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Thin layer chromatographic analysis for various secondary metabolites in the methanolic leaf and root extracts of *Hypochaeris radicata* L.

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ABSTRACT

Hypochaeris radicata, an edible perennial herb is native of Europe has many medicinal uses which include antimicrobial, antioxidant, antiinflammatory and anticancer activities. The study was aimed at to investigate the phytochemical constituents present in the leaf and root extracts of *H. radicata* by thin layer chromatography. The results of the study revealed the presence of phytochemical constituents such as alkaloids, flavonoids, saponins, terpenoids and glycosides in the leaf and root extracts of this species. Further, the thin layer chromatographic profiles may serve as characteristic fingerprint for leaf and root extracts of *H. radicata*. It could be concluded that as the species, *H. radicata* contained rich variety of phytochemicals, it can be used for drug preparations in pharmaceutical industries.

Keywords: *Hypochaeris radicata*, Asteraceae, TLC, secondary metabolites.

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INTRODUCTION

Nature has bestowed on us a very rich botanical wealth and a large number of diverse types of plants. Medicinal and aromatic plants constitute a major segment of the flora, which provides raw materials for use in the pharmaceuticals, cosmetics and drug industries[1]. Plants have been one of the important sources of medicine even since the dawn of human civilization. In spite of tremendous developments in the field of allelopathy during the 20th century, plants still remain one of the major sources of drugs in modern as well as traditional system of medicine throughout the world. Approximately one-third of all pharmaceuticals are of plant origin[2]. There is a growing tendency all over the world shift from synthetic to natural based products including medicinal plants.

The separation and purification of plant constituents are mainly carried out on chromatographic techniques based on their size, shape, or charge[3]. Thin layer chromatography (TLC) is the most widely used for all the simple chromatographic methods for the analysis of mixtures[4]. It is widely adopted for the rapid and positive analysis of drugs and drug preparations. TLC provides a chromatographic drug fingerprint in very short time. It is therefore suitable for monitoring the identity and purity of drugs, and for detecting adulterations and substitutions. It is also used to analyze drug combinations and phytochemical preparations[5].

The plant species, *Hypochaeris radicata* belongs to the family; Asteraceae is an edible perennial herb, distributed in high hills of Nilgiris, the Western Ghats at 2000m above msl. The whole plant is said to be medicinally important by having anti-inflammatory, anticancer, antioxidant[6], antibacterial[7], antifungal[8] and antidiuretic properties. It is being used for the treatment of jaundice, rheumatism, dyspepsia, constipation, hypoglycemia and kidney related problems in traditional medical practice of Tamil Nadu, India[9]. The present study was carried out to evaluate qualitative phytochemical constituents of various secondary metabolites present in the methanolic leaf and root extracts of this species by using TLC.

MATERIALS AND METHOD

Collection and authentication of plant material

The leaf and root parts of *H. radicata* were collected from Kattabettu, Nilgiris, the Western Ghats, India. The plant was authenticated in Botanical Survey of India, Southern Circle, Coimbatore by referring the deposited specimen. The voucher number of the specimen is BSI/SRC/5/23/2010-11/Tech.153.

Extraction of the plant samples

The fresh plant materials were washed with running tap water, shade dried and then homogenized to fine powder. About 50g of leaf and root powders were extracted with methanol (250ml) using soxhlet apparatus at 60-80°C separately. The solvent present in the extracts were reduced under room temperature.

Phytochemical analysis

Thin layer chromatography (TLC)

Methanolic leaf and root extracts of *H. radicata* were subjected to qualitative phytochemical analysis of TLC[5]. It was performed by using silica gel-G as stationary phase in the chromatographic plates of 15x5cm with 3mm thickness to confirm the presence of secondary metabolites. For the separation of phytochemical compounds, the methanolic leaf and root extracts were spotted manually using capillary tube. The spotted plates were put in a solvent chamber which contained various solvent systems to detect the suitable mobile phase. After the separation of phytochemicals, various spray reagents such as 10% ethanolic sulphuric acid, dragendorff reagent, 5% ferric chloride solution, vanillin sulphuric acid reagent, vanillin phosphoric acid reagent and kedde reagent were used to identify the compounds. The colour of the spots was noted and R_f values were calculated by using the following formula:

Retention factor (R_f) = Distance travelled by the solute/Distance travelled the solvent X 100

RESULTS AND DISCUSSION

TLC is the earliest of chromatographic techniques to perform and require simple apparatus[10]. It is a valuable tool for preliminary exploration of many kinds. Similar to paper chromatography, TLC can be used to determine characteristic component-patterns of plant extracts and biochemical preparations[11]. They readily provide qualitative information and it separate small amounts of compounds. Compared to paper chromatography, the special advantages of TLC are the versatility (using different adsorbents), speed (more compact nature of the adsorbent) and sensitivity (only µg amounts of materials can be achieved).

