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Determination of Suitable Agro Climatic Region and Optimum Harvesting Stage by Means of Total Phenolic Content, Total Flavonoid Content and Total Antioxidant Capacity of *Ocimum tenuiflorum* L. (Lamiaceae) Grown in Sri Lanka

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Abstract *Ocimum tenuiflorum* L. (Lamiaceae) is a industrial important medicinal plant,cultivated for its therapeutic values in many Asian countries. Even though the therapeutic properties of *O. tenuiflorum* is mainly depend on its secondary metabolites, presently required plant materials for Ayurveda and traditional systems of medicine are collected from the wild without considering its age and therapeutic properties. Therefore, the present study is undertaken tp compare the important phytochemicals (total .phenolic content (TPC), total flavonoid content (TFC)) and total antioxidant capacity (TAC), of different parts of *Ocimum tenuiflorum*.which were harvested at three consecutive pruning stages from two agro climatic regions in Sri Lanka. TPC, TFC and TAC were performed using colorimetric Folin-Ciocalteu method, aluminum nitrate method and Ferric Reducing Antioxidant Power (FRAP) assay respectively. Data were analyzed using the general linear model (GLM) procedure of Minitab statistical package followed by Duncan's Multiple Range Test (DMRT) for mean separation. Total antioxidant capacity of leaf, bark, flower and seed extracts were significantly (p=0.05) increased from first pruning to third pruning irrespective of agro climatic regions. Antioxidant capacity and Total flavonoid content of different parts of *O. tenuiflorum* cultivated in low country intermediate-Zone (IL_{1a}) and low country dry zone (DL_{1b}) was varied as leaf> bark> flower> seeds. Even though the TPC was significantly varied among different parts as well as pruning stages, remarkable relationship was observed neither agro climatic region nor pruning intervals. Results of the present study are vital important for the determination of suitable agroclimatic region, optimum harvesting stage for higher phytochemical, bioactivity and optimum therapeutic properties of *O. tenuiflorum*.

Keywords: *Ocimum tenuiflorum, Lamiaceae, total antioxidant capacity, total phenoloic content, total flavonoid content*

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1. Introduction

Ocimum tenuiflorum L. (Lamiaceae) is a one of the therapeutically important medicinal plants extensively used in traditional systems of medicine, religious purposes and spiritual sanctity in many Asian countries since historic times. Moreover, *O. tenuiflorum* has been widely used for the treatment of cold, cough, bronchitis, malaria, stomach disorders, inflammation, heart diseases and various forms of poisoning and as an anti-fertility agent [1,2]. It is well-known fact that plant secondary metabolites such as phenols, flavonoids and the antioxidant capacity of plants are considered as essential bioactive compounds which play an important role in therapeutic properties of a plant/ plant material/ herbal drugs. Moreover, these secondary metabolites are influenced by maturity, season, geographical distribution, climatic variations and system of cultivation of a plant [3,4,5]. Even though therapeutic properties of *O. tenuiflorum* mainly depend on its secondary metabolites,

presently required plant materials for Ayurveda and traditional systems of medicine are collected from the wild without considering its therapeutic properties. Furthermore, information on variation of antioxidant capacity, phenolic and flavonoid content in different parts of *O. tenuiflorum* grown in different agro ecological regions is scattered or have not been thoroughly investigated. Therefore, there is an urgent necessity of development of agro technology for *O. tenuiflorum* by means of its phytochemical, antioxidant capacity, and total flavonoid content which are mainly responsible for the therapeutic properties. Present study was undertaken tp compare the important phytochemicals (total .phenolic content (TPC), total flavonoid content (TFC)) and total antioxidant capacity (TAC), of different parts of *Ocimum tenuiflorum*.which were harvested at three consecutive pruning stages from two agro climatic regions (low country intermediate (IL1a) and Low country dry zones (DL1b) in Sri Lanka

2. Materials and methods

2.1. Location and Plant Materials

The experiment was carried out in experimental plots maintained at research farm Wayamba University and Field Crop Research and Development Institute, Mahailluppallama, Sri Lanka. Climatic data of both locations are as follows;

