Comparison of essential oils composition and antioxidative property of leaf extracts of Ocimum sanctum (p) and Ocimumbasilicum.

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Abstract
The present study deals with the total essential oil composition and antioxidant activity of two plants of lamiaceace family. Extraction of total essential oils and volatile essential oils from *Ocimum sanctum* (p) and *Ocimumbasilicum*in four different seasons of 2010 calendar year. Extraction of total essential oil content of plant materials was carried out by Soxhulet extraction and that of volatile oils by steam distillation using Clevenger type apparatus. The percentage composition of eugenol in four seasons was found from GC analysis. The antioxidant activity was studied by DPPH radical scavenging method. Total essential oil and volatile oil contents are more in winter(present study in the month of November) where as very less in summer(in the month of May). Eugenol is the major constituent present in these plants. Methanolic extract of leaves of *OcimumSanctum*(p) and*ocimumbasilicum*showed a very good DPPH radical scavenging activity and the percent of inhibition was 46.91% and 43.1% respectively. This study revealed that extract of leaves of these plants comprise effective potential source of natural antioxidants due to the presence of Eugenol.

Key words: *Ocimumsanctum(p), ocimumbasilicum- total essential oil- eugenol-total phenols-flavonoids-antioxidants-DPPH assay*

1. INTRODUCTION

Ocimum sanctum also known as *Ocimumtenuiflorum*belongs to Lamiaceae family is commonly known as *Tulsi*or *holybasil.* The plant grows wild in India but is also widely cultivated in home and temple gardens and is used for household remediation [1]. *Holy basil* has a strong anise like, slightly musky and lemony taste with a camphoraceous aroma. The dominant aroma component in *holy basil* isEugenol. The more exuberantly flavoured red *holy basil* (redor purple variety) has dark green leaves withreddish purple stems and a purplish cast on theyounger leaves known as *Krishna Tulsi*.

*Ocimumbasilicum*L.is commonly known as *Sweet basil* is a pleasant smelling perennial shrub which grows in several regions all over the world [2,3]. *Sweet basil* is a popular culinary herb and a source of essential oils extracted by steam distillation from the leaves and the flowering tops which are used to flavour foods, in dental and oral products,and in fragrances.[4-9]The stem and leaves of holy basilcontain a variety of constituents that may havebiological activity, including saponins, flavonoids, triterpenoids, and tannins[10]. Theleaf volatile oil[11] contains eugenol, euginal (alsocalled eugenic acid), urosolic acid[12], carvacrol,linalool, limatrol, caryophyllene, methylcarvicol, uteolin, vitexin, isovitexin, orientin,isoorientin, aesculin, chlorogenic acid,aesculetin, caffeic acid[13], beta-carotene[14],Rosmarinic acid, apigenin, cirsimaritin,isothymusin and isothymonin. Two

watersoluble flavonoids:[15] Orientin and Vicenin,ascorbic acid and carotene are also reported[16].
Different parts of *Tulsi*plant such as leaves,flowers, stem, root, seeds etc. are knownto possess therapeutic potentials and have beenused by traditional medical practitioners, asexpectorant, analgesic, anti-cancer, antiasthmatic,antiemetic, diaphoretic, antidiabetic, anti-fertility,hepatoprotective, hypotensive, antistress,analgesic, antihyperlipidemic, antioxidantpotentials in experimental animals[17-22].
The characteristic compounds of *basil* essential oil are linalool, methyl chavicol, eugenol ,estragol ,thymol and *p*-cymen were found[7,23-26].The phenolic compounds and flavonoids such as cinnamic acid, caffeic acid, sinapic acid, ferulic acid, rosemarinic acid, p-coumaric acid ,rutin ,kaempferol and Quercitin in *O. basilicum*L. have also been reported[27].Two minor components of the essential oil of *sweet basil*, juvocimene I and II, have been reported as potent juvenile hormone analogs[28].The leaves and
flowering tops of the plant are perceived as carminative, galactogogue and antispasmodic in folk medicine [29]Chicoric acid was recently identified and quantified in *basil* leaves[30]*Sweet basil* oil possess anticancer, antibacterial , toxic [24,31-39],insecticidal [38,40-42], repellent [43-46],nematicidal[47], fungistatic[35], or antimicrobial [48]activities. The allylphenolic derivative eugenol has a sharp spicy odour reminiscent ofcloves, and is used as a dental analgesic and disinfectant [49].

