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PHYTOCHEMICAL ANALYSIS OF *DRYMARIA CORDATA* (L.) WILLD. EX SCHULT. (WHOLE PLANT) USED BY TEA TRIBES OF ERSTWHILE NAGAON DISTRICT OF ASSAM, INDIA

Rakhi Bhattacharyya*, Krishna Kanta Medhi and Sarat Borkataki

Department of Botany, Nowgong College, Nagaon - 782001, Assam, India.

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Correspondence to Author:

Rakhi Bhattacharyya

Research Scholar,
Department of Botany,
Nowgong College, Nagaon -
782001, Assam, India.

E-mail: rakhibhattacharyya06@gmail.com

ABSTRACT: Ethno-medicinal plants have a significant role in the field of natural product research and drug discovery since ancient times. *Drymaria cordata* (L.) Willd. Ex Schult. belongs to the family Caryophyllaceae is an ethnomedicinal plant used as a home remedy in jaundice by the tea tribes of erstwhile Nagaon district of Assam. The present study deals with the evaluation of the phytoconstituents of *Drymaria cordata* using methanol extract. All the standard phytochemical procedures were followed for the detection of the phytoconstituents. The preliminary phytochemical screening revealed the occurrence of alkaloids, flavonoids, phenols, tannins and saponins. The presence of O-H stretch, C-O stretch, C-H stretch, C-H bend, N-H stretch and N-H bend were confirmed through FT-IR analysis. Several bioactive compounds like Cyclohexan-1,4,5-triol-3-one-1-carboxylic acid, Beta-D-glucopyranose-1,6-anhydro, L-gala-L-ido-octose, 3, 7, 11, 15-Tetramethyl-2-hexadecen-1-ol, n-Hexadecanoic acid, 9, 12 Octadecadienoic acids (Z,Z)-, 9,12-Octadecadienoic acid, methyl ester, Oleyl alcohol and 17-Octadecynoic acid were identified through GC-MS analysis. From this study, it can be concluded that *Drymaria cordata* possesses several medicinally important secondary metabolites. Further phytochemical and pharmacological investigations and isolation of the phyto-compounds is an urgent need which could be responsible for curing jaundice and several other ailments.

INTRODUCTION: Plants are used medicinally and are a rich source of several pharmacologically potent compounds. The therapeutic potentials of plant drugs are being used traditionally since pre-historic times. Plant products are the chief source of pharmaceutical agents used in the traditional remedy¹.

According to the World Health Organization (WHO), due to some economic and geographical constraints, 80% of the population in several Asian and African countries still depends on traditional therapies as their primary healthcare needs². The curative properties of medicinal plants are chiefly due to the presence of various bioactive compounds present in the plants like alkaloids, flavonoids, phenols, saponins, tannins and terpenoids³.

In recent years, evaluation of phytoconstituents is considered to be the most important and promising step in the medicinal plant research⁴. *Drymaria cordata* is an annual spreading herb native to tropical America and is now distributed widely

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throughout the tropics and subtropics of the world⁵. The plant is commonly known as Tropical Chickweed, and Laijabori (in the study area) belongs to the family Caryophyllaceae^{6,7}. It is an important medicinal plant with various remarkable phytoconstituents and several biological activities have been reported like analgesic, anti-bacterial, anti-convulsant, anti-inflammatory, antipyretic, antitussive, anxiolytic and cytotoxic activities⁸. The plant is an ethnomedicinal plant used as a home remedy in jaundice by the tea tribes of Nagaon district of Assam⁷. The study aims to identify and evaluate some biologically active compounds present in the whole plant of *Drymaria cordata*.

MATERIALS AND METHODS:

Collection of the Plant: The whole plant of *Drymaria cordata* was collected from different tea gardens of erstwhile Nagaon district of Assam. The collected plant material of *Drymaria cordata* was authenticated by the Department of Botany, Nowgong College, Nagaon, Assam.

The voucher specimen (RBNG-23) in the form of the herbarium is maintained by using standard methods in the Botany Department of Nowgong College for future references⁹.

Chemicals: In the present study, all the chemicals including the solvents were of analytical grade and are purchased from Sigma Chemical Co. (USA) and Merck Chemical Supplier (India).

Preparation of the Extract: The collected plant material was washed properly. The materials were chopped and dried under shade at room temperature for 2-3 weeks until the weight of the plant is reduced to half of the collected material¹⁰.

