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Identification of pharmacognostic and phytochemical biomarkers to distinguish between *Hedyotis corymbosa* (L.) Lam. and its adulterant, *Glinus oppositifolius* (L.) A.DC.

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ABSTRACT

Hedyotis corymbosa (L.) Lam. is a well-known medicinal herb often confused with a similar looking plant, *Glinus oppositifolius* (L.) A.DC. Micromorphological, pharmacognostic, phytochemical and HPTLC fingerprint differences between these two herbs were identified for the biomarkers aiding in correct identification of the former plant, as well as to detect adulteration. Different parameters were studied for the plant materials in fresh form, powder form and extract form. The study of stomata in fresh plants showed stomata to be paracytic in *H. corymbosa* and anomocytic in *G. oppositifolius*. Microscopic studies of the powders of both plants showed raphides in the former plant, while cystoliths and sphaeraphides in *G. oppositifolius*. Phytochemical analysis of the plants indicated the presence of three flavonols in *H. corymbosa* and two flavones in the other. Two anthocyanins identified in *H. corymbosa* were found absent in *G. oppositifolius*. Phenolic acid content also varied in both plants. The HPTLC fingerprints of both the plants showed enough variation for identification of adulteration.

Keywords: *Hedyotis corymbosa*, *Glinus oppositifolius*, adulteration, pharmacognostic and phytochemical biomarkers.

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INTRODUCTION

Adulteration becomes a very serious problem with crude drugs, and often occurs when a drug is not easily available or when its price is comparatively high. Adulteration, in many cases, may not be intentional. In many cases it could occur due to mistaken identity of the plant. The adulterator chooses a suitable material that is cheap and readily available. Since, flowers form the key tool for identification of a plant, in their absence, the vegetative parts are considered for identification purpose. Similar looking leaves can mislead a person and thus cause wrong identification of the plant. Collection of the wrong plant erroneously by unskilled collectors also is a major reason contributing to the adulteration of the plant of interest. The therapeutic efficiency of the drugs used in these systems depends greatly on the use of proper and genuine raw materials. Due to this reason, the assurance of safety, quality and subsequent efficacy of the medicinal plants and herbal products has now become a major and key issue.[1] Checks on adulteration mainly includes biomarkers identified by micromorphological, anatomical and powder studies, though TLC or HPTLC fingerprinting also is an essential feature. Therefore, in the present study, *Hedyotis corymbosa* and its adulterant *Glinus oppositifolius* were subjected to micromorphological studies, powder characteristics, phytochemical analysis and variation in the HPTLC fingerprints to detect the biomarkers which distinguishes the genuine drug from the other.

Hedyotis corymbosa (L.) Lam. is spreading, suffruticose annual, frequently found especially during monsoon in fields throughout India, Sri Lanka, tropical East Asia to Java and the Phillipines [2]. It is referred to as *Parpatakah*, which is esteemed as a specific remedy for all types of fevers. It overcomes the morbid *pitta* and *kapha*, purifies blood, improves digestion, stimulates the action of liver and cures burning sensation, thirst and skin diseases. The drug is diuretic, anthelmintic, digestive and constipating. The important preparations of the drug are *Amritarishtam*, *Candanasavam*, *Mahatiktaghrtam*, *Jatyadi tailam*, *Aranyatulasyadi* coconut oil etc.[3] The plant is known to clear heat and toxins, activate blood circulation, promote diuresis and relieve stranguria (urinary obstruction). It is also known to act against tumours of the digestive tract lymphosarcoma and carcinoma of the liver and larynx. It is also active against appendicitis, hepatitis, pneumonia, cholecystitis, urinary infection, cellulites and snake bite. Chinese folk medicine describes the plant to treat skin sores, ulcers, sore throat, bronchitis, gynaecologic infections and pelvic inflammatory diseases[4,5,6,7]. *Glinus oppositifolius* (L.) A. DC., *Hedyotis brachypoda* (DC.), *Hedyotis diffusa* Willd., *Polycarpaea corymbosa* (L.) Lamk., *Mollugo stricta* Linn., and *Justicia procumbens* Linn. also are referred to as *parpatakah* in various texts, and they can be used as adulterants for *Hedyotis corymbosa*.