The objective of this research was to examine and compare the % composition of essential oil,% composition of eugenol, total
phenols, flavonoids , antioxidants and DPPH assay of purple variety of *O.sanctum* and *Ocimumbasilicum*.% composition of essential oil and % composition of eugenol of purple*Ocimum sanctum* and *Ocimumbasilicum*were compared in four seasons of 2010 calendar year

2. MATERIALS AND METHODS

2.1 Collection and identification of plantmaterials

All the selected plants were grown in the same geographical conditions in the spacious domestic home garden at Gavaravaram
village, Elurumandal in West Godavari district of Andhra Pradesh is situated between 16.7^{o}North 81.1^{o} East, elevation 22mts 72 feet. Plant material was collected and vouchers of specimens were deposited at the Botany department. Each specimen was labelled, numbered, annotated with the date of collection. In the present study the specimen numbers areM2,N2, M5 and N5. Each specimen was subjected for identification at plant systematic laboratory, Kakatiya University, Warangal, Andhra Pradesh,India.
One kilogram of leaves of each sample was collected, dried under shade, finely powdered in an electric blender (80 mesh) and stored in air tight containers at room temperature in the dark until used.

2.1.1 Preparation of plant extract

One gram of dried leaf powder was grinded with 20ml of 50% methanol and filtered. The filtrate was made up to the volume 50ml
with 50% methanol .This extract was used to analyze the total phenol content , the flavonoids content and the antioxidant capacity.

2.1.2 Soxhulet extraction

Extraction of total essential oil content of plant materials was carried out by soxhuletextraction[50]method. 5gm of dry powder was subjected tosoxhulet extraction with 250ml methanol as solvent, extraction was carried out for 3hrs, 10 cycles and temperature was

maintained at 65°C.This extract was used toanalyze DPPH assay and the antimicrobial activity.

2.1.3 Steam distillation

Extraction of volatile oils from the plant materials was carried out by steam distillation using Clevenger type apparatus [51]. 100g powdered sample was water distilled by using a Clevenger oil arm fitted with condensers through which cooled water was circulated to prevent low volatiles from escaping. The temperature was maintained at 60^0C. The volatile oil was collected and dried over anhydrous Sodium Sulphate and stored at -4°C. 1mg of volatile extract was dissolved in 1ml ofmethanol; from that solution 10µl was taken and made up to 100 µl with methanol. This solution was used for GC analysis. (Same procedure was followed for the preparation of standard eugenol.)

2.2 Gas chromatograph analysis

The essential oils were analyzed using a Shimadzu gas chromatograph model 17 A Japan(2014)at Chandra laboratory, Hyderabad, equipped with flame ionization detector (FID) and DB-Wax capillary column (30mm x 0.32mm, film thickness 0. 5 µm). Injector and detector temperatures were set at 240 and 250^0C, respectively. Column oven temperature was programmed from 40^0C to 220^0C at the rate of 8^0C min-1; initial and final temperatures were held at 3 minutes and 10 minutes, respectively. Helium was used as a carrier gas with a flow of 1.5 mL/ min. A sample of 0.1 µL was injected using slit mode (split ratio, 1:20). Quantification was completed by built-in data-handling software supplied by the manufacturer (spin chrome CFR) of the gas chromatograph. Theresults (composition) were reported as a relative percentage of the total peak area.

