

Pharmacognostical investigation of *Plectranthus hadiensis* (Forssk.) Schweinf. ex Sprenger. and *Plectranthus amboinicus* (Lour.) Spreng

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Abstract *Plectranthus hadiensis* (Forssk.) Schweinf. ex Sprenger. and *Plectranthus amboinicus* (Lour.) Spreng. (Lamiaceae) are two medicinal plants with taxonomic ambiguity. Even though both plants are similar in appearance, their therapeutic properties are different. However, incorrect use of these plants in herbal formulations might adversely effect on therapeutic properties of herbal drugs. Present study describes the comprehensive pharmacognostic aspects of *P.hadiensis* and *P. amboinicus* by means of physical and chemical yield parameters. Morphological, anatomical Thin Layer Chromatographic profiles, essential oil content and composition were carried out according to the established protocols. Plant fresh and dry weights of both species were increased with the maturity while dry fresh ratio was decreased. Physical and chemical yield parameters were optimum at fully matured stage. TLC finger print profiles exhibited nine spots for *P. hadiensis* while five spots for *P. amboinicus* and yellow green (Rf 0.73) spot was characteristic to *P. hadiensis*, while violet-blue (Rf 0.61) and brown- red (Rf 0.55) were characteristic spots for *P. amboinicus*. P-Cymene, Geranyl acetate and geraniol were identified as common compounds for both species. Presence of higher chemical and physical parameters scientifically validates the traditional claims of harvesting of both plants at fully maturity stage. Results of the present study either singularly or as a whole could be incorporated for quality control and standardization of *P. hadiensis* and *P. amboinicus*.

Keywords: *Plectranthus hadiensis*, *Plectranthus amboinicus*, Lamiaceae, Thin Layer Chromatography, Morphology, essential oil

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1. Introduction

Plectranthus hadiensis (Forssk.) Schweinf. ex Sprenger. (Sin. Iriveriya) and *Plectranthus amboinicus* (Lour.) Spreng. (Sin. Kapparawalliya) are widely used medicinal plants in traditional and Ayurveda systems of medicine. Both species are belonging to genus *Plectranthus* in family Lamiaceae [1,2]. Since both plants are belonging to the same genus and family, most of the morphological characters are similar. Due to this similar appearance, people use *P. hadiensis* instead of *P. amboinicus* and vice versa. Incorrect use of these plants in herbal formulations might adversely effect on therapeutic properties of herbal drugs. Moreover, there are more than 100 written recipes in Sri Lankan Ayurveda pharmacopoeia which contain *P. hadiensis* and *P. amboinicus* as major ingredients for the treatment of different ailments. [3,4]. However, according to the existing literature *P. hadiensis* is mainly used for digestive system related diseases such as dysentery, vomiting, diarrhea, acute and chronic congestion of the liver while, *P. amboinicus* is mainly used for whooping cough, malaria [5], laryngitis, bronchitis, pneumonia, after measles, chicken pox, small

pox, asthma and some other diseases related to the respiratory tract, burns, wounds, sores, insect bites and allergies, ringworms, skin ulceration. Although both plant species are widely used an array of traditional and Ayurveda recipes for the treatment of diverse range of ailments, comprehensive pharmacognostic studies are scattered and required for authentication and standardization of *P. hadiensis* and *P. amboinicus*. [6,7,8] Therefore, in the present study, attempts have been made to compare agronomical, morphological and anatomical characters and preliminary phytochemical finger printing of *P. hadiensis* and *P. amboinicus*.

2. Materials and Methods

2.1. Plant Materials

Plant materials of both *P. hadiensis* and *P. amboinicus* were collected from 3-4 months old, well grown healthy plants which have been maintained under similar soil and climatic conditions at institutional medicinal plant research plots. Herbarium specimens of both species were prepared and identified by systematic Botanist at National

Herbarium and deposited at Institutional Herbarium (ITI/HTS 106 and 107).

