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HISTOLOGICAL AND PHYTOCHEMICAL ANALYSIS OF FOUR ETHNOMEDICINAL PLANTS USED TO CURE SKIN DISEASES

SEARCH

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ABSTRACT: Natural plant-derived compounds have been an essential resource in the evolution of drug discovery since the dawn of time. Using plants as a source of medicine is a century-long-standing tradition not merely in India but also in the entire world. North-East India is bestowed with incredible biological richness. The current research is focused on the histological and phytochemical analysis of four potent plant species collected from Goalpara district of Assam that is widely used in treating skin disorders. The four medicinal plants were collected based on traditional knowledge. The plants were evaluated for the identification and histochemical localization of effective phytochemicals following the standard protocols. Qualitative phytochemical analysis revealed the presence of important phytoconstituents responsible for different biological activities, including dermal activity. The histological study further showed the localization of phytochemicals, alkaloids, terpenoids, tannins, phenolics and flavonoids in all four plants. From adults to neonates, skin disorders are a major problem, especially in developing countries. Dermatological problems often deal with costly medications. The present study indicates that the studied plant species could be a dynamic avenue for isolating bioactive chemicals, and novel therapeutics can be developed to combat dermatological problems with potential and cost-effective solutions.

INTRODUCTION: Nature acts as an effective chemist of humankind. Though a vast quantity of plant species was explored, the pharmacognosy of many plant-derived compounds is still untouched and beyond the imagination of even our best researchers 1,2 .

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Since, ancient times, the reliance on plant-derived drugs has led the way for the concept of ethnopharmacology. The history of plant-derived pharmaceuticals is chronicled in ancient Indian writings like the Atharvaveda (2000 BC), the Eber Papyrus (1550 BC) of Egypt, and the Divine Farmer's Herb-Root Classic (3000 BC) of China.

Before the bioactive molecule principle emerged, ancient people accidentally discovered many plantbased drugs and used them from immemorial. For example, the potent anti-malarial drug quinine was derived from the bark of Cinchona ³. According to WHO 80% of people in developing countries depend on traditional plant-based medicine systems ⁴. A substantial number of synthetic medicines are derived from natural harbingers. World Health Organization (WHO) lists 252 medications as fundamental and indispensable, of which 11% are complete of plant origin ⁵. But still, pharmacological screening is yet to be done for most higher plants.

Being one of the world's thirty-six mega biodiversity hubs, India includes four biodiversity hot spots, the Western Ghats, Sundaland, Himalaya, and Indo Myanmar region ^{6, 7}. North-East India is blessed with an embellished covering of rich biological diversity. North-East India is accredited as an ethnobotanical treasure trove of unfathomed therapeutic plants ⁸. Previous studies also reported numbers of medicinal plants used to treat skin diseases in northeastern India ⁹.

The human skin, which functions as the first line of the defense system and the largest organ of our body, plays the most significant role in life processes. Skin is separated into three primary layers: hypodermis, dermis, and epidermis, each with its own set of specialized cells and structures.

Skin performed functions like body shape maintenance, organ protection, body temperature regulation, elimination of toxins from the body by sweat excretion, preservation of body fluids, and percutaneous absorption, among others. Skin diseases are considered a major threat that affects the quality of life. People of every age and gender can be infected with skin diseases. Skin problems are most prevalent in tropical areas of the globe.

The harmful effect of skin disease ranges from physical incapacity to death. Rural people are mostly affected by diverse types of skin diseases. In recent times, skin diseases have gained much importance due to their close association with AIDS/HIV. It was established from clinical studies that more than ninety percent of HIV-infected people experienced mucosal and skin-related issues later in the disease ¹⁰.

In India, the burden of skin diseases has increased significantly in the last few decades. Factors like poor hygiene, lack of basic amenities, and overcrowding enhance the percentage of skin diseases in India. A number of studies suggested the potential application of plant-based therapeutics against various skin disorders. In general, scientific communities engaged in dermatological research seem to have a greater affinity toward producing novel medicines for treating dermatophytic diseases from natural sources, especially from plants. In India, using traditional remedies for skin infections is an entrenched and widely used practice¹¹.

Most people from rural communities depend on traditional phyto medicine to treat skin disorders. Goalpara district of Assam is located on the bank of the river Brahmaputra and is endowed with dense forest cover. These forest regions nurture rich diversity of plant resources. Numbers of endangered as well as rare endemic plants from Goalpara district of Assam were documented in published literature ¹².

