

Pharmacognosy and Phytochemical Screening of some Plant Derived Medicine to Treat Dysmenorrheal Pain by the Rajbanshi Community

Priyankar Roy¹, Palash Mandal^{1*}, Subhasis Panda², Sonia Mitra Roy³, Arunika Subba¹

Priyankar Roy¹,
Palash Mandal^{1*},
Subhasis Panda²,
Sonia Mitra³,
Arunika Subba¹

¹Plant Physiology and Pharmacognosy Research Laboratory, Department of Botany, University of North Bengal, Rajarammohanpur, Siliguri, West Bengal-734013, INDIA.

²Department of Botany, Maulana Azad College, 8, Rafi Ahmed Kidwai Rd, Taltala, Kolkata, West Bengal-700013, INDIA.

³Himalayan International Residential School, Rajganj, Jalpaiguri, West Bengal-735134, INDIA.

Correspondence

Dr. Palash Mandal, PhD

Plant Physiology and Pharmacognosy Research Laboratory, Department of Botany, University of North Bengal, Rajarammohanpur, Siliguri, West Bengal-734013, INDIA.

Phone no : +91 9434886123

E-mail: nbutanypm@gmail.com

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ABSTRACT

Introduction: Dysmenorrhea is a sort of painful menstrual disorder. Several plants like *Allium sativum* L., *Areca catechu* L., *Zingiber officinale* Roscoe, *Crinum amoenum* Ker Gawl. Ex Roxb, *Cuscuta reflexa* Roxb. *Nymphaea rubra* Roxb. Ex Andrews, *Piper nigrum* L., *Citrus limon* (L.) Osbeck were used in appropriate ratio to make herbal formulation to cure dysmenorrheal pain by the traditional healers of Rajbanshi community. **Objective:** By semi-structured questionnaires in the course of scheduled interviews with the local herbal practitioners, four herbal formulations (coded as DYS1, DYS2, DYS3 and DYS4) were recorded. Pharmacognostic characterization was carried out for the authentication of the powder drugs which includes powder microscopy, fluorescence analysis and physicochemical evaluation. The presence of any therapeutic potential in DYS1, DYS2, DYS3 and DYS4 was determined by qualitative evaluation of phytochemicals of various successive solvent extracts based on their polarity. Thin layer chromatography was performed for screening various phyto-active compounds like arbutin, alkaloids, coumarins, cardiac glycoside, etc. **Results:** Powder microscopy revealed the presence of calcium oxalate crystal, stone cells etc. Various fluorescence colors were exhibited by these herbal drugs under UV after reacting with chemical reagents. Physical analyses values were also obtained in a satisfactory way. TLC and qualitative phytochemical analysis showed the presence of active phytoconstituents like arbutin, alkaloids, coumarins, cardiac glycoside etc. **Conclusion:** The results support the use of plants as a traditional medicine for the prevention disorders like Dysmenorrhea. Further evaluation is required for determining the efficacy of those herbal drugs and the active phytoconstituents involved in Dysmenorrheal therapy.

Key words: Dysmenorrhea, Traditional healer, Pharmacognostic evaluation, Antioxidant, Successive solvent extraction, Thin Layer Chromatography.

INTRODUCTION

Plants have created the base of traditional medicine exercised that has been practiced since many years by the people in China, India, and many other countries. Some of the most primitive accounts of the use of the plant as drugs are originated in the Atharva-Veda, which is the origin of Ayurvedic medicine in India.¹ There is an ancient evidence of medicine discovery on Sumerian clay from Nagpur. Twelve very old medicinal formulation have been reported with plant element such as poppy and Mandrake.² Humans have produced medicine from plants and other organisms for centuries. A good number of the drugs used in modern medicines are formed indirectly from medicinal plants.³ and about 90% of raw resources are derived from the wild sources. However traditional therapy is still restricted to certain areas or group of people because of the unavailability of effective scientific communication.⁴ Therefore the requirement of the significance of herbal medicine and study of the knowledge of traditional healing practices by plants is quite essential. Several

active principle constituents are found in those formulations, which could be explained by their function in animal and human system through pharmaceutical and pharmacological study.⁵