2.2. Field Establishment

Same aged well acclimatized potted plants of both *P. hadiensis* and *P. amboinicus* were established in 30x30 cm holes at 45 cm x 45 cm spacing at institutional research field (Lat: 6° 58'; Long: 79° 52', RF 2500 mm, sandy loam soil, Temperature 28±2°C). One kilogram of compost was added to each hole prior planting. Plants of each species were separately established in 100 cm x300 cm beds. Whole experiment contained more than 100 plants from each species. Irrigation, weeding and other aftercare operations were conducted as required. Number of leaves, number of branches, spreading, fresh weight, dry weight and fresh to dry weight ratio was obtained at two weeks interval up to 16 weeks. At least 20 plants were used to obtain growth data.

2.3. Determinations of Morphological Characters

Specimens for both species were collected from the plants cultivated at same soil and environmental conditions as a measure to minimize the impact of environmental factors on the data collected. Distinguished morphological characters of both plants were collected and tabulated. At least twenty individuals per each species were used to record morphological data.

2.4. Anatomical and Powder Microscopic Features

Free hand transverse sections of representative samples of stem, leaf and root were obtained from approximately same aged, healthy, plants from both *P. hadiensis* and *P. amboinicus*. Satisfactory sections were taken through an alcohol series and subsequently strained with 1% safranin in 50% ethanol. Stained material was mounted in glass slides using glycerin. Photomicrographs were taken by using Olympus, Model CX 31 digital microscope. For powder microscopical studies, crushed whole plant powder (3 g) of both plants were separately cleared by boiling with saturated chloral hydrate solution and mounted with drop of glycerin and examined under compound microscope. Line drawing was made for each character as described in literature [9].

2.5. Sample Preparation for Chemical Analysis

Areal samples of both samples were cut into pieces and air dried for three days at room temperature (28±2°C). Samples were coarsely powdered and (5 g) was extracted with 50 mL of dichloromethane by using Soxhlet apparatus. Extract was filtered through Whatman No.1 filter papers and filtrate was concentrated under reduced pressure at 40°C using rotovapour.

2.6. Distillation of Essential Oil

Ground samples of leaf, stem and root of *P. hadiensis* and *P. amboinicus* (200 g per each) were distilled using Clevenger light oil arm for 4 hours. Oil samples were

collected and the volume of the oil was recorded. Results are presented as an average triplicates ±SD.

2.7. GC Analysis of Essential Oil

The chemical constituents of volatile oil were analyzed using a Gas Chromatograph (Shimadzu GC 2010) connected with a 3m x 3mm stainless steel, flame Ionize Detector (FID). Injector temperature 230 °C and detector temperature was 240 °C. Argon was used as carrier gas. Column temperature was programmed from 60 °C to 225 °C and injected volume was 0.3µl. Compounds were identified by retention data and boosting of authentic standard compounds.

2.8. Thin Layer Chromatography of Essential Oil

The thin layer chromatography (TLC) was performed according to the method described in WHO guidelines with some modifications. Briefly concentrated filtrate was dissolved in minimum quantity of dichloromethane and 5 µL of extract was spotted in pre-coated fluorescent silica gel 60A 20 × 20 cm; 0.2 mm thickness, 20 cm x20 cm aluminum plates (Merck KGaA, Darmstadt, Germany). Plates were developed in chloroform: dichloromethane: cyclohexane (3:1:1) and spots were observed by spraying with vanillin- sulphuric acid. The R_f values for each spot were calculated as previously described [9].

2.9. Data Analysis

All quantitative morphological and physical yield (both dry and fresh weight) data are presented as an average of 20 plants and ±SD. Results on essential oil content are presented as mean of triplicate ± SD.

3. Results and Discussion

Medicinal plants are an important source for drugs. However, selection of correct species, use of correct plant part, harvesting of plant material at optimum physical as well as chemical maturity are important to obtain proper quality materials. Moreover, good agricultural practices (GAP), quality control and standardization of raw materials by using multiple techniques, such as morphological, phytochemical, anatomical, microscopical, and chromatographic techniques have been suggested by World Health Organization.

