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Determination of Optimum Maturity Stage for *Ocimum sanctum* L. Grown under Different Growing Systems in Terms of Therapeutically Active Secondary Metabolites

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Abstract *Ocimum sanctum* L. (Lamiaceae) is therapeutically important medicinal plant used in traditional systems of medicine. Present study was undertaken to determine the optimum growth stage in terms of total phenolic content (TPC), total flavonoid content (TFC) and antioxidant capacity (TAC) of leaf and bark extracts of hydroponically and field grown plants of *O. sanctum* in three different maturity stages. Alkaloids, flavonoids, saponins, tannins and steroid glycosides were qualitatively screened through established protocols. TPC, TFC and TAC were determined by colorimetric Folin-Ciocalteu method, aluminum nitrate method and Ferric Reducing Antioxidant Power (FRAP) assay respectively. Leaf and bark extracts of *O. sanctum* grown under both field and hydroponic conditions, at all three maturity stages exhibited the presence of all phytochemicals tested. TPC, TFC and TAC were increased with the maturity. Significantly higher TPC (8.34 ± 0.14 mg GAE /g DW), TFC (132.29 ± 1.45 mg RE /g DW) and TAC (120.02 ± 4.06 mg TE /g DW) were observed in leaf extracts taken from field grown plants at fully maturity stage. The order of increase was just before flowering< just after flowering< fully maturity stage. Presence of higher content of TPC, TFC and TAC in *O. sanctum* leaves at fully maturity stage, scientifically validate the traditional claims of harvesting of *O. sanctum* leaves at fully maturity stage for better therapeutic value. Moreover, presence of all tested phytochemicals in hydroponically grown plants is positive sign of use of hydroponic system as an alternative growing system for *O. sanctum.*

Keywords: *antioxidant capacity, growing systems, Ocimum sanctum, maturity stages, secondary metabolites*

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1. Introduction

Ocimum sanctum L. (Family *Lamiaceae*) is a therapeutically important widely used medicinal plant in traditional systems of medicine for an array of treatments i.e. cold, cough, bronchitis, malaria, stomach disorders, inflammation, heart diseases and various forms of poisoning and as an anti-fertility agent [3,21]. It is known as *Maduruthala* in Sinhala, Holy Basil in English and *Tulsi* in Gujarat. *Ocimum sanctum* is also famous for its religious sanctity, aromatic properties, culinary uses and varied healing properties. Moreover, major chemical constituents in both essential oil and solvent extracts and bioactive compounds such as flavonoids, polyphenols, triterpenes steroids and tannins have been extensively studied by several authors [2,14,18].

Plants have been considered as a rich source of biologically active substances, which are produced due to stress conditions such as water, temperature, radiations and other genetic, environment and agronomic practices [4]. Moreover, therapeutically active substances (Chemical traits) which regulate an important physiological actions on the human body such as polyphenolic compounds, flavonoids, alkaloids, saponins and tannins [8]. Antioxidant, compounds may function as free radical scavengers, complexion agents for pro-oxidant metals, as well as reducing agents and quenchers of singlet oxygen formation [5,9]. Therefore, the importance of the studying for natural anti-oxidants has greatly increased in recent years [12,14]. Hydroponic culture is an alternative culturing system of medicinal and other fruit crops in a small area and has been proven as an alternative system for growing of medicinal plants for their secondary metabolite production [11].

Moreover, study of variation of chemical and bioactive constituents with the maturation process of therapeutically important crops is vital important in order to determine the best harvesting time for optimum therapeutic activity. Even though economically/ physiologically optimal growth stage for physical and chemical maturation of most

of the cultivated crops including *Coriandrum sativum* [13], have been developed, similar studies or information for medicinal plants are scattered due to most of the plants are still harvested from the wild. However, wild harvesting of medicinal plants can be problematic in terms of variation of secondary metabolites, which are mainly responsible for therapeutic properties, and subsequently it will badly effect on quality of drugs produced from wild harvested plant materials generating potential tragic consequences [7]. Therefore, the present study was undertaken to determine the optimum growth stage in terms of therapeutically important secondary metabolites (preliminary phytochemicals, total phenolic and total flavonoid contents) and antioxidant capacity of leaf, bark extracts of hydroponically, and field grown plants of *O. sanctum* in three different maturity stages.