Keeping these points in mind, the current research sought to identify the potent biochemicals as well as analysis of its location in four plant species that are broadly utilized in skin disorders by traditional ethno practitioners of Goalpara district of Assam.

MATERIALS AND METHODS:

Visit the Survey Sites: A detailed survey was conducted for about one year (2018 to 2019) in distinct parts of Goalpara district of Assam. The interview was conducted among the traditional healers who have been practicing phytomedicines for treating skin disorders for a long time.

Different information such as (a) how long they are engaged in the healing profession? (b) are the plants easily available (c) which part of the plant is used mostly in skin diseases. (d) in what form plant material is prescribed as a drug (e) why they are hesitant to disclose the information to others, and (f) their views on the possible advantages of natural healing practices over modern medicines.

Semi-structured questionnaires were prepared during the survey, and the information was collected from thirty key informants. During the survey, bilingual persons effectively communicated effectively with the Bodo and Rabha communities. Local men and women who utilized herbal treatments for skin ailments were questioned for cross verification. **Collection and Authentic Identification of Plant Samples:** In the present study, the plants which were found to be extensively used by the native people of Goalpara district were considered. To identify the plant's relevant literature, Flora of British India and Flora of Assam were utilized ^{13, 14}. Furthermore, updated nomenclatures were adopted by following the database of POWO ¹⁵.

For authentic identification of the experimental plant material, the herbarium was prepared and deposited in the Herbarium of Goalpara College, Assam, India (plant authentication numbers: GC105, GC106, GC107, and GC108).

Processing of the Collected Plant: Collected plant samples were dust-free by gentle washing with water. For drying, the shade dried method was followed at room temperature $(25 \pm 1^{\circ}C)$ under dust-free conditions. After drying, fine powders were prepared by grinding the plant materials.

Distilled water was added to the powdered plant materials, which were subsequently filtered. These plant extract filtrates were assessed for phytochemicals' presence by employing the following standard procedures ^{16, 17}.

Qualitative Phytochemical Analysis:

Test for Alkaloids (Mayer's test): For preparing Mayer's reagent, Mercuric chloride (1.3g) and potassium iodide (5.0g) were dissolved in 60 ml and 10 ml of water, respectively.

These two solutions were combined, and the volume of water was made up to 100 ml. 1 ml of this Mayer's reagent was added to equal the aqueous leaf extract volume. The yellow colour precipitation indicates the containing of alkaloids in the test sample.

Test for Terpenoids (Burchard's test): In a test tube, 2ml of leaf extract and 2ml of acetic

anhydride were blended and then boiled for just a few seconds. Once it had cooled, 1 ml of concentrated sulphuric acid was mixed with the above test sample. The emergence of red colour precipitation showed the presence of terpenoids.

Test for Tannins (Ferric Chloride test): The development of bluish-black colour precipitation on the addition of 3-4 drops of ferric chloride (5%) solution to the aqueous leaf extract shows the positivity of tannins in the test sample.

Test for Flavonoid (Lead Acetate test): For the detection of flavonoid, few drops of 10% lead acetate solution were mixed with the aqueous leaf extract. The formation of yellow-colored precipitation validates the presence of flavonoids in the sample.

Test for Phenols (Ferric Chloride Test): After the addition of 3-4 drops of 5% ferric chloride solution to the aqueous leaf extract, the development of a bluish-black colour precipitate shows the presence of phenolic substances.

Histochemical Analysis: Histochemical analysis was conducted on fresh leaf sections according to standard staining procedures. The fresh leaves, after washing, were cut into small portions, which were then fixed for twenty-four hours in fixative FAA (5 ml of Formalin, 5ml of Acetic Acid, and 90ml of 70% ethanol).

Afterward, these fixed specimens of leaves were stored in 70% ethanol¹⁸. Different reagents were employed for staining as recommended by the individual authors in **Table 1**.

Approximately 15 leaves were free-hand sectioned, stained, and viewed using an OLYMPUS BH-2 compound light microscope (Tokyo, Japan), and images were captured with the Canon A650.