2.2.1 Estimation of total phenolics

Total phenolic content was determined by using Folin-Ciocalteu reagent as previously described [52].Each plant extract (250 µL) was mixed with 5ml of Folin-Ciocalteu reagent and 4 ml of (20%) sodium carbonate, and they were vortexed for 50sec and they were let to stand for 30mins in water bath at 40^0C. The optical density was measured by using systronics(C1117) colorimeter at 680nm. The total phenol content of the extracts was obtained by using the standard curve. The total phenol content was expressed as tannic acid (0.1mg/ml) equivalent in % w/w of the extracts.

Total phenolic content =

$$\frac{\text{optical density of sample} \times \text{concentrationof tannic acid}}{\text{optical density of standard}}$$

2.2.2 Total flavonoids content

The total flavonoid content was determined using the Dowd method [53]. Each plant extract solution (250 µl) was mixed with 0.1ml of 2% aluminium chloride and 1ml of 0.1M potassium acetate, mixed well and allowed to stand for 30min. at room temperature. The optical density was measured by using systronics (C1117) colorimeter at 420nm. Totalflavonoid contents were calculated as quercetin (0.1mg/ml) equivalent from a calibration curve.

Total flavonoids content =

$$\frac{\text{optical density of sample} \times \text{concentrationof quercetin}}{\text{optical density of standard}}$$

2.2.3 Reducing Power Assay

The reducing power of the extracts was measured by using ascorbic acid as previously described[54].Each plant extract solution (250 µl)

was mixed 2.5 ml of phosphate buffer (pH 6.6)and 2.5ml of 1%potassium ferricyanide and were incubated in water bath at 50^0C for 20min.Then the test tube was centrifuged for 10min. at 10,000 rpm . 2.5ml of supernatant were diluted with 2.5 ml water and shaken with 0.5ml of freshly prepared 0.1 % Ferric chloride. The optical density was measured by using systronics (C1117) colorimeter at 680nm. Total antioxidants contentwas calculated as ascorbic acid (0.1mg/ml) equivalent from a calibration curve.

$$\text{Total antioxidants content} = \frac{\text{optical density of sample} \times \text{concentrationof ascorbic acid}}{\text{optical density of standard}}$$

2.2.4 DPPH radical scavenging assay (antioxidant assay)

The following assay procedure was modified from those described by Blois [55] and Govindaragan [56]. Sample stock solutions (1.0 mg/ml) were diluted to final concentrations of 250, 125,50, 25, 10 and 5 µg/ml in methanol. A portion of sample solution (500 µl) was mixed with an equal volume of 6x10-5 M DPPH (1,1-diphenyl-2 picryl-hydrazyl;in methanol) and allowed to stand at room temperature for 30 min. In each experiment methanol (1ml) plus plant extract

solution was used as blank while the DPPH solution alone in methanol was used as control. The absorbance (A) of sample solution was measured by using UV–VIS spectrophotometer 2.2. (double-beam) (SL191 series) at 520 nm, compared with that of control solution (maximum absorbance). The scavenging activity of samples corresponded to the intensity of quenching DPPH. The results were expressed aspercentage inhibition.

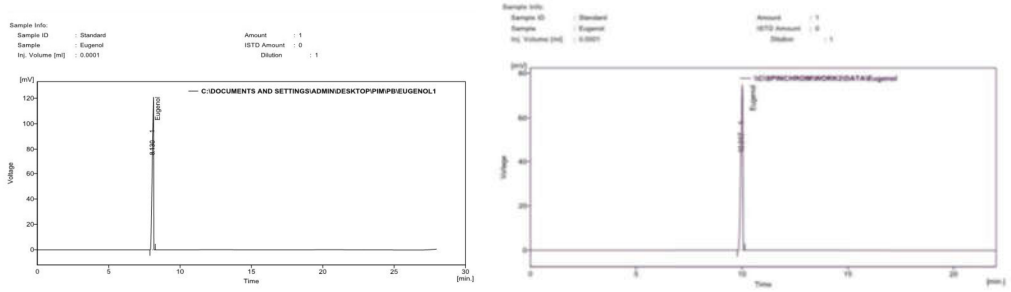
$$\% \text{ inhibition} = \frac{[(\text{A control} - \text{A sample})]}{\text{A control}} \text{X } 100$$

