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Screening of Local and Introduced Varieties of *Pogostemon heyneanus* Benth. (Lamiaceae), for Superior Quality Physical, Chemical and Biological Parameters

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Abstract *Pogostemon heyneanus* Benth. (Lamiaceae) is an aromatic, perfumery important, industrial crop widely cultivated in many Asian countries for its distinguished fragrance and other therapeutic purposes. However, commercial cultivation of *P. heyneanus* was hampered due to lack of high quality planting materials. Purpose of the present study is to explore superior quality *P. heyneanus* variety by means of physical (morphological), chemical (physico-chemical, phytochemical, essential oil content and composition) and biological [total antioxidant capacity (TAC)] parameters in order to establish commercial cultivation. Morphological, physico-chemical and phytochemical analysis were performed according to the methods described in WHO guidelines and other classical texts. The TAC was performed using Ferric Reducing Antioxidant Power (FRAP) assay. Essential oil was analyzed by gas chromatography Mass Spectrometry (GC/MS). Out of 26 morphological characters assessed, 5 characters were *i.e.* plant height, leaf margin, leaf apex, and leaf base and leaf shape polymorphic. All phytochemicals tested were identical to both varieties. However, presence of a prominent spots at Rf 0.12 (dark brown spot), 0.20 (rose colour spot), 0.45 (dark green spot) were characteristic for local variety. Significantly higher total ash content (12.32 %), oil content (0.52%), higher number of compounds in essential oil, patchouli alcohol content (57.0 %) and antioxidant capacity (108.53 ± 2.5 mg Trolox equivalent per g of extract) were reported in introduced variety. According to the results, introduced variety possesses superior quality physical, chemical and biological properties and therefore, introduced variety could be recommended for establishment of commercial cultivation.

Keywords: *Pogostemon heyneanus, Lamiaceae, antioxidant capacity, GC MS analysis, essential oil, Patchouli alcohol*

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1. Introduction

Pogostemon heyneanus Benth (Lamiaceae) is a fragrant, industrially important medicinal and aromatic herb found in Western to Southern in India, Malay Peninsula, Philippine Islands and Sri Lanka. It is known as Gan-kollankola or Kollankola in Sinhala Patraka, Sughandaka in Sanskrit and Pachouli or Java patchouli in English [1]. Patchouli oil is a product distilled from areal parts of *Pogostemon* species such as *Pogostemon cablin*, *Pogostemon heyneanus*, *Pogostemon speciosus* or other *Pogostemon* species grown in different geographic regions in the world. Patchouli oil is distinguished for its deep, earthy and musky fragrance and hence it is mainly used in perfumery industry [2,3]. In addition, patchouli oil is a major component of soaps, cosmetics, fragranced products like paper towels, laundry detergents, and air fresheners. Composition of essential oil (patchouli oil) also varied according to the species. The essential oil obtained from *Pogostemon speciosus* contained α-bisabolol (40.8%), caryophyllene (4.5%), cubebol (5.3%) and [4] while *Pogostemon cablin* contained Patchoulol (40-70%). α-pinene and β-pinene contained as major constituents of *P. heyneanus* [5]. These major phytochemicals and different constituents are responsible for an array of therapeutic properties such as anti-fungal and bacteriostatic properties [6], protection against influenza viral infection [7], anti-tumorigenic activity [8] and anti-inflammatory activity [8,9]. Further, Patchouli oil has been used as insect repellent materials. Although *P. heyneanus* possesses a diverse range of therapeutic effects, industrial potential and traditional uses, commercial cultivation in Sri Lanka is hampered due to unavailability of economically viable superior quality planting materials in order to establish commercial cultivation. Therefore, selection of economically viable superior quality planting materials with desired physical chemical and biological properties is yet to be investigated in order to establish commercial scale cultivation. In the present study we attempted to select superior quality variety of *P. heyneanus* by means of higher oil content, patchouli alcohol content and higher antioxidant capacity in order to generate planting materials for establishment of commercial cultivation in Sri Lanka.

2. Materials and Methods

2.1. Plant Materials

Plants for morphological studies were obtained from six months old *P. heyneanus* mother plants maintained under same soil and climatic conditions. Herbaria for all both varieties were prepared (HTS 50 and HTS 51) and authenticated by comparing with herbarium specimens deposited at National Herbarium at Royal Botanical Garden. Plant materials for phytochemical, physicochemical, essential oil distillation and antioxidant assay were obtained from the above maintained plants.

