

Comparison on Phytochemical and Physico-Chemical Parameters of *Ocimum sanctum* Linn Grown in Different Locations of Sri Lanka

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Abstract

Objectives: To compare the phyto-chemical and physico-chemical differences between green leaves monotype (MT₁) and purple leaves monotypes (MT₂) of *Ocimum sanctum* Linn grown in selected provinces of Sri Lanka. **Methods:** Essential oil was extracted by hydro-distillation using Clevenger type apparatus and analyzed by Gas Chromatography technique. Physico-chemical investigations were done according to WHO guidelines. **Results:** Highest amount of essential oil content was present for both MT₁ and MT₂ of *O. sanctum* collected from Southern Province. Moreover, methyl eugenol was present in *O. sanctum* in a range of 0.2 – 66% and compared to the MT₁ methyl eugenol content was higher in MT₂ of *O. sanctum* collected from all selected provinces. The highest percentage of eugenol was contained in MT₂ of *O. sanctum* and there is no significant dependency of the location. Further, it is very interesting to note that percentages of germacrene- D and β-elemene were both higher in MT₂ of *O. sanctum* in WP and SP respectively. However, there was no significant difference in terms of total ash or water soluble ash or acid insoluble ash between MT₁ and MT₂ collected from four provinces of Sri Lanka. **Conclusion:** Overall results revealed that MT₂ of *O. sanctum* collected from SP is more versatile in terms of oil content and chemical constituents.

Keywords: Methyl Eugenol, *Ocimum sanctum* Linn, Physico-chemical parameters, Sri Lanka.

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INTRODUCTION

Ocimum sanctum Linn (Family: Lamiaceae) is commonly known as Holy Basil or Tulsi and used not only in Ayurveda and Siddha medicines but also in Greek, Roman and Unani systems of medicine. In traditional system of medicine, different parts including leaves, stem, flower, root, seeds and even whole plant of *O. sanctum* have been recommended for the treatment of bronchitis, malaria, diarrhea, dysentery, skin disease, arthritis, eye diseases and insect bites [1, 2]. *O. sanctum* is grown in Asian countries including India, Pakistan, Bangladesh and Sri Lanka. It is a shrub which grows to a height of 50 -60 cm and has typical aromatic smell. It has simple opposite green or purple leaves. Leaves are elliptic –oblong, obtuse or acute, entire or serrate, pubescent on both sides, minutely gland – dotted, base obtuse or acute. The Tulsi flowers are small having purple to reddish color, present in small compact clusters on cylindrical spikes [3-5]. As home remedies, a paste of *O. sanctum* leaves is used to reduce pain and inflammation, heal skin rashes, ring worm affected areas and insect bites, get rid of acne and

pimple. Fresh *O. sanctum* juice is mixed with ginger and honey, help to reduce cough and cold. Regular consumption of *O. sanctum* leaves help to control diabetes and blood cholesterol [6]. Taxonomy of *O. sanctum* given below:

Kingdom	: Plantae
Sub kingdom	: Tracheobionta
Superdivision	: Spermatophyta
Division	: Magnoliophyta
Class	: Magnoliopsida
Subclass	: Asteridae
Order	: Lamiales
Family	: Lamiaceae
Genus	: <i>Ocimum</i>
Species	: <i>O. sanctum</i>

O. sanctum is rich in phytochemicals such as oleanolic acid, ursolic acid, rosmarinic acid, eugenol, carvacrol, linalool, β-caryophyllene (about 8%). The essential oil of *O. sanctum* leaves consists mostly of eugenol (~70%) β-elemene (~11.0%), β-caryophyllene (~8%) and germacrene (~2%) [6, 7]. Scientific investigations have shown antioxidant,

immunomodulatory, antipyretic, anticancer, chemopreventive, radiopreventive, antihypertensive, antidiabetic, hepatoprotective, memory enhancing, antioxidant, analgesic, anti-inflammatory and antimicrobial activities [6, 8, 9].

It is available as a weedy plant in Sri Lanka and locally known as Heenmadurutalā or Madurutalā. A few researches have been carried out to investigate the properties of *O. sanctum* grown in Sri Lanka. Secondary metabolites [10] and essential oil composition [11] of *O. sanctum* were investigated using *O. sanctum* grown in Sri Lanka. However, two monotypes of *O. sanctum* (Plate-1) are present in the country: plants with green leaves monotypes (MT₁) and purple leaves monotypes (MT₂). It is well known that types and amounts of secondary metabolites of plants depend on their environmental conditions. Therefore, an attempt was made to compare the phyto-chemical and physico-chemical differences between MT₁ and MT₂ of *O. sanctum* grown in selected provinces [Western province (WP), North Central province (NCP) Southern province (SP) and North province (NP)] of Sri Lanka.

