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Phytochemical Distribution and Bioactivity of Different Parts and Leaf Positions of *Pimenta Dioica* (L.) Merr (Myrtaceae)

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Abstract *Pimenta dioica* (L.) Merr. (Myrtaceae) is an evergreen aromatic spice widely used in perfumery, food and cosmetic industry in many parts of the world. Present study compared Total Antioxidant Capacity (TAC), Total Phenolic Content (TPC), Total Flavonoid Content (TFC), leaf area (LA), and Fresh to Dry weight ratio of *Pimenta dioica* leaves at different leaf positions (1st, 2nd, 3rd, 4th and 5th leaf positions) and different plant parts (immature leaf, mature leaf and bark). The TAC, TPC and TFC were determined using Ferric Reducing Antioxidant Power Assay (FRAP), modified Folin–Ciocalteu colorimetric method and calorimetric method respectively. Significantly higher TAC 562.38 ± 9.42 (mg TE/g DW), TPC 279.53±7.02 (mg GAE/g DW) were observed in leaf extract obtained from 1st leaf position. However the highest TFC 303.48 ±8.87 (mg RE/g DW) was observed in 5th leaf position. According to phytochemical distribution pattern, significantly higher TAC [619.84 ±11.98 (mg TE/g DW)], TPC [267.53 ± 5.03(mg GAE/g DW)], TFC [305.48 ±8.87 (mg RE/g DW)] were observed in extracts obtained from bud region. The potential of *Pimenta dioica* leaf material and bark as a fabulous raw material for food, perfumery and cosmetic industries. Further harvesting of immature leaves could be suggested for better therapeutic benefits.

Keywords: *Pimenta dioica, Total Antioxidant Capacity, Total Flavonoid Content, Total Phenolic Content*

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1. Introduction

Pimenta dioica (L.) Merr. (Myrtaceae) is well known for its characteristic aroma, therapeutic and culinary qualities which resembles to aroma and flavor of clove, nutmeg cinnamon and hence it is called allspice [1]. Plant is known as, Jamaica pepper, Myrtle pepper and new spice in English [2]. Further, *P. dioica* is small (7-10 m) evergreen tree which is cultivated in Jamaica, Cuba, Haiti, Brazil, Central America, West Indies, Venezuela, Mexico, Honduras, Guatemala and Grenada [3] . Leaves and seeds are widely used as commercial items in traditional medicine for the treatment of diverse range of ailments including flatulence, diarrhea, neuralgia, rheumatism and digestive problems [4]. Moreover, it is valued for its secondary metabolites like essential oil with compositions, oleoresin which is highly utilized in food industry, perfumery and cosmetic industry. These diverse ranges of utilities may be due to presence of therapeutically active secondary metabolites in both seeds and leaves of *P. dioica*. On the other hand, once important plant source found, its commercial planting material production is vital important. Allspice is conventionally propagated through seeds, but the seeds lost their viability soon after harvest [5].

Allspice being a polygamodioeous tree, identification of the functional male and female trees till they flower is difficult and hence, clonal propagation is essential to obtain uniformly high yielding trees [6]. The substances, traditionally referred to as secondary metabolites or phytochemicals, often are differentially distributed among limited taxonomic groups within the plant kingdom [7].

Phytochemicals are chemical compounds that occur naturally in plants. Some are responsible for colour and other organoleptic properties. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids, and phenolic compounds [8]. Secondary metabolites are those metabolites which are not functioning in growth (although they may have survival function). Many of these compounds now have been shown to have important adaptive significance in protection against herbivory and microbial infection, as attractants for pollinators and seed dispersing animals, and as allelopathic agents (allele chemicals that influence competition among plant species). Total phenolic content (TPC), total flavonoid content (TFC) and total antioxidant capacity (TAC) have been renowned as therapeutically active substances which regulate important physiological actions on the human body [9]. Since antioxidant function as free radical scavengers and quenchers of singlet oxygen formation, investigation of natural antioxidants has greatly

increased in recent years [10]. Out of the commonly used spice crops, allspice is distinguished due to its multi aroma and flavor characteristics [2].

Although there are scattered information available on different phytochemicals present in different parts of *P. dioca*, systematic study on phytochemical distribution in different parts and leaf positions are scattered. Therefore, in the present study we compare the distribution of different phytochemicals and bioactivity in different parts and different leaf positions of *P.dioica* in order to determine the most suitable parts for harvesting by means of phytochemicals and bioactivity.