The separation and identification of the phytochemical compounds can made by scanning these chromo-strips with/without detecting reagents, under visible or UV-light. The resulting degree of difference in chromatographic "fingerprints" can essentially be used as 'markers" in standardization of all extract in particular solvent system separating compounds at specific Rf value which differ to other plants extracts. These Rf values are simple, reproducible and thus reliable marker to verify the purity of the crude drugs. TLC has many advantages such as lower cost, short time analysis, the possibility of multiple detection and specific derivatization on the

same plate.

The TLC studies proved the presence of secondary metabolites such as alkaloids, flavonoids, saponins, terpenoids and glycosides present in the methanolic leaf and root extracts of the study species, *H. radicata* (Tables 1 and 2).

Table 1. Qualitative phytochemical analysis by TLC in the methanolic leaf extract of *Hypochaeris radicata.*

Compound	Mobile phase	Total no of bands	Rf value (%)	Colour of the spot/band	Spraying reagent
Alkaloids	Chloroform: Methanol: Glacial acetic acid (83:17:10)	1	0.69	Brown	10% Ethanolic sulphuric acid
	Chloroform: Methanol (30:15)	1	0.23	Brown	Dragendorff reagent
Flavonoids	Ethylacetate: Glacial acetic acid: water (90:10:10)	1	0.62	Gray	5% Ferric chloride solution
	Ethylacetate: Glacial acetic acid: water (80:10:10)	1	0.64	Gray	
	Ethylacetate: Glacial acetic acid: water (100:10:10)	1	0.77	Gray	
Saponins	Chloroform: Methanol (30:5)	3	0.79 0.56 0.92	Violet	Vanillin sulphuric acid
Terpenoids	Benzene: Ethyl acetate (5:95)	1	0.58	Blue	Vanillin phosphoric acid
	Benzene: Ethyl acetate (20:1)	1	0.48	Blue	
	Petroleum ether: Ethyl acetate(40:10)	1	0.74	Blue	
	Hexane: Ethyl acetate (30:10)	2	0.79 0.18	Blue	
Glycosides	Ethyl acetate: Methanol (13:4)	1	0.79	Yellowish orange	Kedde reagent
	Ethyl acetate: Methanol (12:4)	1	0.43	Yellowish orange	

Alkaloids are synthesized form of aminoacids and it contain one or more N atoms as constituents of heterocytes. It is stored in protonated form, mostly in the vacuole, which is acidic. They have multiplicity of host-mediated biological activities, including antimicrobial[12,13], cytotoxic[14], analgesic and antipyretic activities[15].

Table 2. Qualitative phytochemical analysis by TLC in the methanolic root extract of *Hypochaeris radicata.*

Compound	Mobile phase	Total no of bands	Rf value (%)	Colour of the spot/band	Spraying reagent
Alkaloids	Chloroform: Methanol (83:17)	1	0.71	Brown	10% Ethanolic sulphuric acid
	Chloroform: Methanol (30:15)	1	0.21	Brown	Dragendroff reagent
Flavonoids	Ethylacetate: Methanol: water(100:20:12)	1	0.45	Gray	5% Ferric chloride solution
Saponins	Chloroform: Methanol (15:1)	3	0.85	Violet	Vanillin sulphuric acid
Terpenoids	Benzene: Ethyl acetate (20:1)	3	0.49 0.69 0.85	Blue	Vanillin phosphoric acid
	Petroleum ether: Ethyl acetate(40:10)	2	0.77 0.86	Blue	
	Hexane: Ethyl acetate (30:10)	3	0.26 0.67 0.86	Blue	
Glycosides	Ethyl acetate: Methanol (26:8)	1	0.89	Yellowish orange	Kedde reagent

For the detection of alkaloids present in the methanolic leaf extract, two different combinations of chloroform, methanol and glacial acetic acid (83:17:10) and chloroform and methanol (30:15) have been used for the mobile phase. Similarly, two different combinations of chloroform and methanol in the ratio of 83:17 and 30:15 were used for the detection of alkaloids in methanolic root extract. The Rf value of methanolic leaf and root extract were 0.69, 0.23 and 0.71, 0.21 respectively. For this study, 10% ethanolic sulphuric acid and dragendorff reagents are used to detect the presence of alkaloids (Figure 1 & 2.).