3.Result and discussion:

TABLE-1: Total essential content and Percentage composition of eugenol in *O. sanctum(p) and O. basilicum*

Month & Year	Total essential oil content%		Percentage composition of Eugenol	
	O.Sanctum(P)	*O.Basilicum*	*O.Sanctum(P)*	*O.Basilicum*
February 2010	1.85%	1.53%	49.00%	NA
May 2010	1.52%	1.28%	37.969%	6.553%
August 2010	1.61%	1.41%	42.448%	7.747%
November 2010	2.0%	1.7%	61.538%	9.229%

Standard Eugenol chromatogram

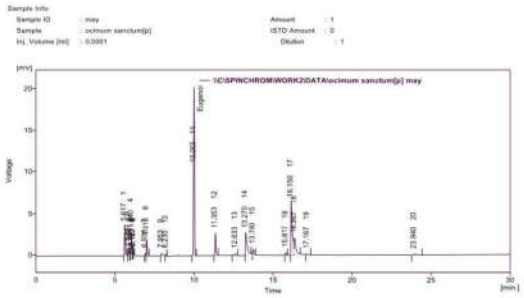


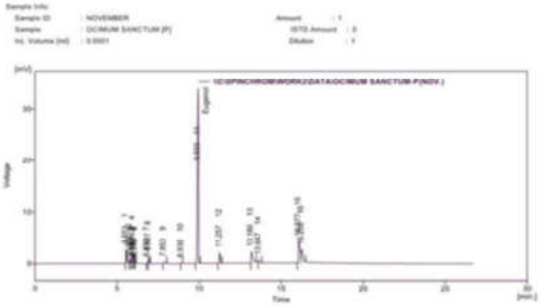
Result Table (Uncal - C:\DOCUMENTS AND SETTINGS\ADMIN\DESKTOP\PIM\PB\EUGENOL1)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]
1	8.130	798.431	120.739	100.000
	Total	798.431	120.739	100.000

Result Table (Uncal - \\C\SPINCHROM\WORK2\DATA\Eugenol)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]
1	10.017	464.988	74.656	100.000
	Total	464.988	74.656	100.000



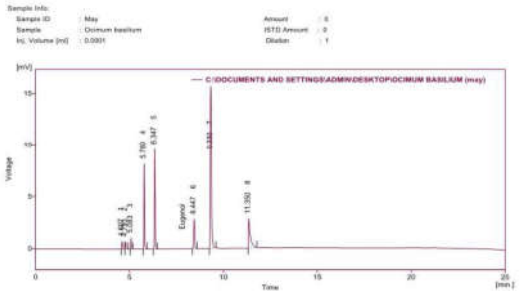


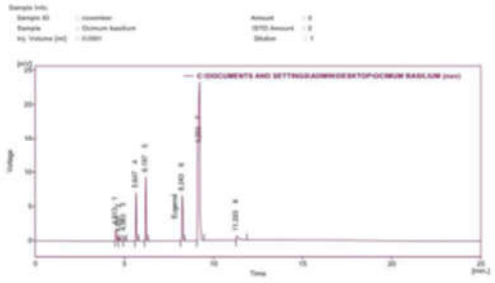
Result Table (Uncal - \\C\SPINCHROM\WORK2\DATA\OCIMUM SANCTUM-P(MAY)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]
1	5.617	12.217	3.550	6.077
2	5.847	2.998	0.983	1.492
3	5.960	0.673	0.329	0.335
4	6.040	6.938	2.527	3.451
5	6.133	0.235	0.141	0.117
6	6.187	0.410	0.181	0.204
7	6.930	1.005	0.352	0.500
8	7.013	5.527	1.863	2.750
9	7.953	2.446	0.319	1.217
10	8.230	1.029	0.306	0.512
11	10.003	76.329	20.028	37.969
12	11.353	10.035	2.632	4.992
13	12.633	1.120	0.222	0.557
14	13.270	19.112	2.697	9.507
15	13.740	2.529	0.596	1.258
16	15.817	1.196	0.332	0.595
17	16.150	39.076	6.329	19.438
18	16.367	16.200	1.992	8.059
19	17.167	0.607	0.105	0.302
20	23.940	1.350	0.124	0.671
	Total	201.032	45.609	100.000

