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Research article

COMPARISON OF BIOACTIVE INGREDIENTS IN OCIMUM SPECIES

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ABSTRACT: With the increasing demand for health care approaches, resurgence of herbal medicines has taken up great dimensions in changing the health care scenario across the globe. However, identification of the correct species of therapeutic importance is of utmost necessity to deliver quality products to the global market. Hence, modern approach in the standardization of single herbal preparations employing sophisticated techniques is the need of the hour. The evaluation of a product in its entirety, so-called "fingerprinting" can be accomplished by appropriate methods, which may include HPLC, GC-MS, HPTLC-densitometry, FT-NIR, high-field NMR or a combination of these techniques. Using chemical fingerprinting, plants can be demarcated on the basis of their species, strain and geographical origin. Chemical fingerprinting of plants, through chromatographic fingerprinting is highly informative which includes its use as an absolute indicator of the chemical characteristics of plants. Adulterants can be distinguished even in processed samples, enabling the authentication of the drug. Herein, in the present study two varieties of *Ocimum species* with green and purple coloured leaves collected from Tirunelvelli district commonly known as "Tulasi" in Tamil or "Holy Basil" in English and widely used in both ayurvedic and siddha drugs was subjected to chemical fingerprinting using HPTLC and GC. Moreover, the secondary metabolities such as polyphenols, tannins, and flavonoids were quantified to check the potency of the crude drug material. The bioactive molecule such as eugenol was found to be varying in both the species and the purple variety was found to contain more of the bioactive molecules. The fingerprinting of chemical profile as well as the quantification of the bioactive molecules in the two varieties of Ocimum species exemplified that fingerprinting using analytical techniques are comprehensive and more informative to identify and authenticate the raw drug and proves to be a tool for standardization of herbal drugs.

Keywords: Ocimum species, Standardisation, HPTLC.

INTRODUCTION

In India more than 70% of the population uses herbal drugs for their health. There is a vast experience-based evidence for many of these drugs. These herbal drugs and Indian medicinal plants are also rich sources of beneficial compounds including antioxidants and components. Chemical constituents with antioxidant activity present in high concentrations in plants determine their considerable role in the prevention of various degenerative diseases (Velioglu et al., 1998). Besides fruits and vegetables, certain plants are recommended at present as optimal sources of such components. The supplementation of human diet with herbs, containing especially high amounts of compounds capable of deactivating free radicals, may have beneficial effects. Throughout recorded history, spices and herbs have been used for flavoring foods and beverages and for medicinal purposes. The preservative effect of many plant species and herbs suggests the presence of antioxidative and antimicrobial constituents (Hirasa and Takemasa, 1998). Among the important constituents in plants, phenolic compounds are mainly involved in the cell defence system against free radicals (Szeto et al., 2002). A number of phenolic compounds with strong antioxidant and antimicrobial activities have been identified in plants, especially in those belonging to the Lamiaceae family. They are of interest to food manufacturers as consumers move towards functional foods with specific health effects (Ozkan et al., 2003). The genus *Ocimum*, a member of the Lamiaceae family, contains 200 species of herbs and shrubs (Simon et al., 1999).

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This species has a long history as culinary herbs, thanks to its foliage adding a distinctive flavor to many foods. It is also a source of aroma compounds and essential oils containing biologically active constituents that possess insecticidal and nematicidal properties (Deshpande and Tipnis, 1997; Chaterje et al., 1982). However, the antioxidative potential of herbs and spices is well correlated with the presence of phenolic compounds due to its redox properties, which permit them to act as reducing agents, hydrogen donators and singlet oxygen quenchers (Caragay, 1992). The major phenolic compounds found in plants are secondary metabolites possessing high antioxidant activity and it is wide spread in the species of Lamiaceae (Gang et al., 2001). Ocimum sanctum Linn. Family Lamiaceae) commonly known as Tulasi or Holy Basil, is widely used in Indian systems of medicine. Atleast 2 varieties of O.sanctum are known, Sri Tulasi/safed Tulasi bearing green leaves and Krishna Tulasi /kali Tulsi bearing dark purple leaves, of which latter is claimed to more potent than the former as per Chunekar In traditional medicine, the plant is used in cardiopathy, blood disorders, skin diseases, etc. Free radicals can initiate or propagate many diseases, such as inflammation, cancer, liver injury and cardiovascular disease. Currently available synthetic antioxidants like butylated hydroxyl anisole (BHA), butylated hydroxy toluene (BHT), tertiary butylated hydroquinon and gallic acid esters, have been suspected to cause or prompt negative health effects. Hence, strong restrictions have been placed on their application and there is a trend to substitute them with naturally occurring antioxidants.