Flavonoids

Flavonoids are phenolic compounds that contain 15 carbon atoms in C_6-C_3-C_6 basic carbon skeleton. They exist in the form of glycosides, accumulated in vacuole and chromoplast. It is used for the colouration of flowers and leaves. They have the properties of antibacterial[16], antioxidant[17], anticancer[18], antiinflammatory, antipyretic and analgesic activities[19].

The TLC studies were performed for detection of flavonoids using the mobile phase of ethyl acetate, glacial acetic acid and water (80:10:10, 90:10:10 and 100:10:10) for methanolic leaf extract and ethyl acetate, methanol and water (100:20:12) for methanolic root extracts. Their Rf values were seen at 0.64, 0.62, 0.77 and 0.45. After derivatization the spots were gray in colour with the spraying reagent 5% ferric chloride (Figure. 3.).

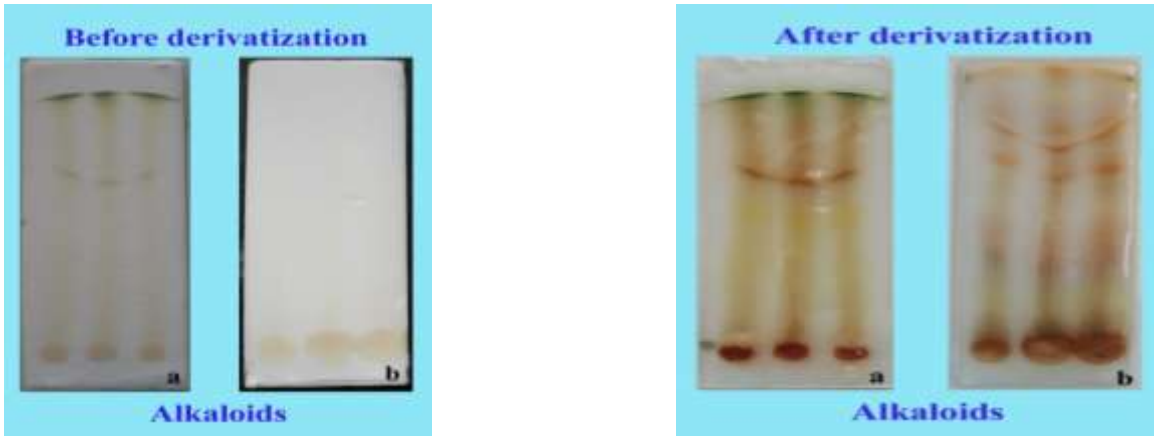

Figure. 1. TLC for detection of alkaloids in methanolic leaf and root extracts of *Hypochaerisradicata.* a-leaf [Chloroform: Methanol: Glacial acetic acid (83:17:10)], b-root [Chloroform: Methanol (83:17)].

Figure. 2. TLC for detection of alkaloids in methanolic leaf and root extracts of *Hypochaeris radicata.* a-leaf, b-root [Chloroform: Methanol (30:15)].

Figure. 3. TLC for detection of flavonoids in methanolic leaf and root extracts of *Hypochaeris radicata.* a-leaf [Ethylacetate: Glacial acetic acid: water (100:10:10)], b-root [Ethylacetate: Methanol: water (100:20:12)].

Saponins

Saponins are glycosidic triterpenoids with foaming characters widely found in plants. These consist of polycyclic aglycones attached to one or more sugar side chains. They are soluble in water and have bitter taste. Three major classes are found as steroid glycosides, steroid alkaloid glycosides and the largest group, triterpene glycosides. There are found in most vegetables, beans and herbs. They have the pharmacological properties like anti-inflammatory and antipyretic activities[20,21]. In the present study, saponins in methanolic leaf and root extracts were determined by using the solvent system chloroform and methanol (30:5 and 15:1 respectively). Three spots detected in leaf extracts were seen at Rf=0.79, 0.56, 0.92 and in root extract the spots were seen at Rf=0.85. They were violet in colour after spraying the vanillin sulphuric acid reagent (Figure. 4.).