Glinus oppositifolius (L.) A. DC. (Syn: *Mollugo spergula* Linn., *Mollugo oppositifolia* Linn.) is a herb similar to *Hedyotis corymbosa* in appearance and leaf characteristics, found distributed throughout India, Ceylon, Tropical Africa and Australia. The plant is also considered stomachic, antiseptic and aperient. It is smeared with castor oil and applied warm for earache. The juice is applied to treat itching and other skin diseases.[8]

The parameters that can aid in rapid identification such as micromorphological studies, powder characteristics, phytochemical analysis and variation in the HPTLC

fingerprints were looked into during the present study. Micromorphology can be used to detect adulteration when the plant is obtained in fresh form. In case of dried plant powder, differences in powder characteristics as well as HPTLC fingerprint profiles can be utilized to ascertain the purity of the given plant powder.

MATERIAL AND METHODS

Hedyotis corymbosa collected from Vadodara, India, was identified and authenticated at Botanical Survey of India, Pune. The voucher specimen of this plant (No.BSI/WC/Tech/2007/734) is deposited at the Herbarium, B.S.I., Pune. *Glinus oppositifolius* was collected from Vadodara, India, identified and authenticated at Department of Botany, M. S. University of Baroda. The voucher specimen of this plant (No. BARO/2007/186) is deposited at the Herbarium, BARO, Department of Botany, M. S. University of Baroda. The plant materials were washed, shade dried for a day and then dried completely in an oven at 38°C. The plants were coarsely powdered using a rotary grinder and stored in airtight plastic containers, and then used for phytochemical analysis and HPTLC fingerprinting. Fresh leaves were used for micromorphological studies.

Micromorphology: Fresh leaves were washed and small fragments of leaves were taken from the middle region of the lamina of mature leaves. Washed leaf fragments were first boiled in 90% alcohol for about 3-5 minutes to remove chlorophyll, then washed 2-3 times with water, then boiled again with 10% KOH solution for 2-3 minutes and washed 4-5 times with water and kept in clean water to remove all traces of the clearing agent[9]. The epidermal layer was peeled off using the help of pointed needle and forceps. The epidermal peels were washed in water, stained with Safranin (0.5%) in water and then mounted in 50% glycerine; the margins of the coverslips were sealed with DPX [10], and the slides were observed under the microscope.

Powder studies: Completely dried plant material was finely powdered and sieved through BSS mesh No. 85. The fine powder obtained was stained using Safranin in water. The stained powder was mounted on a slide and observed under a microscope to locate and identify the characters present. The characters observed were photographed under a Leica DM 2000 microscope connected to a digital Canon camera.

Phytochemical Analysis: Methanolic Soxhlet's extracts of the two plants were individually analyzed for the various classes of phytoconstituents such as flavonoids, phenolic acids, anthocyanins, quinones, alkaloids, tannins, saponins and iridoids using standard phytochemical methods.[11]

HPTLC analysis: About 250 mg of plant powder was placed in a 20 cm³ stoppered test tube, to which 10 cm³ of distilled water was added. The test tube was sealed and subjected to overnight extraction on a rotary shaker. The solution was filtered and the filtrate was subjected to acid hydrolysis using 7% HCl. After hydrolysis the solution was cooled and transferred to a separating funnel. Approximately 2 cm³ of ethyl acetate was added and the separating funnel was shaken thoroughly. The organic layer was separated and the aqueous layer was treated with again with 2 cm³ of ethyl acetate. The organic layer so separated was

pooled with the organic fraction obtained earlier. The pooled ethyl acetate fractions were evaporated on a water bath. The residue obtained was reconstituted in 1cm³ methanol and used directly for HPTLC analysis. Each of the plant extracts were spotted in duplicate on precoated silica gel 60F254 plates (Merck) using CAMAG Linomat V sample applicator. The mobile phase employed was ethyl acetate: toluene (7:3, v/v). The plates were developed in CAMAG twin trough development chambers (10x10) and visualized under short wave UV (254nm) light. This method was used to quantify ferulic acid in *H. corymbosa*, so the scanning of the plate was performed at 310 nm, the λ_{max} of ferulic acid. Densitometric scanning of the plates was performed using CAMAG TLC Scanner 3.