Recently there has been an upsurge of interest in the therapeutic potentials of medicinal plants as antioxidants in reducing such free radical induced tissue injury. Many plant extracts and phytochemicals have shown to have free radical scavenging properties (Larson, 1988 Koleva et al., 2002) but generally there is still a demand to find more information concerning the antioxidant constitutents of plant species. The presence of secondary metabolites phenols, terpenoids and tannins in green and purple tulsi was studied by phytochemical analysis using ethanolic extract. The antioxidant activity of plant might be due to their phenolic compounds (Cook and Samman, 1996) Flavanoids are a group of polyphenolic compounds with known properties which include free radical scavenging, inhibition of hydrolytic and oxidative enzymes and anti-inflammatory action. The presence of polyphenolic compound in green and purple tulsi prompted us to study the free radical scavenging activity. In the present study the quantification of eugenol and secondary metabolites such as polyphenols, tannins and flavonoids were analysed.

MATERIALS AND METHODS

Plant Material:

The leaves of both varieties green and purple tulsi were collected from Tirunelveli and kanyakumari district. The samples were authenticated, and specimens were deposited in Arvind Remedies LTD. The samples were dried in shade, stored at 25°C in air tight containers, and powdered to 40 mesh whenever required.

Anisaldehyde-sulfuric acid reagent-Anisaldehyde (0.5 ml) was mixed with 10 ml glacial acetic acid, followed by 85 mL methanol and 5 ml concentrated sulfuric acid , in that order. Derivatization- The pates were dipped in about 10 ml freshly prepared anisaldehyde-sulphuric acid reagent for 1min and heated for 100 c for 7 min before scanning.

Phytochemical analysis:

Phytochemical analysis was done both qualitatively and quantitatively. The presence of phytochemicals total phenols by Folin Ciocalteau reagent (McDonald et al.,2001). Aluminium chloride colorimetric method for Flavonoids determination (Chang et al.,2002) and total tannins.

In vitro lipid peroxidation inhibition assay:

The extent of inhibition of lipid peroxidation was evaluated by the estimation of thibarbituric acid reactive substances (TBARS level by measuring the absorbance at 532 nm.(Ohkawa et al, 1979). The percentage inhibition of lipid peroxidation was calculated by the formula

%) Inhibition = { (control-test)/control)}/100

Scavenging of nitric oxide radical activity:

It was estimated by use of Griess reagent. The absorbance was read at 546 nm (Green et al,1982)

Calculation

%) NO scavenged= {(control-test)/control) x100

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DPPH radical scavenging activity:

The DPPH assay was performed as described (Koleva et al., 2002). The absorbance was measured at 517 nm and percentage inhibition was calculated

In vitro alpha glucosidase inhibitory activity:

In vitro alpha-glucosidase inhibitory was evaluated by Li et al.,2004. The liberated glucose was measured by GOD-POD method at 546 nm using semi auto analyzer. The inhibitory activity of the extract was calculated as follows

