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Comparison of Phytochemicals Antioxidant Activity and Essential Oil Content of *Pimenta dioica* (L.) Merr. (Myrtaceae) with Four Selected Spice Crop Species

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Abstract *Pimenta dioica* (L.) Merr. (Myrtaceae) (Eng.*Allspice*) is an industrially and therapeutically important, evergreen aromatic spice plant widely used in food, perfumery and cosmetic industries around the globe. Allspice, which tastes like a blend of *Cinnamomum zeylanicum* Blume, *Elettaria cardamomum* (L.) Maton, *Syzygium aromaticum* (L.) Merr. &L.M.Perry and *Myristica fragrans* Houtt. is a common flavoring compound in Asian, Middle Eastern and Jamaican cuisines. However, comparative essential oil content, total antioxidant capacity (TAC) and total phenolic content (TPC) of these similar taste spices is scattered. Therefore, the present study compares the qualitative phytochemical contents, essential oil contents, total antioxidant capacity (TAC) and total phenolic content (TPC) of *C. zeylanicum*, *E. cardamomum*, *S. aromaticum* and *Myristica fragrans* Houtt. with leaf extracts of *Pimenta dioica* using previously published protocols. Results revealed that all tested phytochemicals namely alkaloids, flavanoids, saponins, steroid glycosides and tannins are present in all selected spice species compared. The highest essential oil content was reported from clove buds followed by nutmeg mace, nutmeg seed, cardamom, allspice and cinnamon respectively. Leaf extracts of *P. dioica* exhibited significantly higher total antioxidant capacity (344.9 ± 4.2 mg TE/g DW) and total phenolic content (134.3 ± 7.6 mg GAE/g DW) compared to selected spices except clove. Presence of all tested phytochemicals, comparable amounts of essential oils, greater amount of total antioxidant capacity and total phenolic content undoubtedly demonstrate high potential of *Pimenta dioica* (allspice) as a spice crop for large scale cultivation in Sri Lanka.

Keywords: *pimenta dioica, total antioxidant capacity, total phenolic content, phytochemicals, essential oils*

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1. Introduction

Pimenta dioica (L.) Merr. belongs to the family Myrtaceae and commonly known as Allspice, Pimento, Clove pepper, Jamaican pepper and English spice [1]. *P. dioica* is native to North American subcontinent, widely distributed in West Indies, Mexico, Brazil, Honduras, Jamaica, Grenada, Cuba and Haiti. Dried fruits and leaves of *P. dioica* are used as a valuable spice and a condiment [2]. It is classified as an evergreen shrub which reaches to a height of 10 to 18 m. Since the leaves or berries of *P. dioica* are considered as a source of favorable secondary metabolites, they have been used for an array of human endeavors, such as folk medicine, food spice and perfumery industry and also as a natural pesticide [3]. The essential oil of *P. dioica* is industrially utilized for tanning purposes, meat and canning industries, as tonics, appetizing medicines, flavoring and perfuming agent in cosmetic products. It also has many therapeutic properties such as antimicrobial, antioxidant, analgesic, anesthetic, antiseptic, acaricidal, carminative, muscle relaxant, rubefacient and stimulant. Different plant parts of allspice have been used to relieve rheumatic pains, dental and muscle aches, colds, indigestion, viral infections, bronchitis, depression, sinusitis, nervous exhaustion, cramp, arthritis, fatigue, nausea and hysterical paroxysms [4]. Moreover, its English name allspice may be due to its intricate aroma which is a combination of aroma from spices namely Clove, Nutmeg and Cinnamon. Even though *P. dioica* has an immense potential as a spice crop, information on its comparative bioactive secondary metabolites (essential oil content, total antioxidant capacity (TAC) and total phenolic content (TPC)) with currently used spice crops are scattered. Therefore, current study compares phytochemicals and bioactivity of *Pimenta dioica*, with above mention four selected spices in terms of phytochemicals, total phenolic content, antioxidant capacity, and essential oilscontent.

2. Materials and Methods

2.1. Collection of Samples

Freshly harvested mature leaves of allspice, mature bark of cinnamon, mature green color capsules of cardamom, mace and seed of nutmeg and unopened flower buds of clove were collected as samples from home gardens in Kandy and Gampola areas (similar in soil and climatic conditions) of Sri Lanka during February to March 2015. Herbarium specimen was prepared and deposited in institutional herbarium (HTS 103).