2.2. Morphological Studies

Same aged twenty five plants of each variety which were maintained at same soil and climatic conditions were used for morphological study. Taxonomically significant vegetative characters of both varieties were recorded and tabulated.

2.3. Preparation of Samples for Analysis

Fully matured leaves were harvested from six months old plants and cut into pieces. Then the samples were dried at room temperature (28±2°C) for 5 days. The dried leaves were powdered using an electric grinder (Junke and Staufen, Germany) and used for physicochemical, phytochemical, antioxidant and essential oil extraction.

2.4. Phytochemical, Physicochemical and Antioxidant Activity

2.4.1. Extraction of Plant Materials

Ten grams of powdered plant material of each variety was extracted with 50 mL of methanol by using Soxhlet apparatus. The extract was concentrated at 45°C using rotovapour (Buchi Rotavapour, Type-R-114A29 B-480, and Switzerland).

2.4.2. Phytochemical Screening

The preliminary phytochemical screening tests were carried out for flavonoids, alkaloids, steroid glycosides saponins, and tannins according to the method described by Farnsworth [30].

2.4.3. Quantification of Total Ash

Two grams of air dried materials ignited at 500–600°C until the sample turn into white colour. Then the total ash content of ignited sample was determined as methods described in WHO guidelines [10].

2.4.4. Quantification of Water-Soluble Ash

Previously ignited ash sample was mixed with 25 mL of distilled water and boiled with 25 mL Then it was filtered using a Whatman ashless filter-paper. Insoluble matter was washed with hot water and ignited for 15 min at 450°C. The residue was allowed to cool in a desiccator for 30 minutes [10].

2.4.5. Quantification of Acid-Insoluble Ash

Pre ignite crucible containing ash was gently boiled with 25 mL of HCl. Insoluble matter was collected to Whatman ashless filter-paper and washed with hot water until the filtrate become neutral. Then the acid insoluble matter was transferred to original crucible and ignited to a constant weight at 450°C. Then the residue was allowed to cool in a desiccator for 30 minutes [10].

2.4.6. Quantification of Total Extractable Matter

Hot extraction method - Coarsely powdered samples (4g each) of leaf, stem and flower were separately refluxed with 100 mL of methanol for 1 h. Then the mixture was filtered and total weight was re-adjusted by adding methanol. Then 25 mL of the filtrate was concentrated in a rotavapour (Buchi Rotavapour, Type-R-114A29 B-480, Switzerland) at 45°C. The residue was dried at 105°C for 6 h and allowed to cool for 30 min. The weight was recorded [10].

2.4.7. Cold Maceration Method

Four grams of coarsely powdered, sample was macerated with 100 mL of methanol for 6 h. Then it was allowed to stand for 18 h and filtered. Then 25 mL of the filtrate was concentrated in a rotary evaporator at 45°C. The residue was dried at 105°C for 6 h, cooled in a desiccator for 30 minutes.

2.4.8. Quantification of Total Phenolics

The total phenolic content was determined by using modified Folin-Ciocaltue method (Abeysinghe *et al*). Briefly, 4 mL of distilled water and 0.5 mL of plant extract were mixed in a test tube. Then 0.5 N Folin Ciocalteu reagents (0.5 mL) was added and allowed to react for 3 min. saturated sodium carbonate solution (1 mL) 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).

2.4.9. Determination of Total Antioxidant Capacity (TAC)

Total antioxidant capacity was determined using Ferric Reducing Antioxidant Power (FRAP) assay as described by Benzie and Strain [12]. 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).

2.4.10. Distillation of Essential Oil

Well-dried leaf samples of each variety of *P. heyneanus* separately hydro-distilled in a Clevenger-type distillation apparatus for 8 h. The oil was collected and dried over anhydrous sodium sulfate and stored under refrigeration until analysis. The yield of the oils was calculated based on dry weight of plant materials.