% INHIBITIBITION= {(control-test)/control0} x100

RESULT

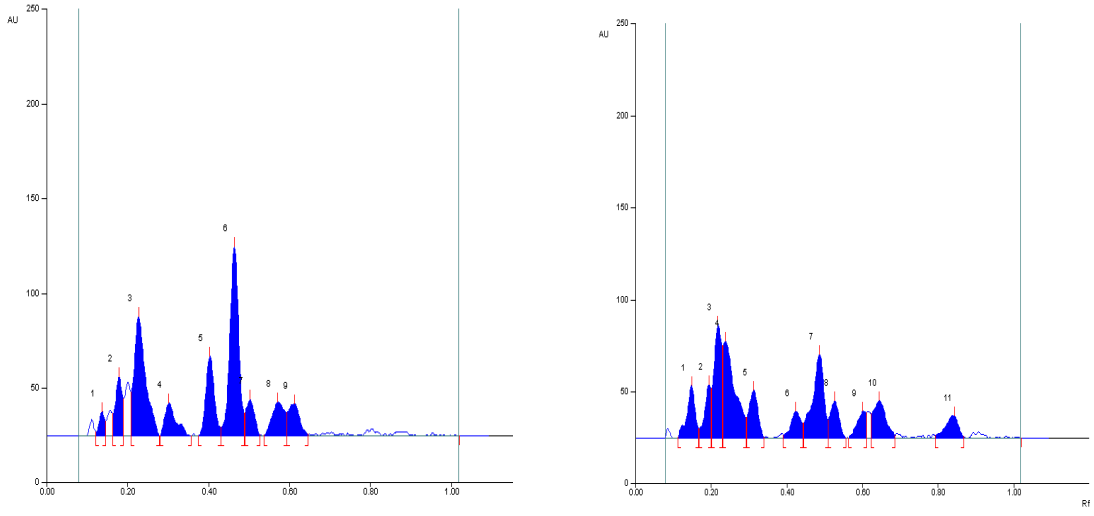
HPTLC profile

The HPTLC finger printing of methanolic extract with green tulsi gave 7 peaks the end Rf is 0.67 whereas the peak was at 8 for purple tulsi, the end Rf was 0.65. For standard the end Rf was 0.67.HPTLC was an invaluable quality assessment tool for the evaluation of botanical materials. It allows for the analysis of a broad number of compounds both efficiently and cost effectively (Table 1 and figure1).

Table 1. Preliminary Qualitative Analysis of compounds present in green and purple tulsi

Chemical test	Green Tulsi	Purple Tulsi
Phenolic compounds	+++	+++
Reducing sugars	++	++
Flavones	+++	+++
Glycosides	+	+
Saponins	+	+
Alkaloids	++	++
Anthroquinones	-	-
Quinones	++	++
Proteins	+	++
Tannins	++	++
Triterpenoids	++	++

+:Presence (+ mild,++ moderate,+++ high),-Absent



Green tulsi **purple tulsi**

Figure 1. HPTLC fingerprint showing the various compounds present in green and purple tulsi

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Bioactivity study - Anti oxidant studies

Lipid per oxidation inhibition assay

The ethanolic extract was subjected to evaluation for anti oxidant activity of lipid peroxidation by the estimation of thiobarbituric acid reactive substances (TBARS) levels by measuring the absorbance at 532 nm (Ohkawa et al, 1979) At all concentration green tulsi was found to have high lipid peroxidation inhibition (Table 2, 3 and 4).

Nitric oxide inhibition assay:

It was estimated by gries reagent. Absorbance was read at 546 nm. Percentage scavenging of nitric oxide radical activity was calculated using vitamin and quercitin as reference compound. Table 5 shows the concentration from 1.5 to 1000 mcg/ml. The concentrations from 1.5 to 250 showed higher inhibitions for purple tulsi whereas for the last two concentration (500mcg/ml & 1000 mcg/ml) purple tulsi showed higher concentration.

Table 2. Preliminary Qualitative Analysis of Primary metabolites Present in green and purple tulsi

Test	Green Tulsi	Purple Tulsi
	% w/w	
Carbohydrates	37.83 ± 2.14	46.48 ± 3.45
Proteins	9.98±0.12	12.13±0.08
Lipids	6.50 ± 0.02	6.30 ± 0.12

Values are mean ± SEM (n=3)

Table 3. Preliminary Qualitative Analysis of Secondary metabolites Present in green and purple tulsi

TEST	Green Tulsi	Purple Tulsi
	%w/w	%w/w
Phenols	7.29±0.01	9.99±0.25
Tannins	3.71±0.08	4.24±0.09
Flavanoids	3.24±0.02	6.14±0.02
Vitamin E	37.01±2.14	28.40±1.12
Vitamin C	24.92±.12	14.31±0.09

Values are mean ± SEM (n=3)

Table 4. Preliminary Qualitative Analysis of Bioactivity compounds Present in green and purple tulsi