2. Materials and Methods

2.1. Location

The experiment was carried out in the experimental plots and laboratory of the Department of Plantation Management, Wayamba University of Sri Lanka, Makandura, Gonawila (NWP) and laboratory of Herbal Technology Section, Industrial Technology Institute (ITI) Colombo from March to July 2014. The experimental plots were situated in the Low Country Intermediate-Zone (IL_{1a}), at an elevation of 25 m above mean sea level [17].

2.2. Chemical and Reagents

2,4,6-trypyridyl-2-try-azine(TPTZ), 6-hydro xy-2,5,7,8-tetramethyl-chroman-2 carboxilic acid (Trolox), Folin-ciocalteu reagent, Gallic acid, Rutin, L. Ferric chloride ($FeCl_3$ $6H_2O$) were purchased from sigma Aldrich Chemical Co. (St. Louis, Mo). All other chemicals used were of analytical grade.

2.3. Cultivation and Collection of Samples

Six weeks old *Ocimum sanctum* seedlings were planted in University research plots plants at 30 × 30 cm spacing. Watering, weeding and manuring were practiced whenever necessary.

Similarly, *Ocimum sanctum* seedlings were established in hydroponic system.

Leaves and bark samples of *O. sanctum* were collected from field and hydroponically grown plants at three different maturity stages (i.e. just before flowering, just after flowering and fully maturity stage).

2.4. Extraction of Samples

Collected bark and leaf samples were air dried for three days at room temperature (28 ± 2°C). Samples were coarsely powdered using motor and pestle and sieved with 0.25 mm mesh. Powdered samples (0.1g) were accurately weighed into a 15 mL centrifuge tube and add 5 mL of 80% methanol. The sample was vortexed for 15 min and placed in a water bath at 60°C for 40 min and vortex procedure was repeated in 10 min interval. Then samples were centrifuged at 4,000 rpm for 5 min and supernatant was decanted into a 15 mL centrifuge tube and the remaining was re-extracted with 5 mL of 80% methanol. Both supernatants were collected and stored at -20°C prior to analysis.

2.5. Qualitative Screening of Phytochemicals

Methanolic extracts of samples were screened for alkaloids, flavonoids, saponins, steroid glycosides and tannins according to the method described by reference [10].

2.6. Quantification of Total Phenolics

The total phenolic content (TPC) was quantified using a modified Folin-Ciocaltue method [1]. Briefly, 4 mL of distilled water and 0.5 mL of plant extract were added into a test tube. Then 0.5 N Folin Ciocalteu reagent (0.5 mL) was added and allowed to react 3 min. One milliliter of saturated sodium carbonate solution was mixed and samples were incubated in a water bath for 2 hr at 30°C. The absorbance was measured at 760 nm using UV visible spectrophotometer (Shimadzu UV-160). Gallic acid was used as the standard and TPC in one gram of dried plant material was calculated and expressed as milligram of Gallic Acid Equivalent (GAE).

2.7. Quantification of Total Flavonoids

Total flavonoid content (TFC) was determined by a colorimetric method described reference [16] with slight modifications. Briefly, 0.5 mL of the plant extract was diluted with 3.5 mL of distilled water. Then 0.3 mL of a 5% $NaNO_2$ solution was added to the mixture. After 6 minutes, 0.3 mL of a 10% Al $(NO_3)_3$. $6H_2O$ solution was added, and the mixture was allowed to stand for another 6 minutes. Two milliliter of 2 M NaOH was added, and the total was made up to 8 mL with distilled water. The solution was well mixed, and the absorbance was measured immediately at 510 nm using UV visible spectrophotometer (Shimadzu UV-160). Rutin was used as the standard and TFC in one gram of dried plant material was calculated in addition, expressed as mg of Rutin Equivalent (RE).

2.8. Determination of Total Antioxidant Capacity (TAC)

TAC was determined using Ferric Reducing Antioxidant Power (FRAP) assay [6]. Methanolic extract (100 µL) was mixed with 900 µL of freshly prepared FRAP reagent of pH 3.6 containing 2.5 mL of 10 mmol/L, 2,4,6-Tripyridyl-s-Triazine (TPTZ), 2.5 mL of 20 mmol/L $FeCl_3$ and 25 mL of 300 mmol/L acetate buffer. Absorbance of the reaction was measured at 593 nm using the spectro photometer (Shimadzu, UV Mini 1240, Japan) after incubating for 4 min. Trolox was used as the standard and TAC in one gram of dried plant material was calculated and expressed as mg of Trolox Equivalent (TE).