Result Table (Uncal - \\C\SPINCHROM\WORK2\DATA\OCIMUM SANCTUM-P(NOV.)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]
1	5.553	8.377	2.704	3.489
2	5.777	2.342	0.774	0.975
3	5.893	0.418	0.212	0.174
4	5.967	5.769	2.193	2.402
5	6.060	0.154	0.093	0.064
6	6.110	0.250	0.111	0.104
7	6.850	0.727	0.274	0.303
8	6.927	4.012	1.391	1.671
9	7.853	1.360	0.286	0.566
10	8.930	0.298	0.094	0.124
11	9.933	147.773	33.853	61.538
12	11.257	7.366	2.043	3.067
13	13.180	14.971	2.203	6.234
14	13.647	2.109	0.485	0.878
15	16.077	26.262	4.194	10.937
16	16.250	17.946	2.494	7.473
	Total	240.135	53.404	100.000





Result Table (Uncal - C:\DOCUMENTS AND SETTINGS\ADMIN\DESKTOP\OCIMUM BASILIUM (may)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]
1	4.607	1.971	0.734	1.325
2	4.793	1.911	0.717	1.284
3	5.083	2.088	1.057	1.403
4	5.780	18.652	8.180	12.533
5	6.347	23.834	9.629	16.015
6	8.447	9.753	2.816	6.553
7	9.330	72.310	15.667	48.589
8	11.350	18.302	2.817	12.298
	Total	148.822	41.617	100.000

Result Table (Uncal - C:\DOCUMENTS AND SETTINGS\ADMIN\DESKTOP\OCIMUM BASILIUM (nov)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]
1	4.513	4.210	1.692	1.770
2	4.697	1.501	0.622	0.631
3	4.983	1.599	0.786	0.672
4	5.647	14.834	7.039	6.238
5	6.197	22.571	9.347	9.491
6	8.243	21.948	6.590	9.229
7	9.203	165.952	23.204	69.783
8	11.293	5.195	0.629	2.184
	Total	237.810	49.911	100.000

3.1 Total essential oil and percentage composition of eugenol

Table 1 shows the yield of total essential oil and percentage composition of eugenol in *Ocimumsanctum(p) and ocimumbasilicum*in four different seasons of 2010 calendar year. It shows that the yield was affected by seasonal changes. The highest amount of the oil in these plants was found in winter i.e in November and very low in summer i.e in May. In *Ocimumsanctum(p)*the percentage composition of essential oil is high in November (2.0%) and low in May (1.52%),where as in *Ocimumbasilicum*the percentage composition of essential oil is high in November (1.7%) and low in May (1.28%). In India the temperature is very high in summer ranging 35-42^{0}c. Low essential oil yield in summer might be attributed to the high temperature and partial evaporation of some constituents of oil can be expected.

From Table 1 it was found that the percentage composition of Eugenol in *Ocimumsanctum(p) and ocimumbasilicum*was high in the month of November (61.538%),(9.229%) and very low in the month of May(37.969%),(6.553%) respectively. Asha and coworkers[57] reported that the oil of *ocimum sanctum* posses

Eugenol(53.10%) as the main compound .Sonia verma[58] reported that in *O. basilicum*the majorconstituents of the oil was linalool (64.35%) and with very low composition of eugenol(3.21%).