Concentration (mcg/ml)	%Inhibition			
	Green tulsi	Purple tulsi	VIT E	Quercitin
1.5	11.83 ±0.11	4.60 ±0.29	43.17±0.03	57.50±0.01
3	17.17±0.37	17.02±0.46	63.37±0.05	70.64±0.02
7	35.95±0.40	21.24±0.01	50.61±0.06	71.68±0.05
15	52.82±0.24	31.52±0.01	64.50±0.02	73.54±0.30
30	53.98±1.07	43.54±0.03	71.77±0.01	74.15±0.05
62	69.82±0.17	45.92±0.38	74.17±0.06	80.02±0.04
125	59.17±0.55	57.80±0.35	79.16±0.07	87.94±0.01
250	62.40±0.16	56.03±0.12	84.34±0.03	88.09±0.03
500	69.06±0.27	65.99±0.14	84.47±0.056	89.98±0.02
1000	80.81±0.40	71.89±0.38	89.16±0.02	89.87±.01

Values are mean ± SEM (n=3)

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Table 5. Analysis of Nitric oxide inhibition Assay in green and purple tulsi

Concentration (mcg/ml)	%Inhibition			
	Green tulsi	Purple tulsi	VIT E	Quercitin
1.5	2.77±0.01	8.14±0.13	90.14±0.04	91.20±0.03
3	5.64±0.01	9.38±0.14	91.38±0.03	96.14±0.05
7	6.74±0.01	12.17±0.09	92.56±0.05	94.71±0.10
15	8.37±0.01	16.49±0.08	84.46±0.01	93.73±0.17
30	12.80±0.02	22.89±0.43	83.63±0.01	92.77±0.11
62	14.16±0.16	24.78±0.59	83.23±0.01	93.00±0.07
125	24.90±0.21	36.34±0.12	61.17±0.22	92.46±0.04
250	60.26±0.48	68.77±0.06	40.77±0.07	86.31±0.07
500	64.26±0.52	55.82±0.42	27.80±0.05	82.72±0.09
1000	71.51±0.96	41.77±0.17	4.64±0.73	81.67±0.09

Values are mean ± SEM (n=3)

DPPH radical scavenging activity:

DPPH is an easy, rapid and sensitive method for the antioxidant screening of plant extracts. The present study investigated the scavenging activity of ethanol extract of green and purple tulsi and expressed in percentage of inhibition of DPPH free radicals using using vitamin and quercitin as standard reference compound. Scavenging activity was observed from 1.5 to 1000 mcg/ml. From table 6, at all concentrations both green and purple tulsi shows almost same DPPH scavenging inhibition.

Antidiabetic study:

It was valuated by Lie et al 2004. From table 7 concentrations from 1.5 to 30 mcg/ml showed maximum inhibition for green tulsi whereas concentrations from 62 to 1000mcg/ml showed maximum inhibition for purple tulsi.

Apha glucosidase inhibitory assay:

Liberated glucose was measured by (GOD-POD) method at 546 nm using semi auto analyzer. From fig 7 showed that there was increase and gradual decrease of alpha glucosidase inhibition in green tulsi when compared to that of purple tulsi (Table 8)

Table 6. Analysis of DPPH Scavenging Assay in green and purple tulsi

Concentration (mcg/ 10 µl)	Green tulsi	Purple tulsi	VIT E	Quercitin
	%Inhibition			
1.5	85.78±0.76	83.75 ±0.14	6.82±1.54	25.45±0.01
3	85.32±0.76	84.80±0.07	7.61±3.84	58.73±1.38
7	85.25±1.45	86.85±0.01	5.75±1.23	81.63±0.15
15	87.48±0.82	88.30±0.42	10.81±0.15	86.16±1.84
30	90.18±1.97	88.35±0.70	12.14±1.54	86.95±0.15
62	91.31±0.88	90.31±0.007	15.58±0.11	82.96±0.31
125	90.25±0.37	91.12±0.02	17.86±0.08	96.81±1.23
250	89.92±0.06	90.78±0.14	36.10±1.23	89.88±0.61
500	90.72±0.71	93.72±0.02	68.05±0.31	63.53±0.46
1000	91.37±0.06	94.63±0.04	90.15±0.15	46.75±1.54