3.1. Agronomic Studies

Some of the important growth and yield parameters of the plant height, plant spreading and number of branches per plant were recorded in two weeks intervals up to 16 weeks. Plant fresh weight, plant dry weight, plant fresh to dry weight ratio of *P. hadiensis* and *P. amboinicus* were recorded in monthly interval and presented in Figure 1. At least 20 plants were randomly selected for the collection of agronomical data.

Results clearly showed that all growth parameters of both plant species were increased with the time forming sigmoid shape growth curve. However, comparatively higher number of shoots per plant were observed in *Plectranthus amboinicus* while spreading was higher in

Plectranthus hadiensis (Figure 1). In addition to the growth parameters, plant fresh weight, plant dry weight, dry to fresh weight ratio of both species were recorded as

major yield components in monthly intervals commencing from eight weeks after planting.

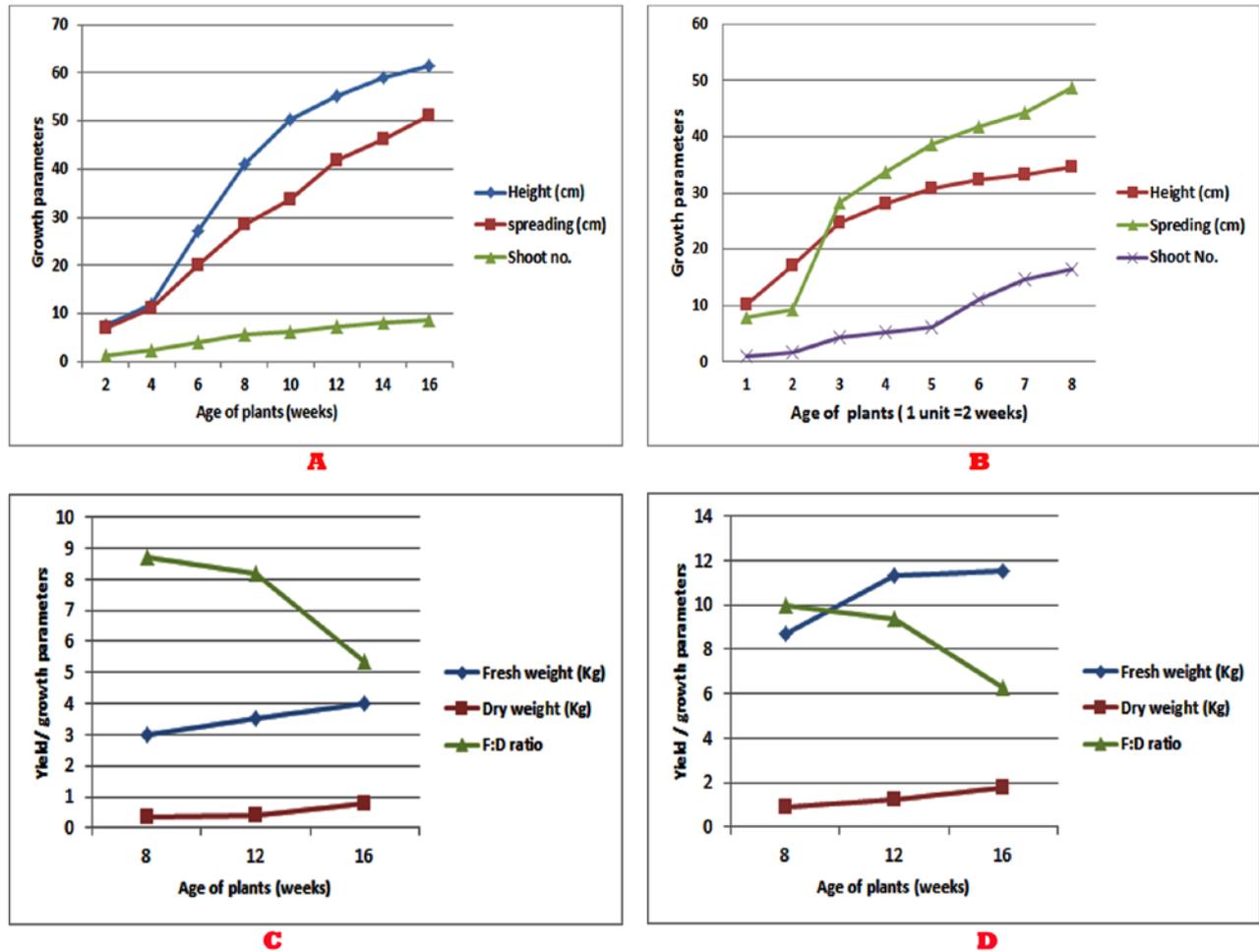


Figure 1. Variation of growth and yield parameters of *Plectranthus hadiensis* and *Plectranthus amboinicus* [A & B = Growth parameters of *Plectranthus hadiensis* and *Plectranthus amboinicus*; C&D= Yield/growth parameters of *Plectranthus hadiensis* and *Plectranthus amboinicus*. Values are a mean of 20 plants]

Plant fresh and dry weights of both species were increased with the maturity. However, fresh materials required for making one kilogramme of dry materials (fresh to dry weight ratio) was decreased with the maturity (Figure 1). Results clearly exhibited that if the plants are harvested at immature stage (before 12 weeks of age), it required higher amount of fresh materials to obtain 1 kg of dry materials (more than 8 kg of fresh materials to make 1 kg of dry materials). However, when plants are harvested after 12 weeks of maturity, it requires only 5.1 kg of fresh materials.