TABLE 1:	HISTOCHEMICAL	TEST WITH	DIFFERENT	REAGENTS
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Reagents	Colour	Secondary metabolites	References			
Mayer's reagent	Grey	Alkaloid	19			
2,4-dinitrophenyl hydrazine reagent	Orange-yellow	Terpenoids	17			
Toluidine blue O stain	Bluish black	Phenols	20			
Neu's test	Yellow/ blue	Flavonoids	17			
Ferric chloride method	Bluish-green	Tannins	21			

RESULTS: Based on the information, four plant samples from three families were collected and

identified that are used by traditional healers in treating skin ailments in the surveyed areas.

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The name of the plant, local name family and parts used for treatments were tabulated in **Table 2**. Mainly leaves, latex, and sometimes the whole

plant was prescribed for treating skin infections. Previously published reports justified the ethnomedicinal use of these plants ^{22, 23, 24, 25}.

Vernacular name	Scientific name	Family	Parts used	Mode of preparation and administration
khorgosh	Senna alata (L.)	Fabaceae	Leaves	To cure the ailments like ringworm and eczema,
	Roxb.			paste of leaves is administered topically; It is
				mixed with seed oil of Pongamia pinnata, used
				externally for any skin disorder
Ahot	Ficus religiosa	Moraceae	Leaves, latex	To address any dermatological issues, paste of
	L.			powdered bark or leaf latex is administered
				topically
Kharua	Streblus asper	Moraceae	Leaves	Paste of leaf is administered topically to treat
	Lour.			measles, swelling in the skin
Harjora	Drynaria	Polipodiaceae	Whole plants,	The fronds are pounded and used as a poultice
	quercifolia (L.)		Leaves	to the skin itches
	J. Sm			

TABLE 2: PLANT SPECIES.	. THEIR COLLECTION	SITES AND MODES	OF ADMINISTRATION

Table 3 lists the phytochemical constituentspresent in aqueous extracts of plant species. Thefindings demonstrated that the four studied plantspossessed therapeutically potent phytochemicals.

From the table, all the phytochemicals that were considered for identification were found to be present in the plant *S. alata*. The medicinal plant *D*.

quercifolia also contains all the phytochemicals except flavonoids. Alkaloids and phenolics were detected in *F. religiosa* without tannins, terpenoids, and flavonoids. *S. asper* contains the phytochemicals terpenoids and flavonoids.

Alkaloids, tannins, and phenolics were not detected in the plant *S. asper*.

Phytochemical profile	Senna alata	Ficus religiosa	Strebulus asper	Drynaria quercifolia
Alkaloids	+ve	+ve	-ve	+ve
Terpenoids	+ve	-ve	+ve	+ve
Tannins	+ve	-ve	-ve	+ve
Phenolics	+ve	+ve	-ve	+ve
Flavonoids	+ve	-ve	+ve	-ve

Histochemical localization of different phytoconstituents of collected plant samples was listed in **Table 4** and **Fig. 1**.

With the use of reagents, secondary metabolites have been detected in the leaf sections of the studied plants. The histochemical study indicated that alkaloids were present mostly in hypodermis and pericycle whereas phenolics were dominantly found in the pericycle, except in the plant *D. quercifolia*.

Terpenoids were predominat in xylems of all the tested plant extracts except *D. quercifolia*. Flavonoids and tannins were detected in different tissues in different medicinal plants studied.

Plant species	Alkaloids	Terpenoids	Phenols	Flavonoids	Tannins
Senna alata (L) Roxb.	Hypodermis	Xylem	Pericycle	Pericycle	Hypodermis, xylem
Ficus religiosa(L)	Pericycle,	Epidermis,	Pericycle,	Cortical	Vascular tissue
	epidermis	xylem	xylem	parenchyma	
Streblus asper Lour	Hypodermis	Epidermis,	Pericycle	Epidermis,	Cortical
		xylem		pericycle	parenchyma
Drynaria quercifolia (L)J. Sm	Pericycle,	Epidermis,	Mesophyll	Mesophyll	Endodermis
	xylem	Hypodermis			



FIG. 1: A-D. ALKALOIDS; E-H. TERPENOIDS; I-L. PHENOLS; M-P. FLAVONOIDS; Q-T. TANNINS; A, E, I, M, Q-SENNA ALATA; B, F, J, N, R-FICUS RELIGIOSA; C, G, K, O, S-STREBLUS ASPER; D, H, L, P, T-DRYNARIA QUERCIFOLIA (ABBREVIATIONS: HY–HYPODERMIS, EP–EPIDERMIS, PE–PERICYCLE, XY–XYLEM, ME–MESOPHYLL CELLS, CO–CORTEX, PH–PHLOEM, EN–ENDODERMIS)

DISCUSSION: All four plant species' leaves were applied externally in treating skin disorders. In the case of *Ficus religiosa*, latex is also used topically in skin infections. The whole plant of *Drynaria quercifolia* is used by traditional healers. In most cases, paste from leaves is used in curing skin disorder like ringworm, itching, swelling, measles *etc*.