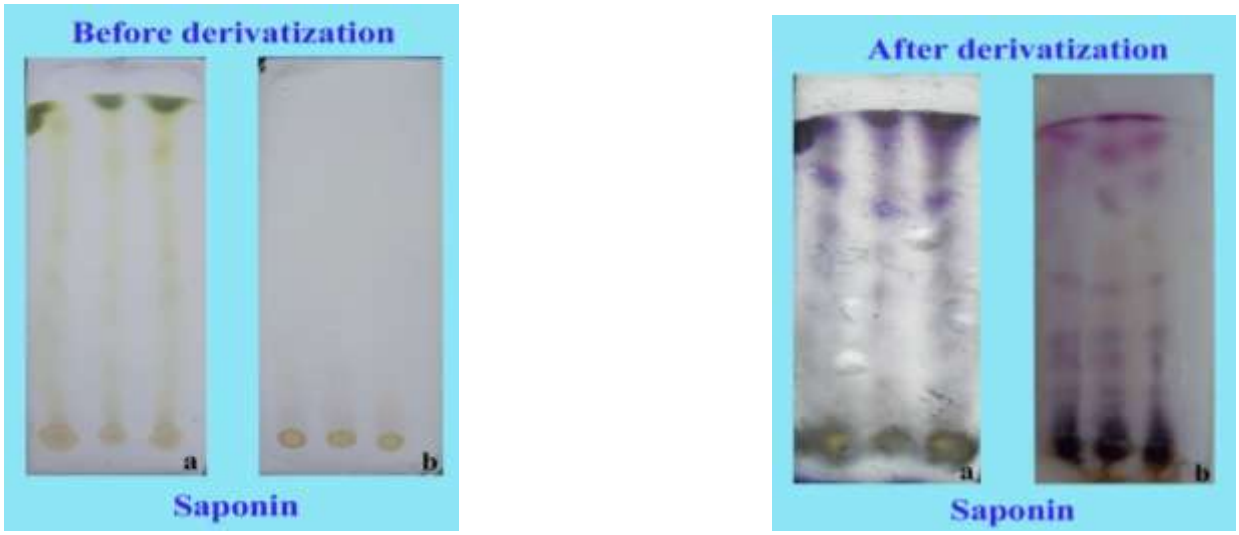

Figure. 4. TLC for detection of saponins in methanolic leaf and root extracts of *Hypochaeris radicata.* a-leaf [Chloroform: Methanol (30:5)], b-root [Chloroform: Methanol (15:1)].

Terpenoids

Isoprenoids are also called as terpenoids and they have found in resins, latex, waxes and oils and they make plants toxic or indigestible as a defense measure against herbivores. They act as antibiotics to protect the plants from pathogenic microbes. It is used commercially as aroma substances for food beverages and cosmetics, vitamins (A,D and E), natural insecticides (e.g. pyretrin), solvents (e.g. turpentine) and as rubber and gutta-percha. They are reported to have antimicrobial, antioxidant[22], anticancer[23] and antiparasitic activities[24]. In the TLC fingerprinting analysis, three different compositions of solvent systems were used for detecting terpenoids for the study species, *H. radicata*..