Table 1. Agro climatic data of two locations

Parameter	Location 1 (IL1a)	Location 2 (DL1b)
Longitude & Latitude	Lat: 7° 22' N; Long: 80° 02' E	Lat. 8^0 07' N; Long. 80^0 28' E
Elevation	25 m above mean sea level	117m above mean sea level
Agro ecological zone	Low Country Intermediate-Zone (IL_{1a})	Low country Dry zone (DL_{1b})
Soil type	Lateritic sub soil	Reddish brown earth
Soil pH	4.97-6.5	6.5 - 7.3
Light duration	8.0	8.1
Temperature Min. and max.	24.2 – 30.5 ºC-	23.5-32.6 ºC
Wind velocity	3.6 Km/h	7.5 Km/h
Humidity	86.5%	81.43%
Annual rainfall	1100-2400 mm/year	650-1100 mm/year

2.2. Chemicals and Reagents

Chemicals of 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, Ferric chloride ($FeCl_3$ $6H_2O$) were purchased from Sigma Aldrich Chemical Co. (St. Louis, Mo). Analytical grade methanol was used throughout the experiment.

2.3. Plant Materials

Plant materials required for determination of TAC, TPC, and TFC were harvested from the same aged, plants cultivated and maintained separately at two agro ecological regions namely low country intermediate zone (IL1a) and low country dry zone (DL1b) in Sri Lanka. Herbarium specimen was prepared and deposited in Institutional Herbarium (ITI/HTS 27). Sample collection was started after 3 months of field establishment and continued up to 6 months. The materials were harvested at the same time of the day. Collected materials were separated into leaf, bark, flowers and seeds. Then they were air dried for three days at room temperature (28 ± 2°C) and coarsely powdered using motor and pestle and sieved with 0.25 mm mesh. Sieved materials were separately packed and labeled.

2.4. Extraction

Powdered sample (0.1g) of each was accurately weighed into a well cleaned, dried tube and 5 mL of 80% methanol was added. 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.

2.5. Determination of Total Phenol Content (TPC)

The total phenolic content (TPC) was determined using modified Folin-Ciocaltue method. Briefly, 0.5 mL of plant extract was mixed with 4 mL of distilled water and 0.5 N Folin Ciocalteu reagents (0.5 mL) was added and allowed to react for 3 min. then 1 mL of saturated sodium carbonate solution was mixed and samples were incubated in a water bath for 2 h 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).

2.6. Determination of Total Flavonoid Content (TFC)

Total flavonoid content (TFC) was determined previously published methodology as described by Liu et al., (2002) with slight modifications. Briefly, 0.5 mL of the plant extract was diluted with 3.5 mL of distilled water and 0.3 mL of 5% $NaNO_2$ solution was added to the mixture. Mixture was incubated for 6 minutes and then 0.3 mL of a 10% Al (NO3)3. 6H2O solution was added. After incubation for 6 minutes 2 mL 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 and presented as mg of Rutin Equivalent (RE) per gram of sample.

2.7. Determination of Total Antioxidant Capacity (TAC)

Total antioxidant capacity was determined using Ferric Reducing Antioxidant Power (FRAP) assay as described by Benzie and Strain, (1996) with some modification. Methanolic extract (100 µL) of extract was mixed with 900 µL of freshly prepared FRAP reagent (pH 3.6 containing 2.5 mL of 10 mmol/L, 2,4,6-Tripyridyl-s-Triazine (TPTZ) solution in 40 mmol/L, HCl plus 2.5 mL of 20 mmol/L FeCl3 and 25 mL of 300 mol/l acetate buffer). Absorbance of the reaction was measured at 593 nm using the spectrophotometer (Shimadzu, UV Mini 1240, Japan) after incubating for 4 minutes. The Trolox was used as the standard solution. Antioxidant capacity was calculated as one gram of dried plant material and expressed as mg of Trolox Equivalent (TE).

2.8. Data Analysis

Field experiments were conducted in complete randomized block design (CRBD) while laboratory experiments were conducted in complete randomized design (CRD). Data on total phenolic content, total phenol content and total antioxidant capacity were analyzed using the general linear model (GLM) procedure of Minitab statistical package followed by Duncan's Multiple Range Test (DMRT) for mean separation. All results were expressed as mean value of at least 5replicated ± Standard Deviation.