Rajbanshis are the fore runner of Koches and are living in Northern Region of West Bengal. Due to a distinctive climatic and ecological provision, a unique Biodiversity hotspot is situated in sub-Himalayan region of North Bengal. Rajbanshis are dominating this area for many years and they are mostly dependent on agriculture. But socio-cultural activities of the Rajbanshi community are directly and indirectly related to the plant resources. They are using various plant parts to make medicinal formulations for alleviating their diseases & disorders since time immemorial.⁶ Previously we have found that the herbal practitioners (Kabiraj or Mahan) of Rajbanshi community exercise a total number of 31 types of plant species from 29 genera and

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24 families for preparation of 10 polyherbal formulations to treat various ailments and disorders like arthritis, lesions with pus formation, wound, dysentery, seasonal fever, liver problem, jaundice, nose bleeding along with frequent urination etc.⁷ Our present study revealed that four polyherbal formulations (coded as DYS1, DYS2, DYS3 and DYS4) was made by mixing the various parts of several plants like *Allium sativum* L., *Areca catechu* L., *Zingiber officinale* Roscoe., *Crinum amoenum* Ker Gawl. ex Roxb., *Cuscuta reflexa* Roxb., *Nymphaea rubra* Roxb. ex andrews., *Piper nigrum* L., *Citrus limon* (L.) Osbeck. For the said treatment of Dysmenorrhea.

Literature survey did not show any information about pharmacognostic studies of the above polyherbs. For that reason, this study was concentrated on the standardization, by measuring various attributes such as powder microscopy, fluorescence analysis and physicochemical studies to explore the presence of active phytoconstituents and qualitative phytochemicals, extracted from different solvents according to their polarity.

MATERIALS AND METHODS

Study Area

Teraí and Duars lie in the Northern half of the state of West Bengal and are surrounded by international boundaries of Bangladesh, Nepal and Bhutan. The area is a combination of the wide-ranging landscape - from the high mountainous region in the north to the vast Gangetic plains in the farthest south. The study area lies at 26.7072°N and 88.3558°E. The annual rainfall is recorded to be about 3900 mm and temperature varies between 7°C to 37°C. The uniqueness of the area has made it one of the treasures of West Bengal with diversified plant and animal populations. The region not only stands out for scenic beauty, along with flora and fauna but also in social atmosphere. The region is a blend of several exclusive tribal communities which makes it rich in culture as well. Some of the major tribes include Rajbanshi, Rabha, Santal, Munda, Oraon, Polia, Lepcha, Toto etc.^{8,9}

Collection and authentication of plant material

Field survey was accompanied with detailed discussions in the form of questionnaires to the medicine men and local people of study areas, as some information was received from them as well. The information includes the mode of consumption, shelf life and ethnic values of the particular plant and its part (s) used. After these detailed discussions, the respective plants were collected properly both in its flowering and fruiting stage. The collected specimens were dried consequently and herbarium sheets were made. Voucher specimen was preserved in the NBU herbarium for further reference.

Sample preparation

Fresh plant parts are collected from the different part of the study area and dried and make the powder to preserve for future use. Polyherbal powder drugs DYS1, DYS2, DYS3 and DYS4 were prepared according to the herbal healers. The polyherbal powder sample was extracted through soxhlet apparatus in seven different solvents based on their polarity. The solvent extracts were heptane (HP), benzene (BZ), ethyl acetate (EA), acetone (AC), butanol (BT), methanol (MT) and water (AQ). The samples were then evaporated and reconstituted in methanol. These extracted samples were used for qualitative phytochemicals. For TLC and pharmacognostic studies, the samples were used in its powder form.

Pharmacognostic characterization

Organoleptic Characters

The colour, odour, taste and texture of the powder of DYS1, DYS2, DYS3 and DYS4 were observed and recorded.

Anatomy

Anatomical parameters have been taken into consideration for the study as they are usually the conserved characters for the specific species. The anatomical study revealed some unique and important characteristic features for the studied species. Transverse section of respective part of the studied plant species were done and treated with different reagents i.e. iodine, phloroglucinol and Toluidine blue stain. Iodine stain was used to check the presence of starch grains. Phloroglucinol stains the lignified tissues, whereas Toluidine blue helps to differentiate xylem and phloem cell wall.