2. Materials and Methods

2.1. Location

The experimental site was situated in the Low Country Intermediate-Zone (IL1a), at an elevation of 25 m above mean sea level [11]. The average annual temperature, relative humidity and solar intensity of the experimental area are 31°C, 78% and 85 kilo lux respectively.

2.2. Planting Materials

Planting materials were collected from 20 years old well grown, pest and disease free mother plant. Plant materials were collected and authenticated with comparing institutional herbarium specimens.

2.3. Measurement of Leaf Area

Leaf area was measured by a leaf area meter (LI-3100C, USA).

2.4. Extraction of Phytochemicals

Leaves were cut into pieces and air dried for three days at room temperature (28±2°C). Then samples (three samples from each category) were powdered using motor and pestle and sieved with 0.25 mm mesh.

Powdered samples (0.1 g) were mixed with 5 mL of 80% methanol vortexed for 15 min. Then it was placed in a water bath at 60°C for 40 min and vortex procedure was repeated in 10 min interval. After centrifugation at 4,000 rpm for 5 min, the supernatant was decanted into a 15 mL centrifuge tube and the remaining was re-extracted with 5 mL of 80% methanol.

2.4.1 Chemicals and Regents

6-hydroxy-2,5,7,8-tetramethy-chroman-2-carboxilic acid (Trolox), Gallic acid, Rutin, 2,4,6-tripyridyl-2-try-azine (TPTZ), Folin-Ciocalteu reagent and ferric chloride ($FeCl_3.6H_2O$) were purchased from Sigma Aldrich Chemical Co. (St. Louis, Mo) and all other chemicals used were analytical grade.

2.4.2. The Total Phenolic Content (TPC)

The total phenolic content was quantified using modified Folin-Ciocalteu method [12]. Briefly, 4 mL of distilled water and 0.5 mL of properly diluted leaf extract were mixed with 0.5 mL of 0.5 N Folin-Ciocalteu reagent (FCR) and allowed to react for 3 min. Then 1 mL saturated sodium carbonate solution was mixed and incubated in a water bath for 2 h at 30 °C. The absorbance was measured at 760 nm using UV visible spectrophotometer (Shimadzu UV Mini 1240 Japan). Gallic acid was used as the standard and data were expressed as mg of Gallic Acid Equivalent (GAE) /g DW.

2.4.3. Quantification of Total Flavonoid (TFC)

Total flavonoid content (TFC) was determined by a colourimetric method, with slight modifications [13]. 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 min, 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 min. Then 2 mL of 2 M NaOH was added, and top up to 8 mL with distilled water. After thoroughly mixing, the absorbance was measured at 510 nm using UV visible spectrophotometer (Shimadzu UV Mini 1240 Japan). Rutin was used as the standard and data were expressed as mg of rutin equivalent (RE) /g DW.

2.4.4. Determination of Total Antioxidant Capacity (TAC)

Total antioxidant capacity was determined using Ferric Reducing Antioxidant Power (FRAP) assay [14]. Methanolic leaf extract (100 µL) was mixed with 900 µL of freshly prepared FRAP reagent of pH 3.6 containing 2.5 mL of 10 mol/L, TPTZ solution in 40 mmol/L, HCl plus 2.5 ml of 20 mmol/L $FeCl_3$ and 25 mL of 300 mol/L acetate buffer. Absorbance was measured at 593 nm using the spectrophotometer (Shimadzu, UV Mini 1240, Japan) after incubating for 4 min. The trolox was used as the standard solution and TAC was expressed as mg Trolox Equivalents (TE) /g DW.

2.5. Statistical Analysis

Statistical comparison of mean values was performed by general linear model (GLM) of ANOVA followed by Turkey multiple range test of the SAS software package (SAS Institute, 1999).

3. Results and Discussions

Spices are products derived from different parts of the aromatic plants *i.e.* leaves, roots, stem, branches, flowers, seeds, stigmas and styles or the entire plant tops which are heavily used by ethnic groups for their flavor, colour, aroma and preservation for foods or beverage worldwide [15]. The secondary metabolites (phenolics and flavonoids) and bioactivity play an important role in plant therapeutic value of medicinal plants. These secondary metabolites support mainly for the natural defense system, and mainly depend on the development stage of plants [16,17]. In the present study attempts were made to compare the variation of total antioxidant capacity, total phenolic content and total flavonoid content of leaves of *Pimenta dioica* at different maturity stages (Table 1). Total antioxidant capacity, was decreased with the maturity of leaves and TAC of 1st 2nd, 3rd leaf positions were significantly different from 4th and 5th leaf positions.