3. Results and Discussion

In the present study attempts were made to investigate the total phenolic content (TPC), total flavonoid content (TFC) and total antioxidant capacity (TAC) of widely used parts (leaf, bark, flower and seeds) of 3 different maturity (pruning) stages [First pruning after 3 months after planting, Second pruning after 4 months of planting; and third pruning after 5 months after planting)] of *Ocimum tenuiflorum* grown in two different agro ecological regions (low country intermediate-zone (IL_{1a}) and low country dry zone (DL_{1b}) in Sri Lanka. All analytical procedures used in the present study were adopted from well accepted, published protocols. A varying level of antioxidant capacity was observed in different part of the plant maintained at both agro climatic regions. As demonstrated in Figure 1, total antioxidant capacity of leaf, bark, flower and seed extracts were significantly (p=0.05) increased from first pruning to third pruning irrespective of agro climatic regions.

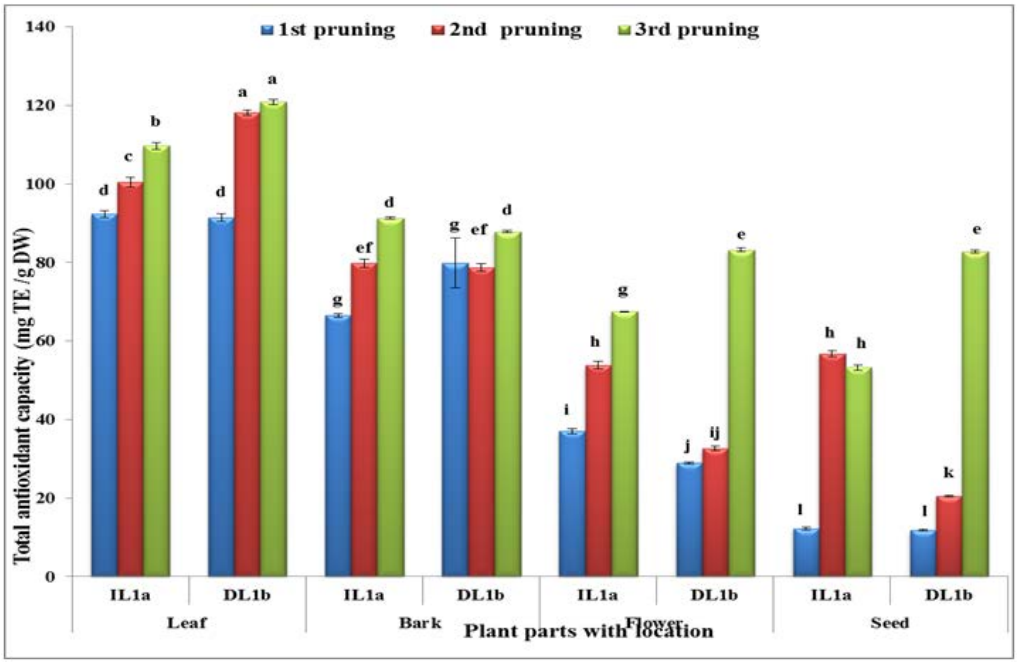

Figure 1. Variation of total antioxidant capacity of different parts of *Ocimum tenuiflorum* L. in 3 pruning cycles grown under two climatic zones [IL1a- low country intermediate zone, DL1b- low country dry zone; Bars denoted by different letters are significantly different at 5% significant level.]

Out of tested plant parts, the highest antioxidant capacity was exhibited from leaf extracts. The total antioxidant capacity of leaf extracts for all three pruning stages were ranged from 92.37±0.86 to 109.69±0.86 (mg TE /g DW) for IL1a while it was varied from 91.48±0.99 to 120.83±0.65 (mg TE /g DW) for DL1b. Comparatively higher or comparable antioxidant capacity was observed in all plant parts obtained from DL1b over the IL1a. Moreover, leaf extract demonstrated the highest antioxidant capacity. Antioxidant potent of different parts of the plant was varied as leaf> bark> flower> seeds. Observed comparatively higher TAC of all plant parts collected from DL1b in might be due to prevailing harsh environment conditions (comparatively high temperature, low rainfall and other conditions) prevailing in DL1b.