In the present study attempts were made to establish some important agronomical data, which are highly essential for establishment of commercial cultivation of *Plectranthus hadiensis* and *Plectranthus amboinicus*. Our study revealed that, major yield components, which are mainly responsible for the physical yield of the plant such as plant height, plant spreading and number of shoots per plants, are maximum at the fully matured stage (4 months after planting) and hence the higher physical yield could be obtained in fully matured stage for both plant species. Previous studies pointed out that *Plectranthus hadiensis* grown in both shade and full sunny conditions gave higher yield in 4 months age [10]. Therefore, our findings are in

the agreement with previous studies. Further, increase of fresh weight and dry weight of materials are in agreement with previous workers, who investigated the increase of seed dry and fresh weight of *Phyllanthus amarus* with the maturity [11].

3.2. Morphological Variations

The plants used for the morphological study were obtained from the well matured, cultivated plants under similar soil and climatic conditions. Therefore, the morphological characters observed could be considered as a true reflection of genetically determined morphology of both species. Despite the fact there were several monomorphic and polymorphic characters were observed, for the present study we have listed prominent polymorphic characters which are highly important for differentiation of both species. Out of listed characters, shape of the stem, pinkish purple stem and petiole, presence of a groove on petiole, coriaceous leaf texture could be highly valued for identification of *P. hadiensis* (Figure 2 and Table 1). Acclimatization or growing of plants in a same soil and climatic conditions prior morphological, anatomical and chemical parameter has been studied [12].



Figure 2. Morphological variations of *Plectranthus hadiensis* and *Plectranthus amboinicus* [A & B- well grown research plot of *Plectranthus hadiensis*; and *Plectranthus amboinicus*; C&D -Flowering branches of *Plectranthus hadiensis*; and *Plectranthus amboinicus*; E &F- Leaves of *Plectranthus hadiensis* and *Plectranthus amboinicus*; G&H- Branches of *Plectranthus hadiensis*; and *Plectranthus amboinicus* showing vegetative characters]

Table 1. Prominent morphological characters of *Plectranthus hadiensis* (Forssk.) Schweinf. ex Sprenger and *Plectranthus amboinicus* (Lour.) Spreng