MATERIALS AND METHODS

Plant Materials

O. sanctum plants (both MT₁: with green leaves and MT₂: with purple leaves) were collected from WP (6.9016° N, 80.0088° E), NCP (8.1996° N, 80.6327° E), SP (6.2374° N, 80.5438° E) and NP (8.8855° N, 80.2767° E) of Sri Lanka at flowering stage. The plants were identified and authenticated by the Curator of National Herbarium, Royal Botanical Gardens, Peradeniya, Sri Lanka. Voucher specimens of each type of *O. sanctum* were deposited in the Institute of Indigenous Medicine, University of Colombo, Rajagiriya, Sri Lanka. The leaves of both MT₁ and MT₂ were separated, washed, shade dried, cut into small pieces and kept in air tight containers separately labeled as WP, NCP, SP and NP until used.

Extraction of Essential Oil

O. sanctum leaves (500 g) of each MT₁ and MT₂ were added to a round bottom, fixed with a Clevenger-type apparatus and essential oil was obtained separately by hydro distillation. The extracted volatile oil was dried over anhydrous sodium sulphate and stored in sealed vials at 4 °C until analysis. The yield of the oil was calculated based on dry weight of plant materials.

Analysis of Essential Oil by Gas Chromatography

The essential oil was analyzed by using Shimadzu 2010 gas chromatograph equipped with FID using capillary column Rtx –wax, gas: Argon (1ml/min), Temperature program (60 °C -225 °C at 5 °C/min), Injector temperature (230 °C) and Detector temperature (240 °C).

Determination of Physico-Chemical Parameters

Physico – chemical parameters such as total ash content, acid insoluble and water soluble ash contents were determined for MT₁ and MT₂ of *O. sanctum* leaves collected from WP, NCP, SP and NP according to methods described below [12].

Total ash

Crucibles (n =3) were cleaned, kept in the oven at 105 °C for 1 h and measured the empty crucible weights. Then, 2 g of accurately weighed sample was placed in a crucible and ignited. Then the sample with the crucible was kept in the muffle furnace (at 500 – 600 °C) until the sample turn into white color. Then it was cooled in the desiccator for 30 min and weighed without delay.

Water soluble ash

Previously ignited crucible with ash was boiled for 5 min after adding 5 ml of distilled water and filtered by using a Whatman ashless filter paper. Same procedure was repeated thrice. Then the ashless filter paper containing water insoluble matter was ignited in the same crucible and kept in the muffle furnace at temperature of 450 °C for 15 min. Then the residue was allowed to cool in a desiccator for 30 min and crucible containing water insoluble ash was weighed without delay.

Acid insoluble ash

Hydrochloric acid (2M, 5 ml) was added to the crucible containing total ash, covered with a watch glass and boiled gently for 5 minutes. The watch glass was rinsed with 5 ml of hot water and added into the crucible. Same procedure was repeated thrice. Acid insoluble matter was collected on a Whatman ashless filter paper and washed with hot water until the filtrate becomes neutral. Then the filter-paper containing acid insoluble matter was transferred to the original crucible and dried on a hotplate and burned in gas cooker and ignited to constant weight at 450 °C in a muffle furnace. Then the residue was allowed to cool in a desiccator for 30 min and weighed without delay.

STATISTICAL ANALYSIS

All data were expressed as Mean ± SEM. All statistical comparison compared through one-way analysis of variance (ANOVA), using Tukey's HSD post hoc test (p ≤ 0.05).

RESULTS AND DISCUSSION

In the present study, phytochemical and physico-chemical comparison was carried out in between two monotypes (MT₁ and MT₂) of *O. sanctum* collected from four locations [Western province (WP), North Central province (NCP) Southern province (SP) and North province (NP)] from the country at flowering stage. In Sri Lanka, only two monotypes (plants bearing green leaves and purple leaves) of *O. sanctum* were present. However, in India, other than green and purple

monotypes, Rama and Shyama monotypes were found among different clusters across the phytogeographical regions [13].