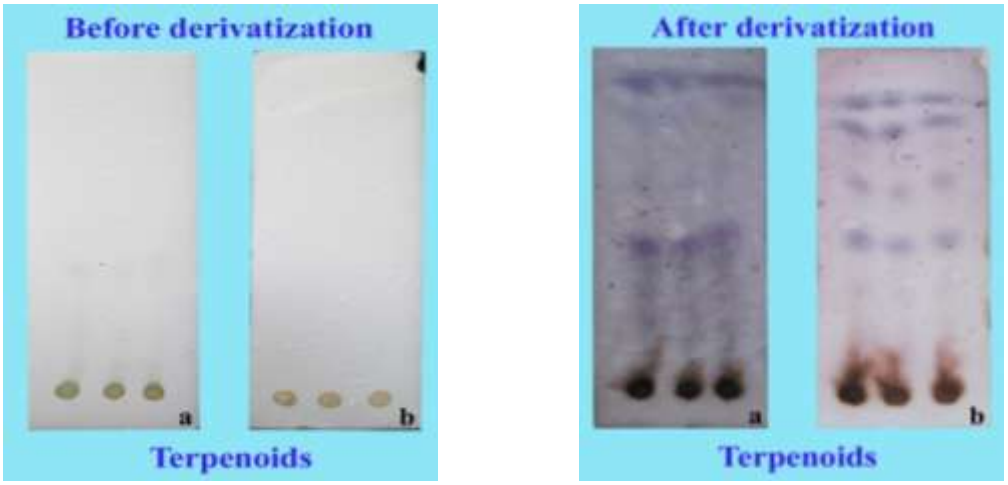

Figure. 5. TLC for detection of terpenoids in methanolic leaf and root extracts of *Hypochaeris radicata.* a-leaf, b-root [Benzene: Ethyl acetate (20:1)].

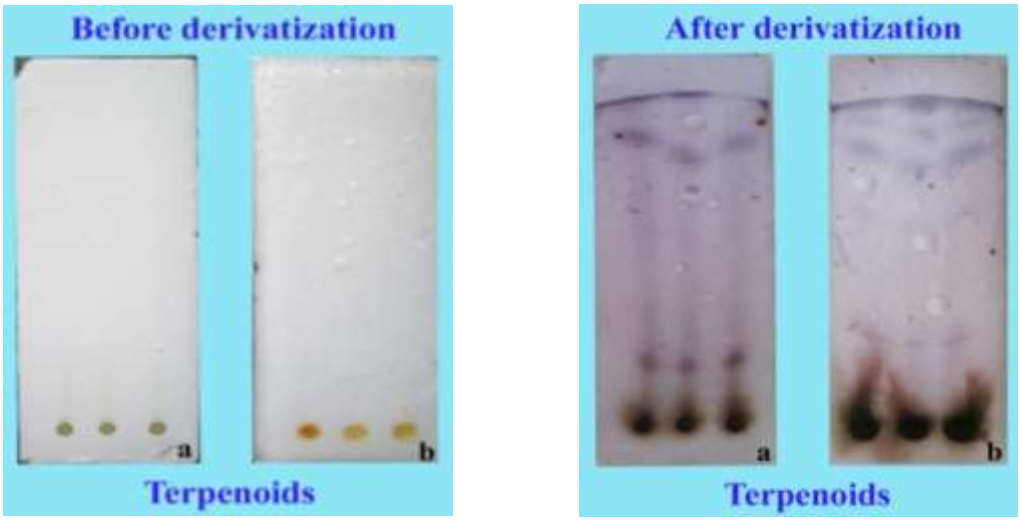

Figure. 6. TLC for detection of terpenoids in methanolic leaf and root extracts of *Hypochaeris radicata*. a-leaf, b-root [Petroleum ether: Ethyl acetate (40:10)].

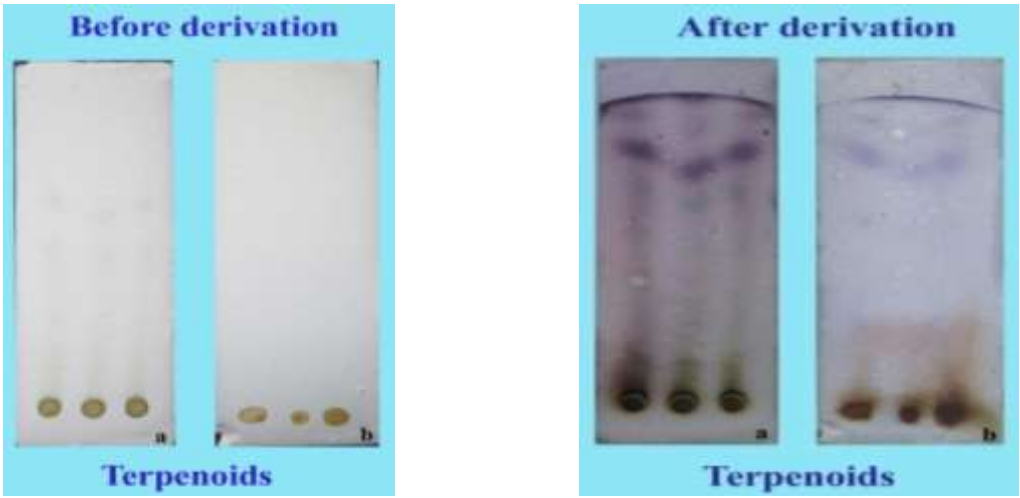

Figure. 7. TLC for detection of terpenoids in methanolic leaf and root extracts of *Hypochaeris radicata.* a-leaf, b-root [Hexane: Ethyl acetate (30:10)].