Character	<i>P. hadiensis</i>	<i>P. amboinicus</i>
1. Nature of the plant	Herb	Herb
2. Shape of the stem	Quadrangular	Circular
3. Colour of the stem	Pinkish purple	Light-Green
4. Colour of the petiole	Pinkish purple	Light-Green
5. Petiole length (cm)	3.0±0.8	1.5±0.2
6. Leaf width (cm)	5.5 ±1.0	3.5±0.7
7. Leaf length (cm)	5±1.1	3.5±1.0
8. Leaf thickness	Medium	Thickness- more
9. Leaf dorsal surface	Apple green	Light green
10. Leaf ventral surface	Apple green	Pale green
11. Groove on petiole	Present	Absent
12. Leaf texture	Coriaceous	Leathery
13. Leaf margin	Dentate	Crenate
14. Leaf apex	Rounded	Pointed
15. Internodal space	2±0.4	3±0.2

Quantitative results are the mean of 20 plants Results are the mean of 3 replicates.

3.3. Anatomical and Powder Microscopic Characters

Results of the anatomical study revealed that the presence of different types of trichomes as main diagnosis anatomical features for both species. Among the trichomes, both simple and glandular trichomes were presence in abaxial and adaxial surface of leaves, stem and petiole cross sections. Some of the observed trichomes included short-stalked and long-stalked capitate trichomes, digitiform trichome, and large spherical headed trichomes. Moreover, with different shapes and sizes, subsidiary cells with wavier outer surfaces and glandular sessile and glandular stalk trichomes with multicellular heads were observed in the stem cross sections. The distinguished features of powder microscopy of *P. hadiensis* and *P. amboinicus* included are presence of simple and glandular trichomes scattered as broken fragments of

alone or attached to epidermal cells of stem, petiole or leaf blade particles, fragments of lower and upper epidermal particles with stomata were observed in both species. Study of anatomical features of genus *Plectranthus* is well famous due to presence of an array of trichomes with special shapes and sizes. Results of the presence study on

trichomes are in agreement with previous workers, who observed short-stalked and long-stalked capitate trichomes, long-stalked capitate trichome with a large spherical head of *Plectranthus* species as taxonomically prominent anatomical characters [13].