The dried samples were powdered using a clean and sterile electric grinder 500 gm of the powdered plant material was placed in a conical glass percolator. Sufficient quantity of 95% methanol is added into the percolator to allow the powdered plant sample to become thoroughly wet. After 24 h the percolate was collected, and this process of extraction was repeated 3 times. The combined methanol filtrates were concentrated in the rotary evaporator, and the extract was calculated for the extractive value. The concentrated plant samples were transferred into an airtight glass container and

stored at Department of Botany, Nowgong College, Nagaon, Assam, for further analysis¹¹.

Preliminary Phytochemical Analysis: The plant's extract was subjected to preliminary phytochemical screening to confirm the presence of the several bioactive compounds^{12,13,14,15}.

Alkaloids:

Mayer's Test: Filtrate was treated with Mayer's reagent and the formation of a white creamy precipitate indicates the presence of alkaloids.

Dragendorff's Test: Filtrate was treated with Dragendorff's reagent and formation of an orange-reddish precipitate indicates the presence of alkaloids.

Flavonoids:

Zinc-Hydrochloride Reduction Test: Plant extract was treated with a mixture of Zinc dust and concentrated hydrochloric acid. The appearance of red color after a few minutes indicates the occurrence of flavonoids.

Shinoda Test: Addition of a few small pieces of Magnesium ribbon to the extract solution in a test tube followed by pouring of concentrated hydrochloric acid dropwise. Formation of pink, crimson red or green color indicates the presence of flavonoids.

Phenols:

Ferric-Chloride (FeCl₃) Test: The extract solution was treated with FeCl₃ droplets gives blue-green color indicates the presence of phenols.

Shinoda Test: The extract solution was treated with few fragments of Magnesium ribbons and followed by drops of concentrated hydrochloric acid. Formation of yellowish color indicates the occurrence of phenols.

Saponins:

Foam Test: About 1 ml of the extract solution was shaken vigorously with little quantity of distilled water. Development of foam indicates the presence of saponins.

Tannins:

Ferric-Chloride (FeCl₃) Test: The filtrate was treated with FeCl₃ droplets gives darks green color which indicates the presence of tannins.

Steroids and Terpenoids:

Salkowski's Test: A fraction of extract was shaken well by treating with few drops of chloroform and concentrated sulphuric acid.

The appearance of red color in the lower layer after some time indicates the occurrence of sterols whereas yellow color indicates the presence of terpenoids.

Analysis by Fourier-Transform Infrared Spectroscopy (FTIR):

FT-IR was used to detect the functional group of the compounds present in *Drymaria cordata*. The FT-IR spectra of methanol extract of *Drymaria cordata* was measured on Alpha FT-IR instrument from Bruker Optics (OPUS 7.5 software) at the Department of Chemistry, Nowgong College (Nagaon), Assam. The absorption spectra were measured between 4000 and 500 cm^{-1} .

Analysis by Gas Chromatography-Mass Spectroscopy (GC-MS):

The methanolic extract of *Drymaria cordata* was analyzed through GC-MS and was performed at Biotech-Park, IIT Campus, Guwahati in Clarus 680 GC & Clarus 600 CMS PerkinElmer, USA. For the analysis of possible active compound present in the plants, 2 μL of the sample was injected in the GC-MS system through auto-sampler in split mode (split ratio 10:1).

The GC-MS system was equipped with the capillary column of 60 m in length and 0.25 mm in diameter and 0.25 μm in film thickness and composed of 5% diphenyl 95% dimethyl polysiloxane and mass ranges from 40-600 amu. Mass Spectra was taken in Electron Impact positive (EI+) mode at 70 eV. The column oven temperature was programmed from 60 $^{\circ}\text{C}$ to 300 $^{\circ}\text{C}$ hold for 10 min. The temperature of the injector was fixed at 280 $^{\circ}\text{C}$ and helium (99.99% purity) was the carrier gas fixed with a flow rate of 1 ml min^{-1} . The total run time of the GC-MS system was 51.83 min.

Identification of the Compounds: Mass spectra were developed by using NIST (National Institute of Standards and Technology) library 2008. The spectrum of the unknown as compared with the patterns of the mass spectra and retention indices of the known present in the databases of NIST library 2008.

RESULTS AND DISCUSSION:

Extractive Value: The color and extractive value of the methanol extract of *Drymaria cordata* is given in **Table 1**.

TABLE 1: PERCENTAGE YIELD OF METHANOL EXTRACT OF WHOLE PLANT OF DRYMARIA CORDATA

Solvent	Part	Colour of extract	Yield (%w/w)
Methanol	Whole plant	Dark green	2.94

Preliminary Phytochemical Screening: The preliminary phytochemical screening of the methanolic extract of *Drymaria cordata* have shown the presence of secondary metabolites like alkaloids, flavonoids, phenols, tannins and saponins, whereas steroids and terpenoids are found to be absent is presented in **Table 2**.