Table 2: Percentage composition of eugenol, total phenols, flavonoids, antioxidants and DPPHscavenging assay.

Name of the plant	% composition of Eugenol	Total Phenols mg TA equivalent/gm (dw)	Total flavonoids mg of quercetin equivalent/gm(dw)	Antioxidants mg of ascorbic acid equivalent /gm(dw)	DPPH %inhibition
O.Sanctum(p)	61.538%	127.3±1.8	0.733±0.01	0.921±0.03	46.91%
O.Basilicum	9.229%	123±1.4	0.604±0.02	1.047±0.01	43.1%

Values are mean ± standard deviation of three samples.
dw: dry weight of the powdered sample

3.2 Total phenols:

The phenols contain hydroxyls that are responsible for antioxidant activity and the radical scavenging effect mainly due to redox properties[59-61] . In the present study *O.Sanctum*(p) and *O.Basilicum*show thevalue of total phenols127.3±1.8 and123±1.4TAequivalent/gram.

Due to the higher phenol content in the leaf, the plant possesses high antioxidant activity and other pharmacological
activities [62].

3.3 Total flavonoids:

The compounds such as flavonoids, which contain hydroxyls, are responsible for the radical scavenging effect in the plants[63,64] .

The flavonoid contents of the extracts in the present study in terms of
quercetin equivalent for *O.Sanctum*(p) and *O.Basilicum* were 0.733±0.01mg/g and 0.604±0.02mg/g respectively.Flavonoidswhich were isolated from the aqueous extract of *Tulsi*have been shown significant anti oxidantactivity, both *in vivo* and *in vitro[*15] .

3.4 Antioxidants:

In the present study the values of antioxidants are given as 0.921±0.03mg/g for *Ocimumsanctum(p)*, and 1.047±0.01mg/g
ascorbic acid equivalent/gm for *Ocimumbasilicum*respectively. According to the recent reports, a highly positive relationship between total phenols and antioxidant activity was found in many plant species[65] . The phenolic compounds may contribute directly to the antioxidativeaction[66]. A number of phenolic compounds with strong antioxidant activity have been identified in *Ocimumbasilicum*plant extracts[67]

3.4 DPPH Scavenging assay

The DPPH molecule contains stable free radical which has been widely used to evaluate the radical scavenging ability of antioxidants.

DPPH stable free radical method is a sensitive way to determine the antioxidant activity of plant extracts [68,69] and measured the
inhibition of reactive species (free radicals)generated in the reaction mixture and these results depend on the type of reactive species
used [70]. In general essential oils exhibited weak to strong free DPPH scavenging activity rang can be shown as(weak: <25%; moderate: 25-50%; strong: >50%) at the tested concentration of 0.1% (v/v).The extracts of*Ocimum sanctum(p)*(46.91%)and*OcimumBasilicum*(43.1%) showed the moderate DPPH radical scavenging activity.Thehigh inhibition of DPPH activity of *Ocimum sanctum* purple in this recentstudy(46.91%%)was lower than the value (71%) reported by LukmanulHakkim*et al* [71] .

4. CONCLUSIONS

Eugenol, (l-hydroxy-2-methoxy-4- allylbenzene), is a phenolic compound, common and major volatile constituent of essential oils
extracted from leaves of the*Ocimum Sanctum*(p),but in *Basilicum* it is minor compound .*OcimumSanctum*(p) and OcimumBasilicum possessed significant antioxidant activity. in*Ocimum Sanctum*(p) it was mainlydue to the presence of eugenol, where as in *Ocimumbasilicum*it is due other phenolic compound like Linalool. These plants are easily available in our surroundings and theprocess of cultivation is also very easy. So we can make use of these plants to extract the costly componentslike eugenol& Linalool. These plants show different medicinal properties. So, still more clinical trials should be conducted to support their medicinal therapeutic uses.

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