2.4.11. Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

GC-MS analysis was carried out on a Hewlett-Packard 6890 Gas Chromatograph fitted with a fused silica HP-5MS capillary column (30m × 0.25mm; film thickness 0.25µm). The oven temperature was programmed from 60 - 280°C at 4°C/min. Helium was used as carrier gas at a flow rate of 2mL/min. The gas chromatograph was coupled to a Hewlett-Packard 6890 mass selective detector. The MS operating parameters were ionization voltage, 70eV; and ion source temperature, 200°C. The constituents of essential oil were identified by means of their GC retention indices (RI) and also by comparing the fragmentation pattern in the mass spectra of the peaks with those reported in the literature and NIST and Wiley MS library databases.

2.4.12. Statistical Analysis

Results of morphological characters were averaged of 20 plants. Data on physico-chemical parameters and antioxidant activity were analyzed by General Linear Model (GLM) ANOVA test followed by Duncan's Multiple Range Test (DMRT). Statistical comparison of mean values was performed by General Liner Model (GLM) of ANOVA followed by Turkey Multiple Range Test using Minitab 15 version and presented means ± SD.

3. Results and Discussion

Present work compared the morphological, phytochemical (alkaloids, flavonoids, saponins, steroid glycosides and tannins & TLC fingerprints), physico-chemical (moisture, total ash, water soluble ash, acid insoluble ash and extractable matter contents) total phenol content (TPC), total antioxidant capacity (TAC), essential oil content and essential oil composition of two varieties of *P. heyneanus* which is industrially potential fragrance plants grown in Sri Lanka.

As shown in Table 1, the most of the characters are monomorphic in both local and introduced varieties. This is quite acceptable as both varieties belonging to same family, genus and species. In spite of huge morphological resemblance these two verities can be authenticated by their prominent polymorphic morphological characters such as plant height, leaf margin, leaf apex, leaf base and leaf texture as well as leaf area (Table 1). Moreover, presence of comparatively larger leaves and higher leaf area value in introduced variety, directly influence on biomass of the plant and finally increase the yield of the crop. The most of the characters we have observed in both varieties are in agreement with previous studies [1,4] which reported the extensive morphological description of *Pogostemon* species.

Plant secondary metabolites play an important role on therapeutic effect of a given plant. Therefore, investigation of phytochemical, physicochemical, antioxidant capacity, essential oil content and composition are vital important in standardization and quality control of medicinal materials [13,14]. As shown in Table 2, current study demonstrated the presence of alkaloids, flavanoids, saponins, and tannins in both varieties of *P. heyneanus*. Conversely, steroid glycosides are absent in both varieties. According to the results, it seems that preliminary phytochemicals present in both varieties are similar.

Table 1. Prominent morphological features of local and introduced varieties of *Pogostemon heyneanus*

Character	Local variety	Introduced variety
Plant habit	Much branched	Much branched
Plant height (cm)	90-110 cm	140 cm – 150 cm
Leaf type	Simple	Simple
Leaf length (cm)	9.5-12.2 cm	11cm – 12cm
Leaf width (cm)	7.2-9.0 cm	9cm – 10cm
Length: width ratio	1.5	1.2
Leaf margin	Double serrate	Serrate
Leaf apex	Acuminate	Acute
Leaf shape	Ovate	Triangular
Leaf base	Acute	Truncate
Leaf arrangement	Opposite	Opposite
Leaf surface colour (upper)	Green	Green
Leaf surface colour (lower)	Light green	Purplish green
Leaf texture	Smooth	Rough
Leaf area	40 cm^2 – 50 cm^2	65 cm^2 – 70 cm^2
No. of veins per leaf	6-10	11- 12
Venation	Pinnate	Pinnate
Leaf trichome (present/ absent)	Present	Present
Petiole length (cm)	3.5-5.8 cm	3.5 cm – 4.0 cm
Petiole colour	Light green	Green
Petiole grove (present/absent)	Present	Absent
Stem colour	Pale green	Purplish green
Intermodal space (cm)	5.75-6.5 cm	3.93-4.20 cm
Stem shape	Quadrangular	Quadrangular
Stem trichome	Present	Present

Results of the physicochemical parameters of two varieties of *P. heyneanus* are presented in Table 3. Moisture content of local and introduced varieties was 11.95±0.03 and 10.08±0.50 respectively. Extractable matter content is one of the most important parameters used for the characterization of botanical drugs [15]. Significantly higher total ash content (12.32±0.68) and extractable matter content (14.48±0.06) was observed in local variety. Since the ash represents the physiological (derived from plant itself such as calcium oxalate and silicate) and non-physiological (accumulated from external environment such as sand, soil, adulterants etc.) impurities, determination of these parameters are vital important in order to maintain the purity of the herbal medicine [15,16,17]. Moreover, acid insoluble ash and water soluble ash content of both varieties were similar. Determination of ash values are vital important in order to maintain the purity of the herbal medicine. Present study reveals the total ash and water soluble ash contents were not significantly ($p<0.05$) different in both varieties. Information on physico-chemical and phytochemical parameters generated through the present study could be incorporated in standardization of quality, purity and sample identification of both varieties of *P. heyneanus*.