2.9. Statistical Analysis

Statistical comparison of mean values was performed by General Liner Model (GLM) of ANOVA followed by Turkey Multiple Rang Test using Minitab 15 version and presented means ± SD.

3. Results and Discussion

This study revealed that alkaloids, flavonoids, saponins, tanins and steroid glycosides are present in both leaf and bark of *O. sanctum* in all maturity stages (Just before flowering, just after flowering and fully maturity) grown under two different growing system (Table 1).

As demonstrated in Figure 1, leaf and bark extracts of *O. sanctum* obtained from both hydroponically and field grown plants exhibited the presence of marked amounts of total phenolics and total flavonoids and TAC at all three maturity stages. Moreover, TPC, TFC and TAC increased with the maturity for both field grown and hydroponically grown plants and the significantly higher (P=0.05) TPC (8.34 ± 0.14 mg GAE /g DW), TFC (132.29 ± 1.45 mg RE /g DW) and TAC (120.02 ± 4.06 mg TE /g DW) were observed in leaf extracts taken from field grown plants at fully maturity stage. The order of increase was just before flowering < just after flowering < fully matunity stage.

Table 1. Qualitative Phytochemical Parameters of *Ocimum sanctum* Grown under Hydroponic System and Field Oonditions

Growing system	Growth stage	Plant part	Alkaloids	Flavonoids	Saponins	Tannins	Steroid Glycosides
Field grown plants	Just before flowering	Leaf	+	+	+	+	+
		Bark	+	+	+	+	+
	Just after flowering	Leaf	+	+	+	+	+
		Bark	+	+	+	+	+
	Fully maturity	Leaf	+	+	+	+	+
		Bark	+	+	+	+	+
Hydroponically grown plants	Just before flowering	Leaf	+	+	+	+	+
		Bark	+	+	+	+	+
	Just after flowering	Leaf	+	+	+	+	+
		Bark	+	+	+	+	+
	Fully maturity	Leaf	+	+	+	+	+
		Bark	+	+	+	+	+

+ : Presence; - : Absence

Increase of TPC, TFC and TAC with the maturity is in agreement with results of previous workers who reported the variation of TPC, TFC and TAC in three *Rubus* species, Coriandrum sativum and *W. somnifera*. [12,13,19] with the maturity. Moreover, it was reported that the higher content of TAC in leaves than the stem and roots.