2.2. Sample Preparation

Collected samples were air dried for five days at room temperature (28 ± 2°C). Electric grinder was used to grind air dried samples and sieved with 0.25 mm mesh.

2.3. Extraction of Samples

2.3.1. Extraction for Total Phenolic Content, Antioxidant Capacity

Ground samples (0.1 g) were accurately weighed into a 15 mL centrifuge tube and 5 mL of 80% methanol was added. Then samples were vortexed for 15 min. and placed in a water bath at 60°C for 40 min Vortex procedure was repeated in 10 min intervals. Then samples were centrifuged at 4,000 rpm for 5 min and supernatant was decanted into a 15 ml centrifuge tube. The residue was re-extracted with 5 mL of 80% methanol. Both supernatants were pooled and stored at - 20°C prior to analysis.

2.3.2. Extraction for Cytotoxicity and Phytochemicals Screening

Powdered plant material (5 g) was extracted with 250 mL of methanol using Soxhlet apparatus for 6 hr and extract was filtered through cotton wool. Filtrate was dried over anhydrous sodium sulfate and concentrated under reduced pressure at 50°C using Rotorvapour (Buchi Rotavapour, Type-R-114A29 B-480, Switzerland).

2.4. Qualitative Screening of Phytochemicals

The phytochemical screening tests were performed as described by [5], with slight modifications. Methanolic extracts of samples were screened for alkaloids, flavonoids, saponings, steroid glycosides and tannings.

2.5. Quantification of Total Phenolic Content

Total Phenolic Content (TPC) was quantified using modified Folin-Ciocaltue method [6]. Briefly, 0.5 mL of methanolic plant extract was diluted with 4 ml of distilled water. Then 0.5 N Folin-Ciocaltue reagent (0.5 mL) was added. After the mixture was allowed to react for 3 min., 1 mL of saturated Na_2CO_3 solution was added. Samples were incubated in a water bath for 2 hr at 30°C. Absorbance was measured at 760 nm using the spectrophotometer (Shimadsu, UV Mini 1240, Japan). Galiic acid was used as the standard and 80% methanol was used as the control.

2.6. Determination of Total Antioxidant Capacity

Total Antioxidant Capacity (TAC) was determined using the FRAP assay [7] with slight modifications. Briefly, the FRAP reagent was freshly prepared by mixing 25 mL of 300 mM Sodium Acetate buffer (pH 3.6), 2.5 mL of 10 mM TPTZ solution, and 2.5 mL of 20 mM Ferric Chloride solution. The absorbance at 593 nm was read 4 min. after mixing of 100 µLplant extract with 900 µL of FRAP reagent, using spectrophotometer (Shimadzu, UV Mini 1240, Japan). Trolox was used as the standard and 80% methanol was used as the control. The measurement was compared to a standard curve of prepared trolox solutions and expressed as mg Trolox Equivalents (TE)/g dry weight (DW) of samples.

2.7. Distillation of Essential Oil

Ground samples (50 g) were separately hydro-distilled in a Clevenger-type distillation apparatus for 6 hr. and the oil was collected. Volume of oil was recorded and essential oil content was calculated as a percentage based on dry weight of samples.

2.8. Thin Layer Chromatography

Thin Layer Chromatography (TLC) was performed according to the method described in WHO guidelines with slight modifications. 5 µL of extract was spotted in TLC plates (Pre-coated silica gel 60 A 20 × 20 cm; 0.2 mm thickness). Extracts were developed in Cyclohexane: Dichloromethane: Ethyl acetate: Methanol (5:1:4:0.8) mobile phase and observed under UV 366 nm and after spraying with Vanillin-Sulfuric acid. The Rf values and color of the spots were recorded.

2.9. Statistical Analysis

Data are given as means ± Standard Deviation (SD). Statistical comparisons were made using analysis of variance (ANOVA) with GLM procedure followed by Turkey Test (SAS Institute, 1999).