Table 2. Comparative phyotochemical analysis of two *Pogostemon heyneanus* varieties grown in Sri Lanka

Metabolite	Local variety	Introduced variety
1. Saponins	+	+
2. Alkaloids	+	+
3. Tanins	+	+
4. Flavanoids	+	+
5. Steroid Glycosides	-	-

(+) =Presence (-) =Absence

Table 3. Physico-chemical parameters of different variety of *Pogostemon heyneanus* Benth

Parameter	Plant Variety	
	Introduced variety	**Local Variety**
Total ash	10.61 ± 0.05^{b}	12.32 ± 0.68^{c}
Water soluble ash	5.41 ± 0.39^{b}	6.24 ± 0.09^{b}
Acid insoluble ash	0.78 ± 0.43^{a}	0.38 ± 0.08^{a}
Moisture content	11.95 ± 0.03^{b}	10.08 ± 0.51^{c}
Extractable matter		
Hot Extraction	11.54 ± 0.06^{b}	14.48 ± 0.06^{c}
Cold Extraction	12.42 ± 0.09^{a}	11.59 ± 0.42^{a}

Results are the means of three replicates; ±SD, Means followed by same letter are not significantly different at 0.05 levels.

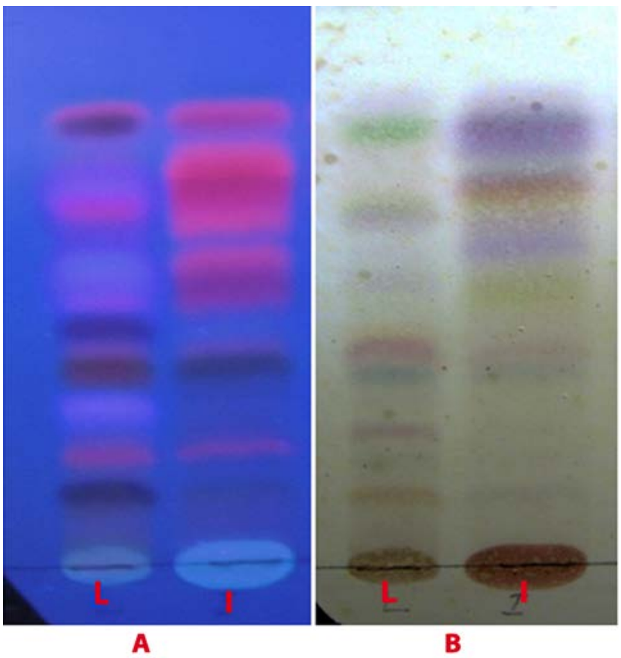

Figure 1. (A) Thin layer chromatographic profiles of two varieties of *Pogostemon heyneanus* Benth. under UV 366 nm; B. Thin layer chromatography of two varieties of *Pogostemon heyneanus* Benth. after spraying vanillin sulfate (L)-local variety, (I) – introduced variety [Solvent system chloroform: cyclohexane diclorомethane: methanol (1:1:2:0.2)]

Thin Layer Chromatography is a widely used technique for screening of herbal materials and herbal products and to differentiate between herbal species due to its simplicity, cost effectiveness, less time consuming and the ability of changing the solvent system [18,19]. Further, use of TLC fingerprint pattern for determining the identity, stability, and consistency of traditional medicine and identification of adulterants included to Panax *species have been investigated* [20,21]. In the present study we have observed TLC finger prints of leaf extracts of two varieties of *P. heyneanus* under UV 366 and after spraying with Vanillin sulfuric acid. TLC profile observed under UV 366 nm of local variety exhibited 11 spots while introduced variety showed only 9 spots. Out of these, seven spots were common for both varieties. Presence of similar spots of both varieties is obvious because both are belong to same genus and species. Nevertheless, spots appeared on Rf 0.12 (dark brown spot), 0.20 (rose colour spot), 0.45 (dark green spot) are specific to local variety (Figure 1). When consider the TLC profile sprayed with vanillin sulfuric acid also exhibited higher number of spots from local variety. These varietal specific spots could be used for authentication of these two varieties. Thin layer chromatographic profile have been successfully used for authentication of *Acmella oleraceae* [22], *Munronia pinnata* and *Andrographis paniculata* [23], for authentication of field grown and hydroponically grown *Acmella oleracea* [24] and *Gyrinops walla* [25].