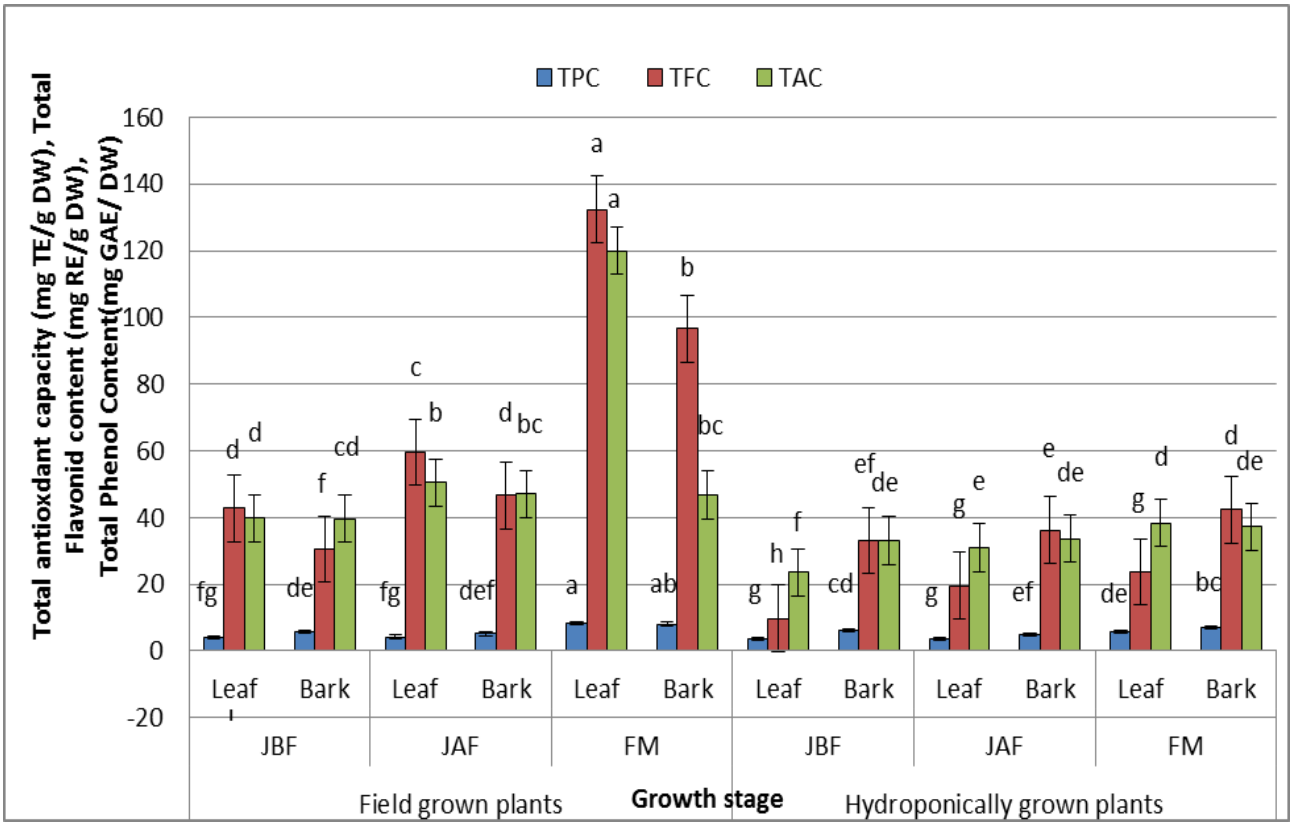

Figure 1. Total phenolic content, total flavonoid content and total antioxidant capacity of *Ocimum sanctum* at different maturity levels under hydroponic and field conditions

GAE = Gallic acid equivalent; TE = Trolox equivalent; RE = Rutin equivalent; DW = Dry weight; JBF = Just before flowering; JAF = Just after flowering; MS = Maturity stage

The higher content of TAC, TPC and TFC contents of both leaf and bark extracts in field grown plants were significantly higher than hydroponically grown plants (Figure 1). Plant secondary metabolites play an important role in relation to biotic (defensive role against herbivore, pest and diseases and attraction of pollinators etc.) and

abiotic stress conditions such as variation of temperature, water, micro and macro nutrition and light intensity [15]. Accordingly, higher content of TPC, TFC and TAC observed in leaf, bark extracts of field grown plants may be due to high stress conditions in field grown plants, and in contrast, lower content of TPC, TFC and TAC in hydroponic culture may be due to less stress conditions. *Moreover*, the bark extracts of hydroponically grown plants exhibited significantly higher TPC, TFC and TAC at all maturity stages when compared to the leaf extracts (Figure 1).

4. Conclusions

Present study demonstrated the distribution of total phenolic, total flavonoids and total antioxidant capacity of leaf and bark extracts of field grown and hydroponically grown *O. sanctum* in 3 different maturity (just before flowering, just after flowering, fully maturity) stages for the first time in Sri Lanka. The order of increase of TPC, TFC and TAC was just before flowering < just after flowering < fully maturity for both field grown and hydroponically grown plants. Presence of higher content of TPC, TAC and TFC of fully maturity stage, clearly validate the traditional claims of harvesting of *O. sanctum* at fully maturity stage. Moreover, presence of marked total phenolics, total flavonoids, and TAC in leaves at fully maturity stage also scientifically validates the use of leaves of *O. sanctum* at fully maturity. Presence of all tested phytochemicals in hydroponically grown plants is positive sign of use of hydroponic system as an alternative growing system for *O. sanctum.*

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Statement of Competing Interests

No competing interests.

List of Abbreviations

TPC: total phenolic content ;TFC total flavonoid content ;TAC :antioxidant capacity; FRAP :Ferric Reducing Antioxidant Power; NWP: North western Province; ITI: Industrial technology institute; IL: Low Country Intermediate-Zone; GAE: Gallic acid equivalent; TE: Trolox equivalent; RE : Rutin equivalent; DW : Dry weight

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