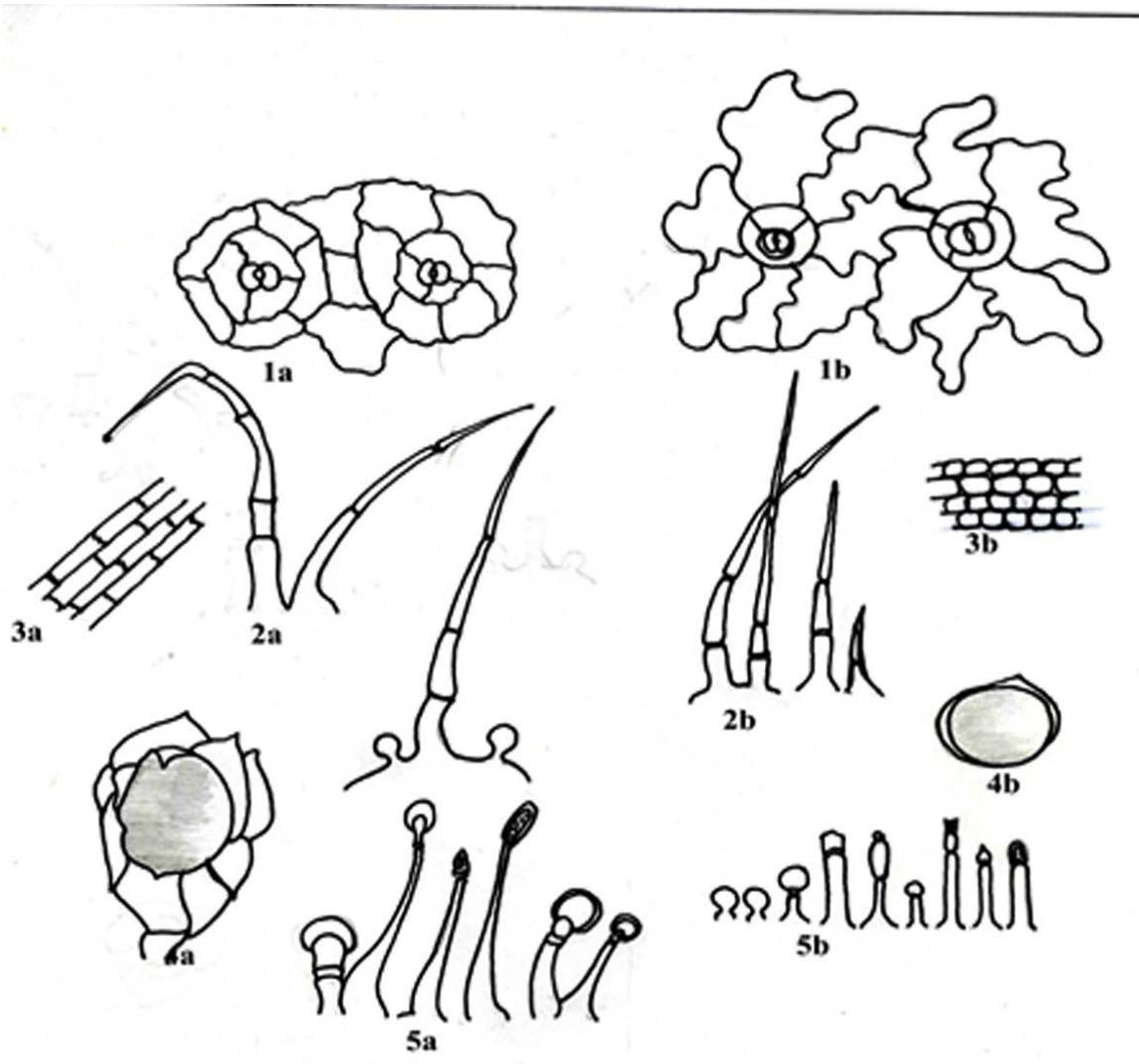


Figure 3. Main anatomical characters of *Plectranthus hadiensis* (Forssk.) Spreng. and Schweinf. *Plectranthus amboinicus* (Lour.) and ex Sprenger. [A- *Plectranthus hadiensis*; B- *Plectranthus amboinicus*; 1a & b - stomata, 2a & b- Multicellular trichomes, 3 a & b- cork tissues, 4 a & b Oil glands, 5a & b- glandular multicellular trichomes (x 40)]

3.4. Determination of Essential Oil Content

Essential oil content of different parts of the plants (roots, stems and leaves) of both *Plectranthus hadiensis* and *Plectranthus amboinicus* at different maturity stages with commercial samples are presented in Table 2.

Essential oil content and composition of medicinal plants play an important role in therapeutic properties. As demonstrated in Table 2, essential oil content of different parts (leaf, stem and roots) of *Plectranthus hadiensis* and *Plectranthus amboinicus* increased with the maturity. The higher essential oil contents of both species were reported in roots. Essential oil content different parts of both species harvested after 5 months of maturity approximately similar to essential oil content obtained from market samples. Further, essential oil content was increased as leaf < stem < roots. Results of the present study

on essential oil content of both *P. hadiensis* and *P. amboinicus* are in agreement with previous studies conducted for both essential oil content of *P. hadiensis* and *P. amboinicus* elsewhere [14,15]. Presence of higher content of essential oil at 5 months aged maturity clearly validates the traditional claims of harvesting of both species at fully maturity stage. Moreover, root essential oil content was comparatively higher in *P. amboinicus*. It was varied from 0.75-1.34% in roots. Meanwhile previous workers pointed out that *Plectranthus* species are essential-oil-rich and it was more than 0.5% in dry matter basis [16].

As demonstrated in Table 3, p -Cymene, Geranyl acetate and geraniol were identified in both species harvested after 3 and 4 months maturity. Since both plants are belonging to the same genus, presence of common compounds is unavoidable.