As demonstrated in Table 1, Total phenolic content and total flavonoid content were decreased up to 3rd leaf

position. However, they were significantly increased at 5th leaf position. Meanwhile leaf area was increased up to 4th leaf position and decreased at 5th leaf position (Table 1). Presence of higher content of TPC, TFC and TAC in immature leaves and bud, might be due to the less cellulose and pectin in immature leaves and the active components present in tender parts are more soluble than those present in mature parts. Observed high TAC, TPC and TFC in immature leaf and bud, extracts are in agreement with previous studies [3].Who investigated the higher TAC, TPC and TFC in immature and mature *Pimenta dioica* leaves and who investigated immature and mature tea (*Camellia sinensis)* leaves [18].

Table 2 demonstrates the distribution of total antioxidant capacity, total phenolic content, total flavonoid content in different parts (leaf bud, immature leaf, mature leaf and bark) of *P. dioica*.

The results clearly demonstrated that the highest TAC, TPC and TFC were contained in leaf bud. Order of decrease of total antioxidant capacity was leaf bud>immature leaf>mature leaf>bark.

The highest TAC was observed in bud (619.84±11.98 mg TE/g DW) and the lowest TAC was observed in bark (317.67±13.14 mg TE/g DW). As shown in Table 2, TFC, bud potion significantly different from bark potion, and bark showed higher significant difference than immature and mature leaf.

The highest TPC and TFC were observed in bud (267.53±5.03 mg GAE/g DW and 305.48±8.87 mg RE/g DW respectively) whereas the lowest TPC and TFC were observed in mature leaf (131.53±3.06 mg GAE/g DW and 221.90±3.30 mg RE/g DW respectively). The correlation value between TAC and TPC was R^2=0.9462.

Table 1. Total antioxidant capacity (TAC), total phenolic content (TPC), total flavonoid content (TFC), leaf area, and dry weight:Fresh weight ratio of leaves of *Pimenta dioica* at different leaf positions

Leaf positions	TAC (mg TE/g DW)	TPC (mg GAE/g DW)	TFC (mg RE/g DW)	Leaf area(cm^2)	Dry weight:Fresh weight ratio
1st	562.38±9.42[a]	279.53±7.02 [a]	296.43±1.89[a]	0.6685±0.0002	37.80:100
2nd	552.36±1.63[a]	206.87±2.31[b]	291.90±1.49[a]	13.8515±0.0090	41.91:100
3rd	546.12±8.69[a]	154.20±8.72[c]	259.52±6.44[b]	39.209±0.113	49.51:100
4th	522.82±2.86[b]	154.20±5.29[c]	269.53±1.80[b]	50.4705±0.00305	52.12:100
5th	501.41±11.04[c]	152.87±1.15[c]	303.48±8.87[a]	42.082±0.01033	53.59:100

Values within a column followed by different letter are significantly different (P<0.05), GAE-Gallic acid equivalent; TE- Trolox equivalent; RE- Rutin equivalent.

Table 2. Total antioxidant capacity (TAC), total phenolic content (TPC), total flavonoid content (TFC) of *Pimenta dioica* at different plant parts

Parts	TAC (mg TE/g DW)	TPC (mg GAE/g DW)	TFC (mg RE/g DW)
Bud	619.84±11.98[a]	267.53±5.03[a]	305.48±8.87[a]
Immature leaf	395.99±11.10[b]	192.20±8.00[b]	225.48±5.95[c]
Mature leaf	325.80±10.29[c]	131.53±3.06[c]	221.90±3.3°C
Bark	317.67±13.14[c]	143.53±8.33[c]	245.71±1.43[b]

Values within a column followed by different letter are significantly different (P<0.05); GAE-Gallic acid equivalent; TE-Trolox equivalent; RE-Rutin Equivalent.

4. Conclusions

Present study investigated phytochemicals distribution and bioactivity in different parts and different leaf positions of *Pimenta dioica*. All tested bioactive compounds were distributed in all tested parts and the highest bioactive compounds were reported from leaf or leaf bud positions. Further, present study clearly indicated that leaf can be used as raw materials for industrial purposes instead of fruits.

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