Table 4. Percentage composition and comparison of oil profiles of two varieties of *Pogostemon heyneanus*

Peak No.	Compound	Compound %	
		Introduced variety	Local variety
13.77	α -pinene	-	14.25
14.98	β- pinene	-	30.99
16.88	Limonene	-	2.38
17.62	Ethanone	-	16.87
26.90	1,3-butanedione	-	1.71
26.94	β-Pachoulin	4.19	-
28.01	Seychellene	14.79	-
28.38	β- caryophyllene	2.15	2.20
28.71	α -guaiene	-	2.38
29.36	Guaia	14.79	-
31.21	Nerolidol	-	15.49
33.39	Patchouli alcohol	57.12	7.14

Essential oil content of local variety was lower (0.4 %) than the introduced variety (2.4%). Results on oil compositions of local and introduced varieties are shown in Table 4. A total of five constituents from introduced variety and nine constituents from local variety which represent over 90% of the oil constituents were identified according to mass spectrum of each constituent.

Out of identified constituents, β- caryophyllene and patchouli alcohol were shared constituents of both species. Patchouli alcohol (57.12%), Guaia (14.79%) and Seychellene (14.79%) were identified as major constituents of essential oil of introduced variety, while β- pinene (30.99%), ethanone (16.87%) and nerolidol (15.49%) were major constituents of local variety. In the commercial point of view, cultivation of patchouli with higher content of Patchouli alcohol exists higher demand in the global market. Therefore, it could be suggested to commence commercial cultivation with introduced variety of *Pogostemon heyneanus* by means of Patchouli alcohol.

Results of the current study are in agreement with Sundaresan *et al*., who investigated the compositional analysis of essential oil of 2 species of *Pogostemon* [26] Moreover, compounds identified in the present study are major compounds found in patchouli oil [26,27,28].

Table 5. Total Antioxidant Capacity (TAC) and Total Phenolic Content (TPC) of local and introduced varieties cultivated in Hydroponic system and field planted

Variety	TAC (mg Trolox per g of extract)	TPC (mg Gallic acid Equelent per g of sample)
Local variety	6.88 ± 0.26	0.83 ± 0.01
Introduced variety	108.53 ± 2.5	6.41 ± 1.10

Polyphenols are group of secondary metabolites which is responsible for an array of physiological properties [29]. Total polyphenolic content and antioxidant capacity play a significant role on therapeutic value of the medicinal plants. Antioxidants and phenols are mainly responsible for the defense mechanisms of a plant [30]. In the present study, TPC and TAC of leaf extracts of two varieties of *P. heyneanus* were compared. Results clearly demonstrated the presence of significantly higher TPC (6.41 mg Gallic acid Equelent per g of sample) and antioxidant capacity (108.53 mg Trolox per g of extract) in leaf extract of introduced variety. Results of the present study are in agreement with Abdullah Hussain *et al*. [31], who reported the higher antioxidant capacity in essential oil of *Pogostemon* species.

Results of morphological, phytochemical, essential oil content and composition, total phenolic content and antioxidant capacity clearly demonstrated the superior herbage yield, high oil content, higher patchouli alcohol content, higher antioxidant and phenolic content in introduced variety over the local variety. Therefore, in the commercial point of view establishment of commercial scale cultivations using introduced variety could expect higher commercial value.

4. Conclusion

Present study clearly demonstrates high oil content, antioxidant capacity and Total phenolic content and higher content of patchouli alcohol in introduced variety in the first time in Sri Lanka. Therefore, introduction of commercial scale cultivation of *P. heyneanus* by using introduced variety could be suggested. Information generated through the present study could be incorporated for quality control and standardization of *P. heyneanus* and upgrade the Sri Lankan pharmacopeia.

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