Table 2. Essential oil content of different parts of *Plectranthus hadiensis* (Forssk.) Schweinf. ex Sprenger. and *Plectranthus amboinicus* (Lour.) Spreng with maturity

Age (Months)	Plant part	<i>P. zeylanicus</i>	<i>P. amboinicus</i>
2	Leaf	0.4 ± 0.07	0.27 ± 0.021
	Stem	0.50 ± 0.71	0.14 ± 0.028
	Root	0.75 ± 0.14	0.41 ± 0.019
3	Leaf	0.37 ± 0.14	0.45 ± 0.021
	Stem	0.53 ± 0.04	0.40 ± 0.28
	Root	0.84 ± 0.07	1.00 ± 0.141
4	Leaf	0.71 ± 0.03	0.49 ± 0.035
	Stem	0.72 ± 0.02	0.6 ± 0.016
	Root	0.91 ± 0.03	1.13 ± 0.021
5	Leaf	0.71 ± 0.01	0.57 ± 0.06
	stem	0.78 ± 0.05	0.84 ± 0.08
	Roots	1.34 ± 0.14	1.36 ± 0.30
Commercial sample	Leaf	0.86 ± 0.24	0.57 ± 0.042
	stem	0.76 ± 0.01	0.84 ± 0.021
	Root	1.14 ± 0.23	1.20 ± 0.28

Results are the mean of 3 replicates, ± = indicates the standard deviation.

Table 3. GC analysis of essential oil

Retention time (min)	Compound	Compound %			
		<i>P. hadiensis</i>		<i>P. amboinicus</i>	
		3 Months	4 Months	3 Months	4 Months
4.5	Unknown	ND	2.91	ND	0.49
6.4	Unknown	ND	ND	0.17	2.44
6.5	Unknown	0.13	1.42	ND	0.32
8.15	p-Cymene	0.25	2.17	0.08	0.56
13.6	Unknown	0.25	ND	0.11	0.33
15.8	Unknown	0.65	2.79	0.12	0.47
18.65	Geranyl acetate	0.90	2.68	ND	ND
20.0	Unknown	0.55	2.63	0.06	0.20
21	Geraniol	0.42	3.73	0.03	0.10
25.5	Unknown	0.51	ND	0.71	1.68

ND= not detected

As demonstrated in Table 3, p-Cymene, Geranyl acetate and geraniol were identified in both species harvested after 3 and 4 months maturity. Since both plants are belonging to the same genus, presence of common compounds is unavoidable.

3.5. Thin Layer Chromatography of essential Oil and Dichloromethane Extracts

Thin Layer chromatography is the widely used analytical technique in herbal materials or drugs standardization process due to its reliability, cost effectiveness and simplicity.

In the present study, we attempt to compare the TLC fingerprinting pattern of essential oil of areal parts of *P. hadiensis* and *P. amboinicus* at different maturity stages (2, 3, and 4 months after planting) with commercially available samples (market samples) in order to study the proper maturity stage with higher secondary metabolite (essential oil) content. As demonstrated in Figure 4, TLC finger print profiles observed after spraying with vanillin sulphuric acid exhibited nine spots for essential oil of areal parts of *Plectranthus hadiensis* while five spots for *Plectranthus amboinicus* at all maturity stages. Further, all these spots were also present in essential oil distilled from samples purchased from open market. Out of these spots, a prominent, yellow green (R_f 0.73) spot was characteristic to *P. hadiensis*, while violet-blue (R_f 0.61) and brown-red (R_f 0.55) were characteristic spots for essential oil of *P. amboinicus*. Moreover, intensity of spot colour of TLC profiles was increased with the maturity. Therefore, TLC fingerprinting technique could be easily incorporated for authentication of market raw materials of both species in the process of quality control and standardization of raw materials as well as finished products containing *P. hadiensis* and *P. amboinicus* as ingredients. Use of TLC fingerprint profiles for standardization of *Plumbago indica* [17], *Acmella oleraceae* [18], *Gyrinops walla* [19], *Munronia pinnata* and *Andrographis paniculata* [20] have been extensively used. Further increase of the colour intensity might be due to increasing of secondary metabolites with the maturity stage [21].