Powder microscopy

The colour, odour, taste and texture of the powder of DYS1, DYS2, DYS3 and DYS4 were observed and recorded.¹⁰ Microscopic examination was carried out in a standard method.¹¹ A small amount of powder of DYS1, DYS2, DYS3 and DYS4 were taken on glass slide, mounted on glycerin and observed under microscope. For the observation of lignified tissues, powder was stained with alcoholic solution of phloroglucinol followed by concentrated HCl. Similarly the powder was also stained with N/10 iodine solution to observe the starch granules and for the identification of fixed oil and fats, Sudan III were used.

Fluorescence analysis

Fluorescence study of the dried leaf powder was performed using standard procedure.^{11,12,16} A small quantity of the sample was treated using fluorescence reagents (such as 1 N HCl, 50% H₂SO₄, FeCl₃, iodine solution, acetic acid glacial, 0.1 N NH₄OH, 1% CH₃COOH). The powder samples after treatment with various chemical reagents were subjected to fluorescence analysis. Observations were made under visible light and under UV light of short (254 nm) and long wave length (365 nm) separately.¹²

Physicochemical parameters

Coarse powder of the plant root was used to perform quality control parameters such as total ash, acid insoluble and water soluble ash, water and alcohol soluble extractive values and loss on drying.¹³ three determinations were carried out for each parameter.

Preliminary phytochemical studies

The DYS1, DYS2, DYS3 and DYS4 powder extracted in various solvents were subjected for preliminary phytochemical screening to observe the presence or absence of phytoconstituents like tannin, triterpenoids, amino acid, steroids, cardiac glycosides and flavonoids by the standard methods.¹⁴⁻¹⁷

Thin layer chromatography

TLC was performed to analyze the variation in bioactive chemical constituents.¹⁸ Readymade TLC plates (coated with silica gel 60 F254 on aluminum sheets) purchased from Merck Germany were used. The powdered sample was extracted with different procedures for the identification of each of the active constituents i.e. anthraglycosides, arbutin, cardiac glycosides, flavonoids, bitter principles, saponins, coumarins and alkaloids. The mobile phase solvent systems used were ethyl acetate: methanol: water (100:13.5:10) for the detection of anthraglycosides, arbutin, cardiac glycosides, bitter principles and alkaloids. The mobile phase ethyl acetate: formic acid: glacial acetic acid: water (100:11:11:26) was used for flavonoids identification and for the identification of saponin, the solvent system of chloroform: glacial acetic acid: methanol: water (64:32:12:8) was used while for the identification of coumarin, toluene : ethyl acetate (93:7) was used. The developed chromatograms were analyzed for the presence of drug constituents by spraying with suitable group reagent. The chromatograms were then

observed under UV-254 nm and UV-365 nm light. Photographs were taken with DSLR Nikon camera (D-3200) and the R_f values were calculated with the following formula:

$$R_f = \frac{\text{Distance traveled by solute}}{\text{Distance traveled by solvent}}$$

RESULTS

Organoleptic evaluation

The powder of DYS1, DYS2, DYS3 and DYS4 were brown in colour and rough in texture. DYS1 and DYS4 were slightly bitter in taste, whereas DYS2 and DYS3 were partially sweet in nature. In DYS1, a characteristic pungent smell was observed, as garlic was used in that formulation in appropriate ratio according to the herbal healer's prescription.