The mobile phases identified for both leaf and root extracts were benzene and ethylacetate (20:1), petroleum ether and ethyl acetate (40:10) and hexane, ethylacetate (30:10). The separated peaks were identified at Rf values for leaf extract 0.48, 0.74, 0.79 and 0.18. In methanolic root extract, three spots were detected in benzene, ethyl acetate (Figure. 5.) their Rf values were seen at 0.49, 0.69 and 0.85, two spots were detected in petroleum ether and ethyl acetate (Figure. 6.) their Rf values were seen at 0.77 and 0.86 and also three spots were detected in hexane and ethylacetate (Figure. 7.) with Rf values were seen at 0.26, 0.67 and 0.86. After spraying vanillin phosphoric acid reagent, spots turned blue in colour

Glycosides

Glycosides consist of a sugar group called, glycone chemically attached to a non-carbohydrate compound is known as aglycone or a genin. Glycosides have the properties of antiinflammatory[25], antiproliferative[26] and anxiolytic activities[27]. The TLC was well resolved for methanolic leaf and root extracts of the species, *H. radicata*. For detection of glycosides the solvent system ethyl acetate and methanol were used. For leaf extract two different combinations 13:4 and 12:4 for root extract one combination 13:4 were used. Rf value of methanolic leaf extracts were 0.79 and 0.43 and root extract was 0.89. Yellowish orange colour was obtained by spraying the Kedde reagent (Figure. 8.).

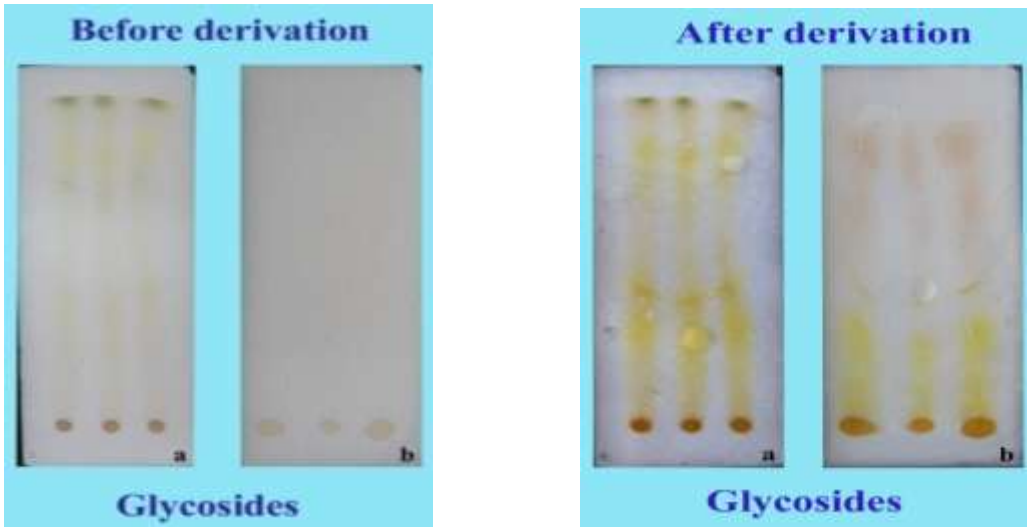

Figure. 8. TLC for detection of glycosides in methanolic leaf and root extracts of *Hypochaeris radicata.*

a-leaf, b-root [Ethyl acetate: Methanol (13:4)].

CONCLUSION

The TLC study of leaf and root extracts of *Hypochaeris radicata* confirmed the presence of various secondary metabolites such as alkaloids, flavonoids, saponins, terpenoids and glycosides.

Presence of wide spectrum of phytochemical constituents proved the traditional medicinal usage of this species. Further investigation on isolation and purification of phytochemical compounds and pharmacognosy are suggested before commercializing this species for pharmaceutical industries.

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