RESULTS

The characters observed in the micromorphology and in the powders of the two plants are shown in Plate 1 and 2. Phytochemicals obtained from both the plants are compared in Table 1. Similarly the HPTLC chromatograms of methanolic extracts of *H. corymbosa* and *G. oppositifolius*, prepared under similar conditions are presented in Figures 1 and 2.

DISCUSSION

Micromorphology: *Hedyotis corymbosa* clearly exhibited the presence of paracytic stomata, while *Glinus oppositifolius* possessed anomocytic stomata (Plate 1). Both these stomatal types are very different from each other and can thus help in differentiating between the two plant species. *Hedyotis corymbosa* showed no vein terminations since the veins formed a continuous network within the leaf. *Glinus oppositifolius*, on the other hand, showed 3-5 vein terminations per square millimetre. This forms an important identification tool to differentiate between the two species, since the leaves of both the plants appear to be very similar, which increases chances of misidentification.

Powder study: Leaf of *Hedyotis corymbosa* showed the presence of serrated edges, which were also observed as fragments in the whole plant powder (Plate 2). These serrations were absent in case of *Glinus oppositifolius*. The former plant showed presence of a large number of raphides in its powder, which were absent in *Glinus oppositifolius*. The calyx of *Hedyotis corymbosa* showed a net-like arrangement in the epidermal cell walls, which was absent in case of *Glinus oppositifolius*. The whole plant powder of *Glinus oppositifolius* showed the presence of a number of characters such as rhomboidal calcium oxalate crystals, starch grains and sphaeraphides, all of which were found absent in case of *Hedyotis corymbosa*. These characters can help in differentiating between the powders of the two similar looking plants. Another very interesting character observed in the *Glinus oppositifolius* powder was the presence of cystoliths, none of which were observed in case of *Hedyotis corymbosa*.

Phytochemical Analysis: Analysis of the methanolic extracts of *Hedyotis corymbosa* and *Glinus oppositifolius* revealed many chemical differences (Table 1). Only the phenolic acids showed similarity, while other classes showed many variations which could help in differentiating between the extracts of the two plants. *H. corymbosa* showed the presence of flavonols such as Quercetin, 3'-Methoxy quercetin and 3', 4'-Dimethoxy quercetin while flavonols as a whole were absent in *Glinus oppositifolius*. Instead the latter plant contained

flavones such as 4'-Methoxy apigenin and 4', 7-dimethoxy apigenin. (*H. corymbosa* was found devoid of flavones.) The glycoflavone Vitexin is reported only in *Glinus oppositifolius*. Glycoflavones were found absent in *H. corymbosa*. Vanillic, syringic acid, *p*-hydroxy benzoic, *p*-coumaric, ferulic and caffeic acids were the phenolic acids found common in both plants. However, melilotic acid was found present only in *H. corymbosa*. Cyanidin and pelargonidin were the anthocyanidins located in *Hedyotis corymbosa*. *G. oppositifolius* did not show the presence of any such pigmentation. Iridoids were found in all the parts of *H. corymbosa*, on the basis of qualitative tests using Trim-Hill reagent, and were found absent in *G. oppositifolius*. Other coloured pigments such as quinones and betacyanins were found absent in both plants. Tannins, hydroxy coumarins and saponins were found absent in both plants. However, qualitative tests showed the presence of alkaloids in both the plants.

H.P.T.L.C. Fingerprinting: Though the methods of extraction and chromatographic conditions for both plants were kept identical, the HPTLC chromatograms obtained when the HPTLC plate was scanned at 310 nm, the λ_{max} of the reference standard used for the analysis of *Hedyotis corymbosa*, showed immense variations. *Hedyotis corymbosa* exhibited bands at R_f 0.41, 0.48, 0.54, 0.65, 0.74 and 0.85 (Figure 1). On the other hand, the extract of *Glinus oppositifolius* showed just two intense bands at R_f 0.49 and 0.57, apart from bands of lower intensity at R_f 0.25, 0.37, 0.41, 0.75 and 0.87 (Figure 2). The peak at R_f 0.48/ 0.49 is common to both the plants. Since the chromatographic conditions were common for both the plant extracts, the peak should correspond to that of ferulic acid in the plants. Ferulic acid was identified during the phytochemical analysis of both the plants. However, the intense peak at R_f 0.57 in case of *Glinus oppositifolius* extract was absent in case of *Hedyotis corymbosa*. This peak could be taken as an important identification factor to differentiate between the two plants. Thus both the chromatograms showed enough variation to differentiate between the two plant extracts. However, the HPTLC fingerprint for *Glinus oppositifolius* needs to be developed separately as a quality control parameter for the plant. The chromatogram displayed for the plant in Figure 4 is only for the purpose of comparison with *Hedyotis corymbosa*, using the chromatographic conditions of the latter.