TABLE 2: PRELIMINARY PHYTOCHEMICAL SCREENING OF WHOLE PLANT OF DRYMARIA CORDATA

Phytochemicals	Test	Presence/Absence
Alkaloids	Mayer's test	+
	Dragendorff's test	+
Flavonoids	Zinc-chloride test	+
	Shinoda test	+
Phenols	Ferric-chloride test	+
	Shinoda test	-
Tannins	Ferric-chloride test	+
Saponins	Foam test	+
Terpenoids	Salkowski's test	-
Steroids	Salkowski's test	-

FT-IR Analysis: The IR spectroscopy of *Drymaria cordata* showed the occurrence of alcohols, amines, alkanes, carbohydrates, ethers and phenols. The peak with strong and broad intensities at around 3340 cm^{-1} indicated the presence of O-H stretching band and referred to the presence of alcohols and carbohydrates. N-H stretching vibration may also be present in the system which may be overlapped with the broad O-H stretching band corresponds the amines. The peak at around 1655 cm^{-1} indicated the N-H bend (1° amines). Two strong peaks near 2920 cm^{-1} and 2800 cm^{-1} may be assigned to the C-H asymmetric and symmetric stretching vibrations respectively and revealed the occurrence of alkanes.

The C-H bending vibration was confirmed by the wavenumber obtained at around 1460 cm^{-1} . The strong peak obtained at 1030 cm^{-1} (having a shoulder peak near 1100 cm^{-1}) indicated the

presence of C-O stretching vibration also indicate the alcohols and ethers is shown in **Table 3** and **Fig. 1**.

TABLE 3: FTIR ANALYSIS OF DRYMARIA CORDATA (WHOLE PLANT)

Functional groups	Vibrations	Peaks (cm ⁻¹)
Alcohols	O-H stretch	3340
	C-O stretch	1100, 1030
Alkanes	C-H stretch	2920, 2800
	C-H bend	1460
Amines	N-H stretch	3340
	N-H bend	1655
Carbohydrates	O-H stretch	3340
	C-O stretch	1100, 1030
Phenols	O-H stretch	3340

FTIR (Fourier Transform Infrared Spectroscopy) is a high-resolution analytical techniques to identify and elucidate the pattern and structure of the chemical compounds. The FT-IR analysis also indicated the presence of some primary and

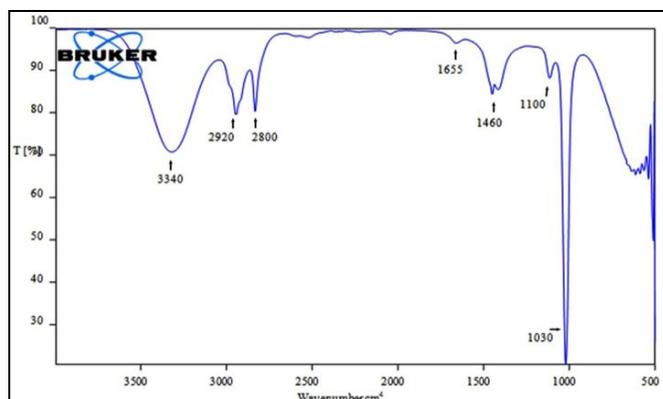


FIG. 1: FTIR SPECTRUM OF DRYMARIA CORDATA (WHOLE PLANT)

secondary metabolites. The IR spectrum of *Drymaria cordata* revealed the presence of alkaloids due to N-H stretching and N-H bend, free phenols, and flavonoids due to O-H stretch. The occurrence of phenols is also due to the C-O stretch. The FT-IR analysis predicted the occurrence of the functional groups like O-H stretch, C-H stretch, C-H bend, C-O stretch, N-H stretch and N-H bend. All the compounds obtained from FT-IR (except carbohydrates) belong to the secondary plant metabolites as per researcher explanations^{16,17}.

GC-MS Analysis: Phyto-constituents of *Drymaria cordata* were analyzed through GC-MS also. The phytochemical components in *Drymaria cordata* by GC-MS fingerprinting revealed the presence of 9 bioactive compounds having various pharmacological properties.