As demonstrated in Figure 2, the highest total flavonoid content was observed in leaf extract in both agro climatic regions. Total flavonoid content of leaf extracts of DL_{1b}

was ranged from 110.47±0.44 -146.36±0.25 mg RE /g DW while it was ranged from 110.74±1.99 -112.74 ±0.10 mg RE /g DW in leaf extracts obtained from IL_{1a} in all three pruning stages. Moreover, significantly higher total flavonoid contents of leaf, flower and seeds were observed in third pruning stage irrespective of their agro climatic conditions. Variation of TFC of different parts were as leaf> bark>flower > seeds. However, increased TFC was observed in seed extract collected from $DL_{1b.}$ Moreover, comparatively higher TFC was observed in DL_{1b} compared to IL_{1a} (Figure 2).

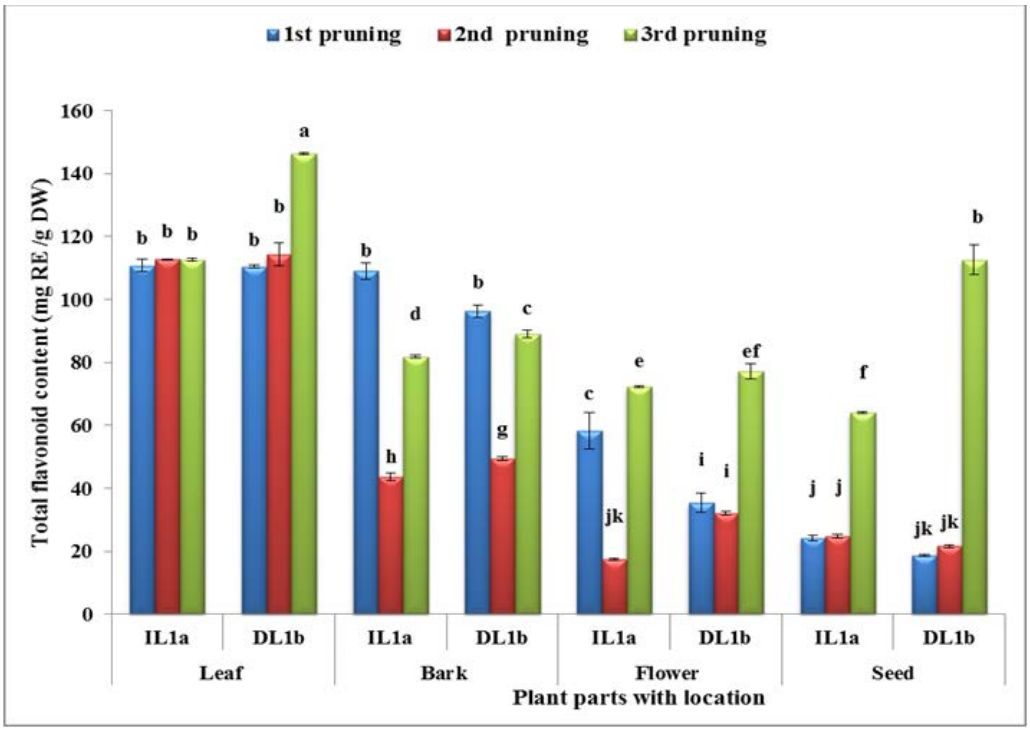

Figure 2. Variation of total flavonoid content of different parts of *Ocimum tenuiflorum* L. in 3 pruning cycles grown under two climatic zones [IL1a- low country intermediate zone, DL1b- low country dry zone; Bars with different letters are significantly different at 5% significant level.]