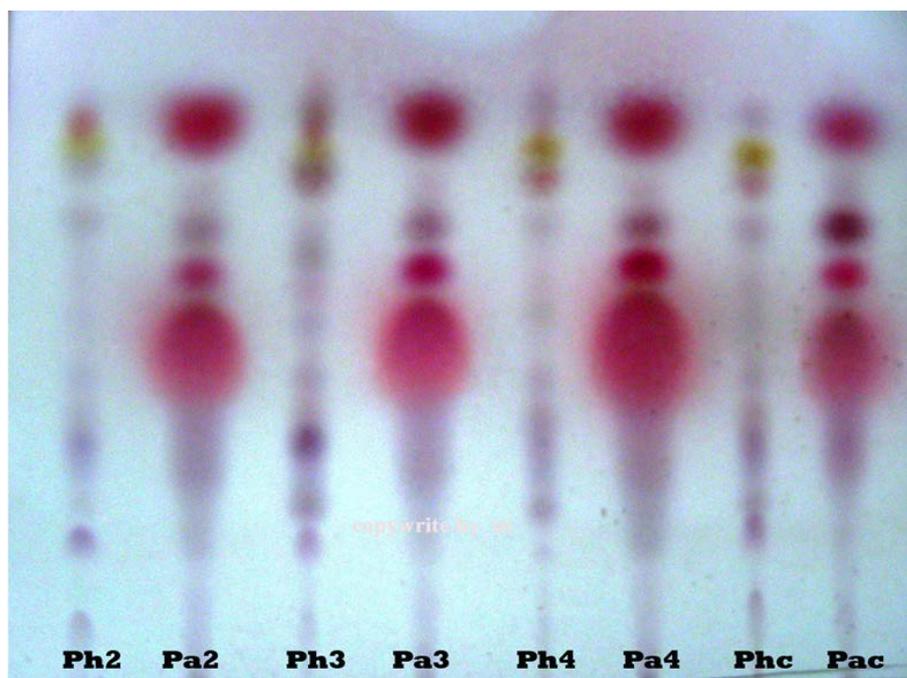


Figure 4. Thin Layer Chromatogram of essential oil of *Plectranthus hadiensis* and *Plectranthus amboinicus* at different maturity stages. [Ph₂ & Pa₂ = leaf essential oil of *Plectranthus hadiensis* and *Plectranthus amboinicus* after two months of transplanting; Ph₃ & Pa₃ = leaf essential oil of *Plectranthus hadiensis* and *Plectranthus amboinicus* after three months of transplanting; Ph₄ & Pa₄ = leaf essential oil of *Plectranthus hadiensis* and *Plectranthus amboinicus* after four months of transplanting; Phc & Pac = leaf essential oil of *Plectranthus hadiensis* and *Plectranthus amboinicus* collected from open market solvent system Methanol 1: cyclohexane 1: dichloromethane 4]

According to the WHO guidelines good Agricultural and Collection Practices (GAP), proper identification of a plant species, quality control and standardization of herbal materials are considered key issues in the herbal drug standardization. In the present study we investigate the variation of major yield and growth parameters with the maturity, key taxonomically important morphological and anatomical features, powder microscopic analysis, essential oil content with maturity, TLC profile of essential oil with the maturity of *Plectranthus hadiensis* and *Plectranthus amboinicus* by using recommended protocols. Our results on morphological variations, anatomical and powder microscopic analysis and chemical analysis are in agreement with previous studies [1,22]. Therefore, results of the present study either singularly or as a whole could be incorporated for standardization and quality control of *P. hadiensis* and *P. amboinicus*.

4. Conclusions

Results of the present study on morphological, anatomical, powder microscopical, TLC and essential oil contents are key parameters of quality control and standardization of *P. hadiensis* and *P. amboinicus*. Therefore, results of the present study either singularly or as a whole could be incorporated for quality control and standardization of either raw materials finished products containing *P. hadiensis* and *P. amboinicus* as raw materials. Further, presence of higher physical growth of the plant, higher content of essential oil and TLC spots with higher colour intensity scientifically validate the traditional claims of harvesting of both plants at fully maturity stage (after 12 weeks of age).

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