-1}$)

This trait had significant positive association with leaf chlorophyll (0.834), β carotene content (0.878), carbohydrate content (0.824) and per cent oil from leaf (0.844); highly significant positive association with aroma score of leaf (0.949) and ascorbic acid content (0.957).

Ascorbic acid in leaf (mg 100 g $^{-1}$)

Significant and positive correlation of this trait was observed with leaf chlorophyll (0.872) and carbohydrate (0.856) whereas, leaf β carotene content (0.937), phenols (0.957) and aroma score of leaf (0.934) showed highly significant positive association with ascorbic acid in leaves.

Aroma score for fresh leaves

The character exhibited significant and positive correlation with leaf chlorophyll content (0.813), and per cent oil from leaf (0.811).

Protein content in leaf (%)

Protein content in leaf had no significant correlation with any of the biochemical traits.

Per cent oil from leaves (Dry weight basis)

Phenols content in leaf (0.844) and aroma score of leaves (0.811) showed significant positive association with per cent oil from leaves.

It is evident from these results that oil percentage was positively and significantly associated with phenols and aroma scores. This might be because of the reason that the total phenolic compounds in the leaf would have maintained a dynamic equilibrium with fatty acids and essential oil principles which were phenolic in nature.

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