3. Results and Discussion

Spices are plant products derived from many parts of the aromatic plant: bark, buds, flowers, fruits, leaves, rhizomes, roots, seeds, stigmas and styles or the entire plant tops which are heavily used by ethnic groups for flavour, colour, aroma and preservation of food or beverages worldwide ([8]). However, information on phytochemicals and bioactivity of Sri Lankan grown allspice are lacking. In the present study attempts have been made to compare the key phytochemicals and bioactivity parameters of qualitative phytochemical contents, total antioxidant capacity (TAC), total phenolics content (TPC), and essential oils content of mature leaves of *P. dioica* with commonly used spices such as *C. zeylanicum*, *E. cardamomum*, *S. aromaticum* and *M. fragrans.*

As demonstrated in Table 1, all tested phytochemicals namely alkaloids, flavanoids, saponins, steroid glycosides

and tannins are present in all spice species tested. Presence of phytochemicals tested is in agreement with Faria et al 2014 [9] who investigated the pharmacognostic parameters of *P. dioica*.

Table 1. Qualitative phytochemical parameters of selected spices

Plant Species	Alkaloids	Flavonoids	Saponins	Steroid Glycosides	Tannins
Allspice	+	+	+	+	+
Clove	+	+	+	+	+
Cinnamon	+	+	+	+	+
Cardamom	+	+	+	+	+
Nutmeg – mace	+	+	+	+	+
Nutmeg – seed	+	+	+	+	+

+: Presence; - : Absence.

Plant secondary metabolites namely TPC, bioactivity viz TAC and essential oil content and its composition play an important role in spice value of a crop. As demonstrated in Table 2, significantly higher (P=0.05) TAC was observed in clove (974.340 ± 28.47) followed by allspice (344.917 ± 4.19), cinnamon, nutmeg and cardamom respectively. However, essential oil content was varied as clove> nutmeg> cardamom> allspice> cinnamon (Table 2). Present study revealed that allspice had a significantly higher TAC and total phenolic values when compared to all selected spice species except clove. The higher contents of TAC and TPC in this study are an agreement with the findings of [10], who reported higher content of TAC and TPC in allspice leaves. Moreover, significantly higher TAC in clove than in cinnamon is in agreement with [11], who compared the essential oil composition of 12 different spices available in market in Croatia.

Table 2.Total phenolic content (TPC), total antioxidant capacity (TAC) and essential oil percentage of selected spices

Plant Species	TPC (mg GAE/g DW)	TAC (mg TE/g DW)	Essential oils percentage (%)
Allspice	134.3 ± 7.6^{b}	344.9 ± 4.2^{b}	1.4
Clove	310.4 ± 8.5^{a}	974.3 ± 28.5^{a}	18.6
Cinnamon	67.5 ± 0.5^{c}	104.9 ± 5.3^{c}	0.9
Cardamom	3.5 ± 0.1^{e}	3.2 ± 0.2^{e}	2.9
Nutmeg – mace	28.4 ± 0.3^{d}	91.5 ± 3.4^{cd}	10.8
Nutmeg – seed	34.8 ± 0.4d	82.4 ± 2.9^{d}	3.7

Means in a column with the same letters are not significantly different at 0.05 level
GAE= gallic acid equivalent; TE= trolox equivalent; DW= Dry weight.

Thin layer chromatography (TLC) is the widely used analytical technique in herbal drug standardization process due to its simplicity, rapidness and cost effectiveness ([12]). In the present study, we compared the TLC fingerprints patterns of allspice with other selected spices. Interestingly, it was revealed that all tested spices showed common spots (R*f* 0.16 light purple; R*f* 0.43 dark purple; R*f* 0.60 brown; light brown spots at R*f* 0.69 and R*f* 0.83). Presence of common spots is quite acceptable as most of the spices contained similar aroma and chemical composition. In contrast, presence of species specific spots (R*f* 0.09- light purple spot; 0.43 orange purple spots) also will help in standardization and authentication process. Use of TLC finger prints has been established as a reliable tool for identification and authentication of medicinal and aromatic plants including *Acmella oleraceae* ([13]); *Gyrinops walla* ([14]); *Munronia pinnata* and *Andrographis paniculata* ([15]).

Therefore, the present study is in agreement with previous work conducted in elsewhere.

4. Conclusions

According to the results, allspice possesses all tested phytochemicals and high value of phytochemicals and bioactivities including TAC and TPC and. Thin layer chromatography fingerprinting also clearly exhibited the presence of some common spots in all tested spices. These comparable results clearly validate the use of leaves of allspice by communities living in North Americans as a spice. Further, since all spice possesses all tested phytochemicals in comparable or greater amounts with conventionally used spice crops, it could be suggested for large scale cultivation of allspice as an industrial crop in Sri Lanka.

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