Values are mean ± SEM (n=3)

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Table 7. Analysis of antidiabetic inhibition in green and purple tulsi

Concentration (mcg/ml)	Green tulsi	Purple tulsi
	%Inhibition	
1.5	44.49±0.14	37.68±2.75
3	66.52±1.30	47.54±1.88
7	74.93±2.75	51.30±1.45
15	78.81±0.58	52.75±2.75
30	86.81±0.87	61.16±3.10
62	75.51±0.14	95.07±2.90
125	66.23±0.72	81.30±1.59
250	60.14±0.43	68.684±0.72
500	53.62±0.87	62.61±2.03
1000	52.90±2.30	55.65±0.87

Table 8. Analysis of Alpha Glucosidase inhibitory assay in green and purple tulsi

Concentration (mcg/ml)	GREEN TULSI	PURPLE TULSI
	%Inhibition	
1.5	28.03 ± 3.02	13.51±1.07
3	36.92±2.27	26.78±1.09
7	39.83±0.53	28.13±1.09
15	38.28±2.34	30.01±0.94
30	50.97±0.16	31.49±1.27
62	50.68±0.34	39.18±0.76
125	44.97±0.31	30.25±0.81
250	48.64±2.26	27.70±0.11
500	43.40±0.38	27.37±0.39
1000	40.61±0.34	30.60±0.02

DISCUSSION

In the present study purple and green tulsi was taken for analysis. Test was performed in which both the plants showed similar activity for DPPH scavenging. Green tulsi showed maximum activity for two tests namely , lipid peroxidation test, alpha glucosidase test, whereas purple tulsi gave good results for presence of carbohydrate, protein, phenols, tannin, flavonoids, nitric oxide inhibition test, alpha amylase, and eugenol contents. Tulsi has potent antioxidant properties whereby it neutralizes dangerous biochemicals that contribute to degenerative diseases and premature aging (Deshpande and Tipnis, 1997). The study also showed the beneficial effects of tulsi's antioxidants on blood glucose levels. The TAA compound in the Ocimum accession extracts (Javanmardi et al., 2002), it is rather difficult to characterize every compound and assess or compare their antioxidant activities. Each herb generally contained different phenolic compounds, and each of these compounds possesses differing amounts of antioxidant activity. Medicinal plants are being used by large proportion of Indian population. The reasons for this include a) True improvement of diseases conditions after herbal treatment b) Harmful side effects and high cost of the other forms of treatment. Emerging trends in antioxidant research point to the fact that low levels of phenolics (and other phytochemicals) and low values of antioxidant indices in plants do not translate to poor medicinal properties. Mineral elements, other secondary plant metabolites not detected or evaluated and vitamins contribute to the synergy of phytochemicals that confer medicinal properties on plants.

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The present investigation indicates that a plant of low economic value, it is not worthless. Its use in traditional medicine attests to this. There are prospects for its commercial utilization especially in view of its abundant and widespread nature. The toxic compounds in the plant could be removed through appropriate extraction and processing methods making extracts and products from the plant safe for the utilization of animal and man. Finding ways of profitably utilizing it may be the best option left. Further work is in progress in our laboratory along these lines. So far as plant phenolics constitute one of the major groups of compounds acting as primary antioxidants or free radical terminators, it was reasonable to determine their total amount in the selected plant extracts (Cook and Samman, 1996). The therapeutic potential of natural medicinal plants as an antioxidant in reducing such free radical induced tissue injury, suggests that many plants have antioxidant activities that can be therapeutically useful (Kanatt et al., 2007).

According to our study, the high phenolic content in *O*cimum sp can explain its high free radical scavenging activity. This study reveals that tested plant materials have moderate to significant antioxidant activity and free radical scavenging activity. The result of the present study suggests that selected plants can be used as a source of antioxidants for pharmacological preparations which is which is very well evidenced by the present work. Furthermore, all the plant extracts were found to exhibit a good anti-oxidant activity in the selected in vitro antioxidant assays. Potential activity of all these extracts in the tested antioxidant assays is a promising factor for their application as an effective preservative for the food and cosmetic industries.

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