Highest amount of essential oil content was present for both MT₁ and MT₂ of *O. sanctum* collected from SP (Table-1). However, amount of essential oil content in MT₂ (2.1 ±0.2%) was greater than that of MT₁ (1.5 ±0.2%) of SP. According to Dharmadasa and co-workers [11] percentage of essential oil content in *O. sanctum* aerial parts were in the range of 1.4 – 1.5%. In contrast, volatile oil percentage of *O. sanctum* grown in India was in between 0.7% [14] and volatile oil 0.8% [15]. Essential oil composition of *O. sanctum* is given in Table 2 and in the present study, mainly focused on four prominent volatile compounds (eugenol, methyl eugenol, β- elemene, β-caryophyllene and germacrene-D) which known to present in *O. sanctum*. Methyl eugenol is the major compound in *O. sanctum* [1, 7] and reported to be the most effective naturally occurring male-fruit fly attractant [16, 17]. Overall results revealed that methyl eugenol was present in *O. sanctum* in a range of 0.2 – 66%. Compared to the MT₁ methyl eugenol content was higher in MT₂ of *O. sanctum* collected from WP, NCP, SP and NP. The highest amount of methyl eugenol was present in MT₂ of *O. sanctum* collected from WP and SP. The percentage of β-caryophyllene was in a range of 13.3 – 29.9% in the entire tested *O. sanctum* and there was no

marked difference among the MT₁ and MT₂ or with their location. The highest percentage of eugenol was contained in MT₂ of *O. sanctum* and there is no significant dependency of the location. Further, it is very interesting to note that percentages of germacrene-D and β-elemene were both higher in MT₂ of *O. sanctum* in WP and SP respectively. Therefore, MT₂ collected from SP was rich in methyl eugenol and β-elemene. Similar study was conducted to compare the two monotypes (*O. sanctum* Shyama and *O. sanctum* Rama) of *O. sanctum* grown in India and results revealed that percentages of methyl eugenol and e-caryophyllene were different among the two monotypes [18].

There was no significant difference in terms of total ash or water soluble ash or acid insoluble ash between MT₁ and MT₂ collected from four provinces of Sri Lanka (Table-3). The total ash usually consists of both physiologic ash and non-physiologic ash (eg.carbonates, phosphates, silicates and silica). Acid insoluble ash indicates contamination with silica, for example, earth and sand [19]. Further, we have collected *O. sanctum* leaves during the flowering stage and therefore, maturities of the leaves are in same age. Thus, chemical or physico-chemical variations are due to the geological or environmental factors in four provinces of Sri Lanka.

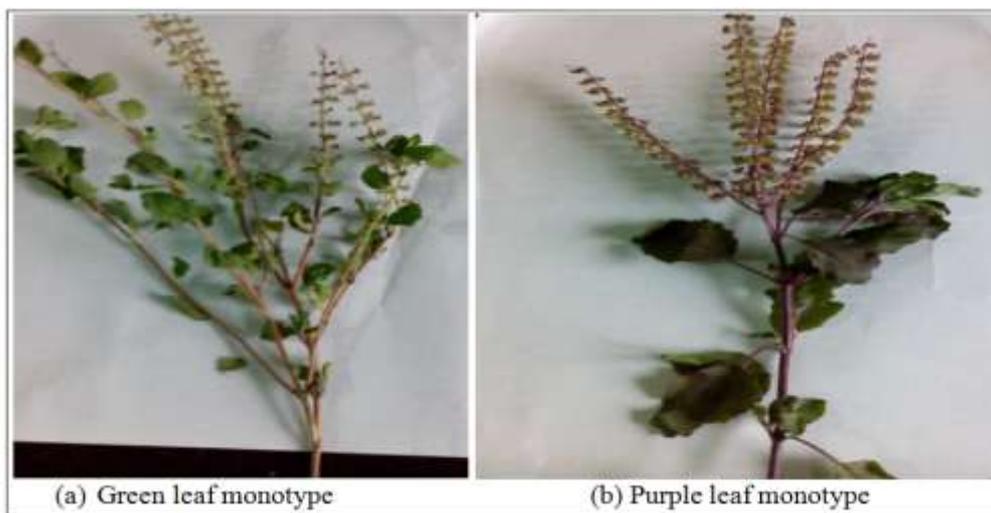


Plate-1: Green and purple leaf monotypes of *Ocimum sanctum*

Table-1: Percentages of volatile oil contents in green leaf variety (MT₁) and purple leaf variety (MT₂) of *Ocimum sanctum* grown in Sri Lanka

Provinces	Volatile oil content (%) of green variety (MT ₁)	Volatile oil content (%) of purple variety (MT ₂)
Western Province	0.7 ±0.2 ^b	1.6 ±0.1 ^b
Southern Province	1.5 ±0.2 ^a	2.1 ±0.2 ^a
North Central Province	1.0 ±0.1 ^b	1.6 ±0.2 ^b
North Province	0.6 ±0.3 ^b	1.4 ±0.2 ^b

Data expressed as Mean ± SEM, n=6

^{a,b} Alphabetical superscripts in a column indicate significant differences between oil content of *Ocimum sanctum* grown in different provinces, p < 0.05