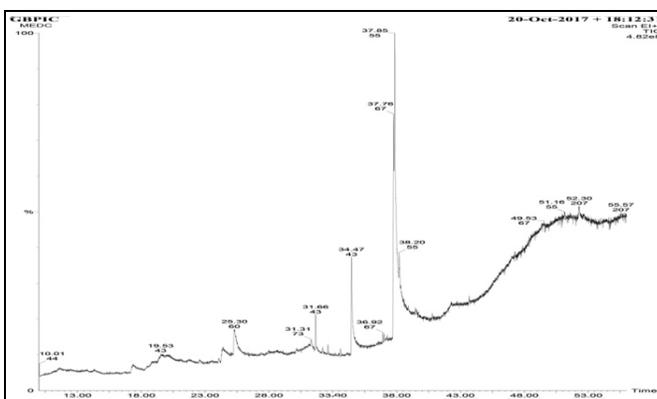


FIG. 2: GC-MS SPECTRUM OF DRYMARIA CORDATA (WHOLE PLANT)

TABLE 4: GC-MS ANALYSIS OF DRYMARIA CORDATA (WHOLE PLANT)

Peak	Retention time	Name	Peak area%	Molecular weight	Molecular formula
1	19.53	Cyclohexan-1,4,5-Triol-3-one-1-carboxylic acid	1.56	190	C ₇ H ₁₀ O ₆
2	25.30	Beta-D-glucopyranose, 1,6-anhydro	4.24	162	C ₆ H ₁₂ O ₅
3	31.31	L-gala-L-ido-octose	0.85	240	C ₈ H ₁₆ O ₈
4	31.66	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	1.67	296	C ₂₀ H ₄₀ O
5	34.47	n-Hexadecanoic acid	7.14	256	C ₁₆ H ₃₂ O ₂
6	36.92	9,12-Octadecadienoic acid (Z,Z)-	2.55	280	C ₁₈ H ₃₂ O ₂
7	37.76	9,12-Octadecadienoic acid, Methyl ester	12.023	294	C ₁₉ H ₃₄ O ₂
8	37.85	Oleyl alcohol	29.86	268	C ₁₈ H ₃₆ O ₂
9	38.20	17-Octadecynoic acid	6.95	280	C ₁₈ H ₃₂ O ₂

The major components obtained were Oleyl alcohol (29.86%) and 9,12-Octadecadienoic acid, methyl ester (12.02%) with minor compounds like n-Hexadecanoic acid (7.14%), 17-Octadecynoic acid

(6.95%), Beta-D-glucopyranose, 1, 6-anhydro (4.24%), 9, 12-Octadecadienoic acid (Z,Z)- (2.55%), Cyclohexan-1, 4, 5-triol-3-one-1-carboxylic acid (1.56%), 3,7, 11,15-Tetramethyl-2-

hexadecen-1-ol (1.67%) and L-gala-L-ido-octose (0.85%). The results are tabulated in **Table 4** and **Fig. 2**. The molecular structures of the phyto-

constituents identified from GC-MS analysis were given in **Fig. 3**.