Alkaloids, terpenoids, tannins, phenolics, and flavonoids are considered important phytochemicals in their biological activities ^{26, 27}. The liquid extract of *Sana alata* exhibited all these significant biochemicals. On the other hand, except for flavonoids, all other four phytoconstituents are present in *Ficus religiosa*. In the case of *Strebulus asper* only terpenoids and flavonoids are present.

According to the phytochemical study, *Drynaria quercifolia* contained flavonoids, phenolics, terpenoids, and alkaloids. In earlier investigations, alkaloids play a vital role in curing skin disorders ²⁸. Alkaloids like piperine are widely used in skin inflammation. Piperine is also used in atopic dermatitis ²⁹.

Other alkaloids like Pseudoephedrine (PSE) are used to improve skin dehydration ³⁰. Magnoflorine (MAG) is a well-known alkaloid that is used to treat various skin infections ³¹. Likewise, other alkaloids Indirubin, Esculetin, Peiminine, Tryptanthrin are also used in curing skin ailments. Terpenoids such as Astaxanthin, Fucoxanthin, Platycodin D, Igalan, and Elemol are the established phytochemicals widely used to treat skin disorders ³². Phenolics like polyphenols help to prevent the proliferation of skin disorders ³³. The antioxidant property of phenolics and flavonoids help in rejuvenating the skin cells. Apart from that, phenolics have anti-inflammatory and antimicrobial characteristics making them used full in the fight against skin infections ³⁴. Histochemistry is the science of the localization of organic molecules inside the cells using the colours of the molecules. It acts as a potent marker in decoding crucial biosystematics problems. To avoid adulteration, it is crucial to determine the location of plant derivatives in their organs utilized for developing pharmaceuticals ³⁵.

In the current histochemical studies, alkaloids were observed in the hypodermis of S. alata and S. asper, epidermis, and pericycle of F. religiosa, pericycle and xylem in D quercifolia. Terpenoids were observed in the epidermal tissue of all the plants except S. alata. Likewise, terpenoids were also observed in the xylem of all plant species studied except D. quercifolia, which it is present in the hypodermal cells. Phenols were observed in the pericycle of S. alata and S. asper; mesophyll cells in D. quercifolia; xylem, and pericycle in F. religiosa. Flavonoids were observed in the cortex of F. religiosa; pericycle of S. alata; epidermis and pericycle of S. asper; mesophyll cells of D. quercifolia. Tannins were observed in the hypodermis and vascular tissue of S. alata; vascular tissue of F. religiosa; cortical cells of S. asper and endodermal cells of D. quercifolia.

The analysis showed that all the plant species have different phytochemical constituents as they showed variation from each other in colour tests. Even each plant showed a specific location for each phytoconstituents in their cells or tissues of the same organ. The histochemical study supported the results of the phytochemical study with few exceptions. S. asper showed the presence of phenol in histochemical analysis by developing bluishblack colour in the pericycle region. Still, the phytochemical analysis of leaf extract did not show the presence of this compound. This may be due to the lack of solubility of the compound or due to the inefficiency of the non-polar solvents in penetrating the cell wall. The presence of different phytoconstituents with specific locations helps understand the basic characteristics and features and serves as biological markers for systematic identification.

CONCLUSION: Besides furnishing fundamental requirements for life, plants also provide effective biologically active phytochemicals for health promotion and prevention of diseases. Plantderived drugs are considered as most significant bioactive molecule against deadly diseases. Skin ailments are becoming widespread not merely in adults but also children as well. The fast-growing pace of the disease and the costly medical procedure worsen the disease burden. High throughput screening of the collected plant species can contribute to developing potent drug molecules for treating skin ailments. Furthermore, proper conservation of plant resources should be addressed. The accumulation of bioactive constituents varied with season and location. For that, a systematic study of the collected samples in different seasons is another important matter of concern. Our study demonstrated that the four medicinal plants studied can be used as a precursor for the formulation of medication to treat skin diseases with minimum or no side effects, unlike chemical drugs.

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