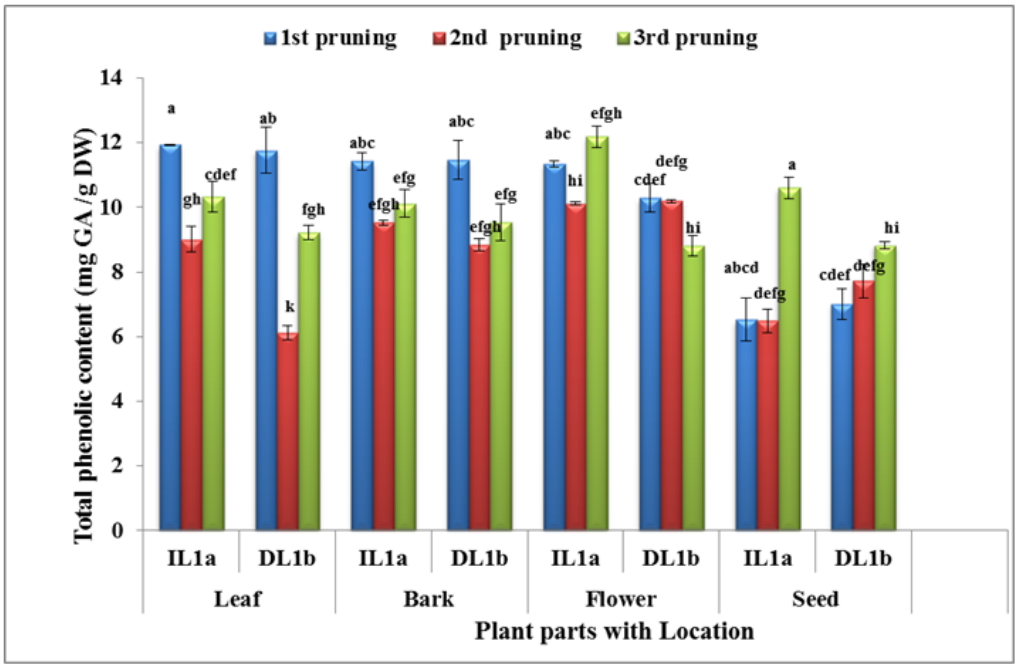

Figure 3. Variation of total phenolic content of different parts of *Ocimum tenuiflorum* L. in 3 pruning cycles grown under two climatic zones [IL1a- low country intermediate zone, DL1b- low country dry zone; Bars with different letters are significantly different at 5% significant level.]

As shown in Figure 3, significantly (p=0.05) higher total phenolic content was reported in leaf extracts followed by bark, flower and seeds irrespective of climatic regions. Even though the TPC was significantly varied among different parts as well as pruning stages, remarkable relationship was observed neither agro climatic region nor pruning intervals. However, TPC, TAC, TFC of seed extracts exhibited continuous increase from first pruning to third pruning irrespective of agro climatic region (Figure 1, Figure 2, Figure 3).

Results of the present study, clearly demonstrate the distribution of total antioxidant capacity (TAC), total

phenol content (TPC) and total flavonoid content (TFC) of leaf, bark, flower and seeds of *O. tenuiflorum* grown in two different agro climatic regions of Sri Lanka.

Even though the secondary metabolites present in the plants are not of paramount significance for plant life, they are greatly important to adopt changing environmental conditions and overcome the stress constraints [8]. Moreover, content and composition of secondary metabolites are greatly depending on soil, climatic, growing season and maturity stages of a plant [9]. Generally, secondary metabolites such as TAC, TPC and TFC, which are mainly responsible for the defense mechanism and help to overcome stress conditions of plants [8]. In the present study, we observed comparatively higher content of TAC and TFC in DL_{1b} might be due to prevailing harsh environment conditions (comparatively high temperature, low rainfall and other conditions). Variation of secondary metabolites with the location, climatic conditions and cultivars for Rasberry cultivars [10], *Cleome chelidonii* L.[11] have been investigated and found that the secondary metabolite contents are varied with the environmental factors. Moreover, previous studies indicated a significant differences in polyphenolic concentrations, in apple fruits grown in different regions in New Zealand [12]. Further, the observed higher content of TAC and TFC in leaf extracts are in agreement with previous studies which investigated the higher content of TAC in leaf extracts of *Withania somnifera* [13], *Ocimum sanctum* [3], *Munronia pinnata* [14]. Absence of remarkable pattern in total polyphenolic content in all parts of *Ocimum tenuiflorum* cultivated in both agro climatic regions are in agreement with previous studies which pointed out that the total phenolic content did not exhibit marked changed with the maturity of black berry fruits [15].

4. Conclusion

Present study compared the variation of TPC, TAC and TFC of leaf, bark, flower and seed extracts in three continuous pruning stages of *Ocimum tenuiflorum* cultivated in two different agroclimatic regions for the first time in Sri Lanka. The highest TAC and TFC were observed in leaf extracts irrespective of their agro climatic regions and pruning stages. Antioxidant potent and total flavonoid content were varied as leaf> bark>flower > seeds. Comparatively higher TAC was observed in $DL1_b$. Information generated through the present study are vital important for the determination of suitable agroclimatic region, optimum harvesting stage, suitable plant parts and for higher phytochemical, bioactivity and optimum therapeutic properties of *O. tenuiflorum*.

Conflict of Interest

Authors declare that there is no conflict of interest.

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