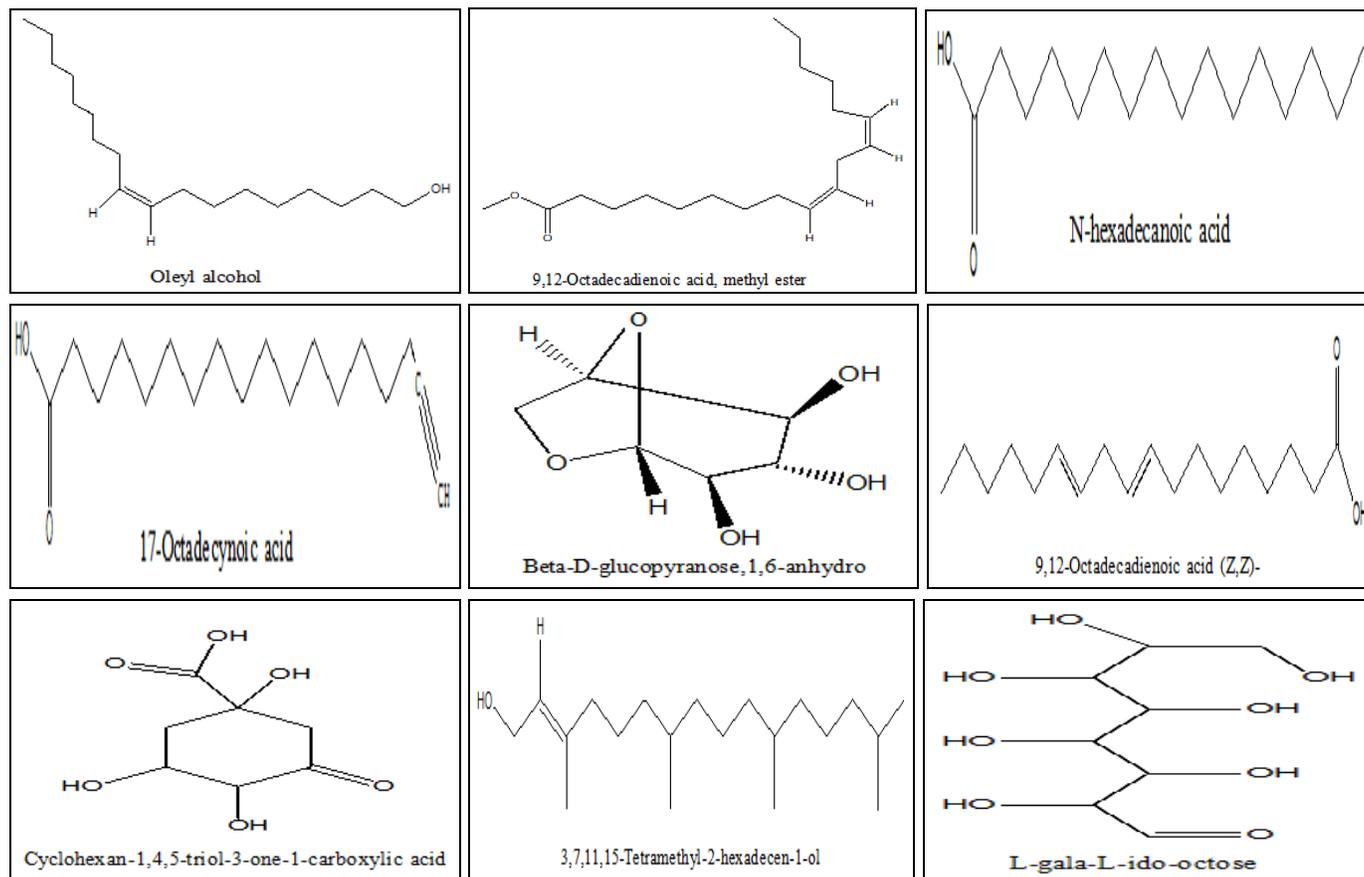


FIG. 3: MOLECULAR STRUCTURE OF THE PHYTO-CONSTITUENTS IDENTIFIED FROM GC-MS ANALYSIS

The major and minor compounds detected through GC-MS possess numerous biological activities. Beta-D-glucopyranose, 1, 6-anhydro also known as Levoglucosan acts as a preliminary material for the synthesis of stereoregular polysaccharides and have anti-human immunodeficiency virus and blood coagulant activities¹⁸. L-gala-L-ido-octose is an important compound needed for memory drug production¹⁹. 3, 7, 11, 15-Tetramethyl-2-hexadecen-1-ol mainly known as Phytol have several biological activities like anticancer, anti-inflammatory, antioxidant, antimicrobial, diuretic and hepato-protective²⁰. n-Hexadecanoic acid (Palmitic acid) possesses 5-alpha reductase inhibitor, anti-androgenic, antioxidant, hypocholesterolemic, lubricant, nematocide, pesticide and mosquito larvicide activities^{20, 21}. The 9, 12-Octadecadienoic acid (Z, Z)- (Linoleic acid) obtained from GC-MS analysis has many biological activities like anti-acne, anti-androgenic, anti-arthritic, anti-coronary, anti-eczemic, anti-

histamine, anti-inflammatory, cancer preventive, 5-alpha reductase inhibitor, hepatoprotective, hypercholesterolemic, insectifuge and nematocide²⁰. Oleyl alcohol (cis-9-octadecen-1-ol) and 9, 12-Octadecadienoic acid, methyl ester (Methyl linoleate) were the major compounds obtained in the study have the anti-tumor or anti-cancer properties^{22, 23}. The 17-octadecynoic acid though not a major component identified through GCMS analysis has been reported to possess antihypertensive properties²⁴.

CONCLUSION: The preliminary phytochemical screening and FT-IR results revealed that the methanolic extract of *Drymaria cordata* consists of various bioactive compounds like alkaloids, flavonoids, phenols, tannins, saponins and carbohydrates. Some major and minor constituents have been analyzed through GC-MS having several pharmacological properties. The result validates several therapeutic uses and importance of

Drymaria cordata and supports the authenticity of the tea tribal people of the study area. Further phytochemical and pharmacological studies are an urgent need with *Drymaria cordata* to identify the unknown functional groups and compounds and isolation of the bioactive compounds which are responsible for prevention and curing jaundice and other ailments.

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CONFLICT OF INTEREST: The authors declare no conflict of interest.

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