CONCLUSION

Magnitudes of differences were observed between *Hedyotis corymbosa* and its adulterant *Glinus oppositifolius*. The plant *Hedyotis corymbosa* contained paracytic stomata, absence of vein terminations, serrated edges on leaves, raphides in plenty, net-like arrangement of the epidermal cells in calyx, absence of cystoliths, rhomboidal calcium oxalate crystals, starch grains and sphaeraphides, anthocyanins and iridoids. *Glinus oppositifolius* showed the presence of anomocytic stomata, 3-5 vein terminations per square millimetre, distinct cystoliths, rhomboidal calcium oxalate crystals, starch grains and sphaeraphides, glycoflavones. It also showed absence of anthocyanins and iridoids. These differences can play a key role in proper regulation of collection and authentication of both the plant species, as well as detect adulteration. Irrespective of whether the plant is provided in the form of fresh material, powder, or extract, diagnostic characters for each case have been identified for both plants.

Plate 1: Stomata in lower epidermis of *Hedyotis corymbosa* and *Glinus oppositifolius*

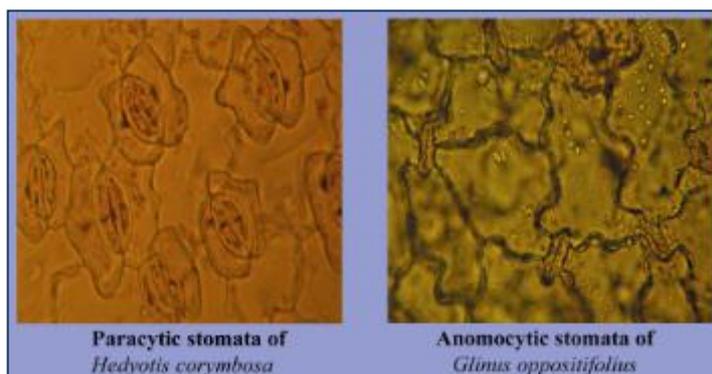
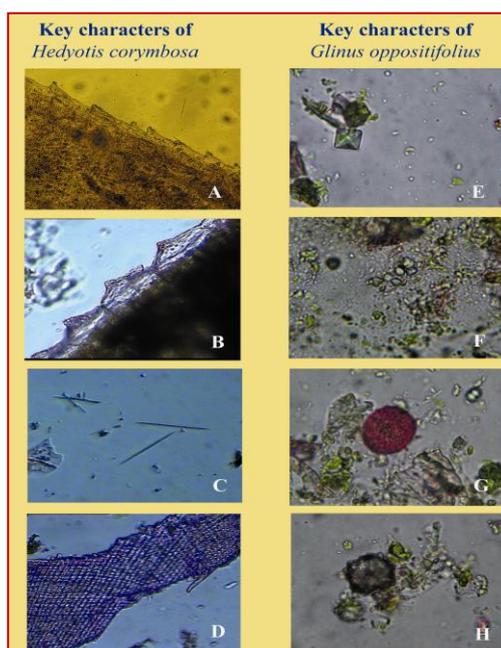


Plate 2: Characters observed in powder of *H. corymbosa*: Serrated edges of leaf (A, B), Raphides (C), Epidermal cells of the calyx (D). Characters observed in powder of *G. oppositifolius*: Prismatic calcium oxalate crystals (E), Starch grains (F), Cystolith (G), Sphaeraphide (H).