Table-2: Some selected volatile oil percentages of green leaf variety (MT₁) and purple leaf variety (MT₂) of *Ocimum sanctum* grown in Sri Lanka

Specimen	Methyl eugenol %	β -Caryophyllene%	Eugenol%	Germacrene- D%	β -Elemene%
Western Province	5.5 – 9.4 (MT ₁)	22.3 – 28.7 (MT ₁)	1.9 – 6.0 (MT ₁)	8.8 – 9.1 (MT ₁)	0.6 – 8.2 (MT ₁)
	0.2 – 66.0 (MT ₂)	20.5 – 28.5 (MT ₂)	5.2 – 6.8 (MT ₂)	3.8 – 11.6 (MT ₂)	0.6 – 13.0 (MT ₂)
Southern Province	0.8 – 0.5 (MT ₁)	13.3 – 21.6 (MT ₁)	0.6 – 4.6 (MT ₁)	3.3 – 10.2 (MT ₁)	1.9 – 8.8 (MT ₁)
	15.9 – 64.7 (MT ₂)	21.3 – 29.9 (MT ₂)	2.8 – 5.8 (MT ₂)	12.6 – 18.3 (MT ₂)	12.2 – 13.4 (MT ₂)
North Central Province	7.2 – 7.4 (MT ₁)	19.7 – 24.8 (MT ₁)	5.3 – 6.1 (MT ₁)	7.6 – 12.3 (MT ₁)	0.6 – 2.8 (MT ₁)
	0.2 – 51.4 (MT ₂)	23.3 – 25.5 (MT ₂)	6.7 – 6.8 (MT ₂)	10.7 – 11.0 (MT ₂)	1.9 – 2.0 (MT ₂)
North Province	17.2 – 18.0 (MT ₁)	23.9 – 24.0 (MT ₁)	1.9 – 2.2 (MT ₁)	7.9 – 8.2 (MT ₁)	6.5 – 6.7 (MT ₁)
	31.9 – 32.1 (MT ₂)	27.2 – 27.4 (MT ₂)	5.0 – 5.5 (MT ₂)	7.5 – 8.5 (MT ₂)	7.4 – 7.8 (MT ₂)

Data expressed as Mean \pm SEM, n=6**Table-3: Percentages of physico-chemical parameters of green leaf variety (MT₁) and purple leaf variety (MT₂) of *Ocimum sanctum* grown in Sri Lanka**

Provinces	Physico-chemical parameters of green variety (MT ₁)		Physico-chemical parameters of purple variety (MT ₂)	
Western Province	Total ash	11.6 \pm 0.8	Total ash	10.2 \pm 0.5
	Water soluble ash	7.1 \pm 0.8	Water soluble ash	6.6 \pm 0.7
	Acid insoluble ash	\geq 0.01	Acid insoluble ash	\geq 0.01
Southern Province	Total ash	10.5 \pm 0.7	Total ash	9.8 \pm 0.5
	Water soluble ash	7.8 \pm 0.4	Water soluble ash	6.0 \pm 0.7
	Acid insoluble ash	\geq 0.01	Acid insoluble ash	\geq 0.01
North Central Province	Total ash	9.8 \pm 0.9	Total ash	11.6 \pm 0.8
	Water soluble ash	7.6 \pm 0.4	Water soluble ash	7.6 \pm 0.7
	Acid insoluble ash	\geq 0.01	Acid insoluble ash	\geq 0.01
North Province	Total ash	10.1 \pm 0.4	Total ash	10.0 \pm 0.8
	Water soluble ash	6.8 \pm 0.6	Water soluble ash	6.0 \pm 0.5
	Acid insoluble ash	\geq 0.01	Acid insoluble ash	\geq 0.01

Data expressed as Mean \pm SEM, n=6

CONCLUSION

An attempt was taken for the first time, to compare the phyto-chemical and physico-chemical differences between MT₁ and MT₂ of *O. sanctum* grown in four different geological areas of Sri Lanka. Methyl eugenol, the most important phyto-constituent is significantly highest in MT₂ of *O. sanctum* than that of MT₁. Therefore, overall results revealed that MT₂ of *O. sanctum* collected from SP is more versatile in terms of oil content and chemical constituents.

COMPETING INTERESTS DISCLAIMER

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

Authors' Contributions

This work was carried out in collaboration among all authors and equally contributed for the research concept and work plan. Author HRDF conducted the experiments and Author LDAMA and HRDF prepared the manuscript. Author WMSSKK provided relevant literature. All authors read and approved the final manuscript.

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