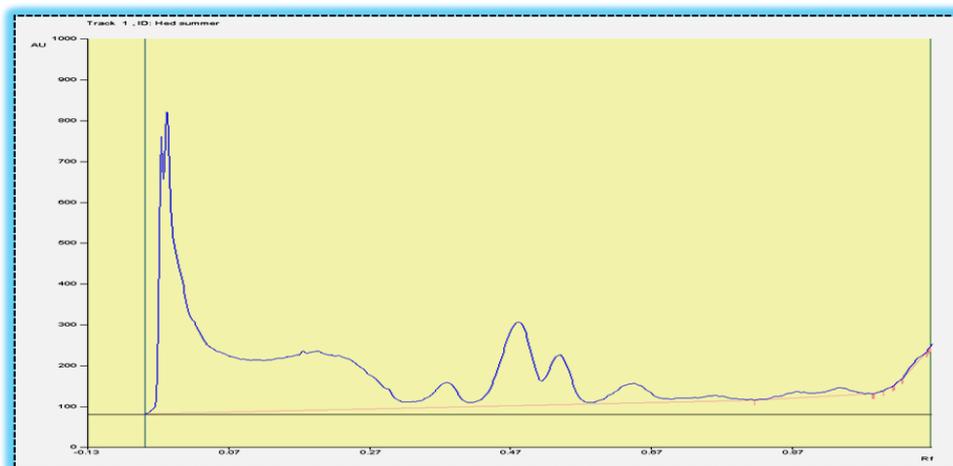


Figure 1: HPTLC chromatogram of *Hedyotis corymbosa*

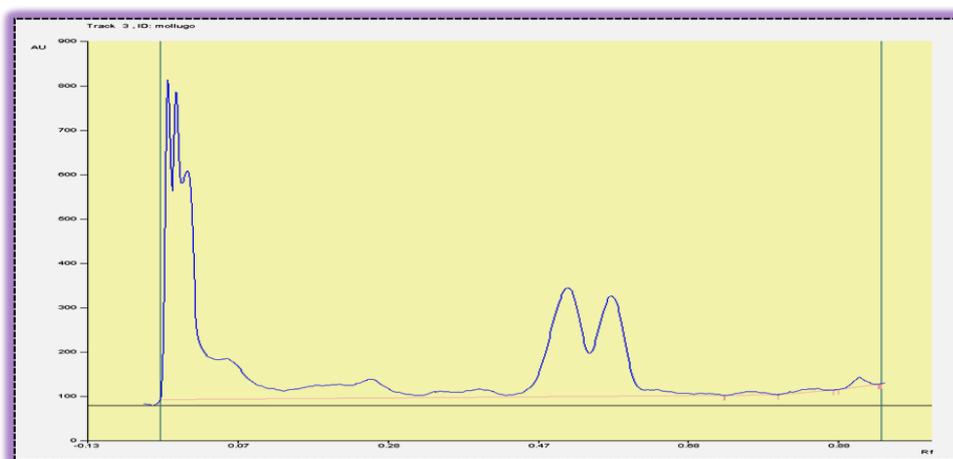


Figure 2: HPTLC chromatogram of *Glinus oppositifolius*

Table 1: Phytochemicals identified in *Hedyotis corymbosa* and *Glinus oppositifolius*

Class of phytochemical	<i>Hedyotis corymbosa</i>	<i>Glinus oppositifolius</i>
Flavonols	Quercetin, 3'-Methoxy quercetin and 3', 4'-Dimethoxy quercetin	-
Flavones	-	4'- Methoxy apigenin and 4', 7-Dimethoxy apigenin
Glycoflavones	-	Vitexin
Phenolic acids	Vanillic acid , Syringic acid , <i>p</i> -Hydroxy benzoic acid , <i>p</i> -Coumaric acid, ferulic acid , gentisic acid and caffeic acid	Vanillic acid , Syringic acid , <i>p</i> -Hydroxy benzoic acid , <i>p</i> -Coumaric acid, ferulic acid and caffeic acid
Anthocyanins	Cyanidin and pelargonidin	-
Betacyanins	-	-
Hydroxycoumarins	-	-
Alkaloids	+	+
Tannins	-	-
Saponins	-	+
Iridoids	+	-
Quinones	-	-



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