Anatomy

Presence of starch grains were confirmed in species like *Citrus limon*, *Crinum amoenum*, *Zingiber officinale* and *Nymphaea rubra* (Figure 1 A-D). Starch grains were stained with dark bluish-purple colour and were mostly present within the thick walled parenchymatous cells either near the cortex or pith. Starch grains were of various shape and sizes with prominent centered hilum in case of *Nymphaea rubra*. Sclerenchymatous cells occur in patches in *Areca catechu*, confirmed through phloroglucinol staining (Figure 1 E). Presence of collenchymatous cells was seen in *Nymphaea rubra* (Figure 1 F). Sclerenchymatous fiber cells were also found in *Piper nigrum* (Figure 1 K). In case of *Zingiber officinale*, prominent oil glands were observed (Figure 1 L). *Areca catechu*, when treated with toluidine blue stain, showed xylem and phloem wall differentiation along with sclerenchymatous fiber cells embedded within the phloem tissue. The cortical cells of *Allium sativum* were thick walled and showed prominent differentiation when stained with Toluidine blue (Figure 1 H). Within the cortical region of *Nymphaea rubra*, some of the cells showed clear wall thickening and could be concluded as islet of sclerenchymatous cells (Figure 1 I). In case of *Piper nigrum* fruit coat, presence of thick walled osteosclereides was observed in rows above mesophyll cells (Figure 1 J).

Powder microscopy

Powder microscopy has revealed some specific features and helps in authentication of the studied herbal drugs. In case of DYS1, presence of acicular type of calcium oxalate crystals and triangular starch grains with concentric striae were confirmed (Figure 2 A and B). In DYS2 starch grains of various shapes with central hilum and astrosclereids were observed. Literature showed the presence of astrosclereid in *Nymphaeaceae* family.¹⁹ The astrosclereid is usually eight armed with somewhat prominent basal cells, arms are thick without any lumen and were broad-based to narrow tapering ends (Figure 2 C and D). DYS3 revealed the presence of pitted xylem vessels and round shaped uniform starch grains (Figure 2 E and F). In DYS4, elongated stone cells were visible but starch grains were absent (Figure 2 G and H). The four formulations have specifically shown the presence and absence of certain features which are significant tools for standardization of these powdered formulations.

Fluorescence analysis

Different fluorescent colours were exhibited after treating the powdered drugs with different chemical reagents (Figure 3). The colours were detected under visible and UV light and were identified by using the standard colour chart of German RAL Institute and noted down in Table 1. Characteristic blue and green colours were observed under UV light when these herbal drugs were interacted with acidic, alkaline or

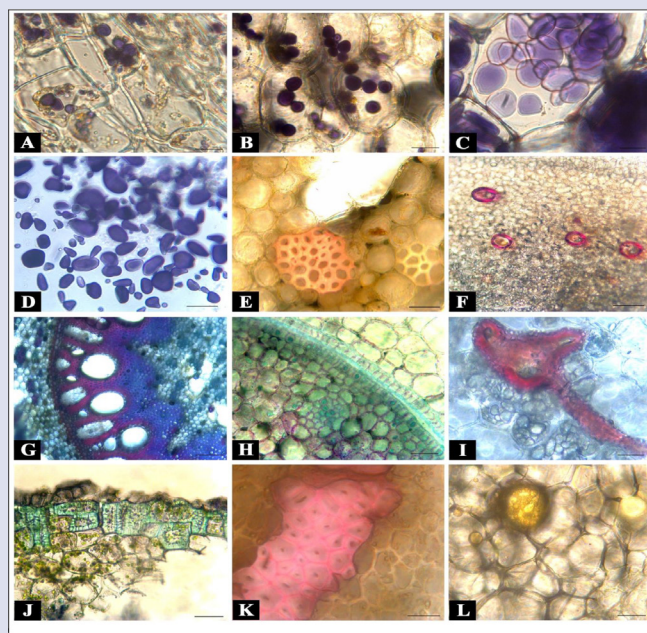


Figure 1. A-D: Starch grain: A. *Citrus limon*, B. *Crinum amoenum*; C. *Zingiber officinale*, D. *Nymphaea rubra*, E. Sclerenchymatous patch in *Areca catechu*; F. Collenchymatous cells in *Nymphaea rubra*; G. Lignin deposition in xylem and sclerenchymatous patches within phloem in *Areca catechu*; H. Lignin deposition in cell wall of cortical cells in *Allium sativum*; I. Sclerenchymatous cells in *Nymphaea rubra*; J. Lignin deposition in Sclerenchymatous fiber cells in *Piper nigrum*; K. Sclerenchymatous patch in *Citrus limon*; L. Oil gland in *Zingiber officinale*: Scale bar: 330µm (660x)

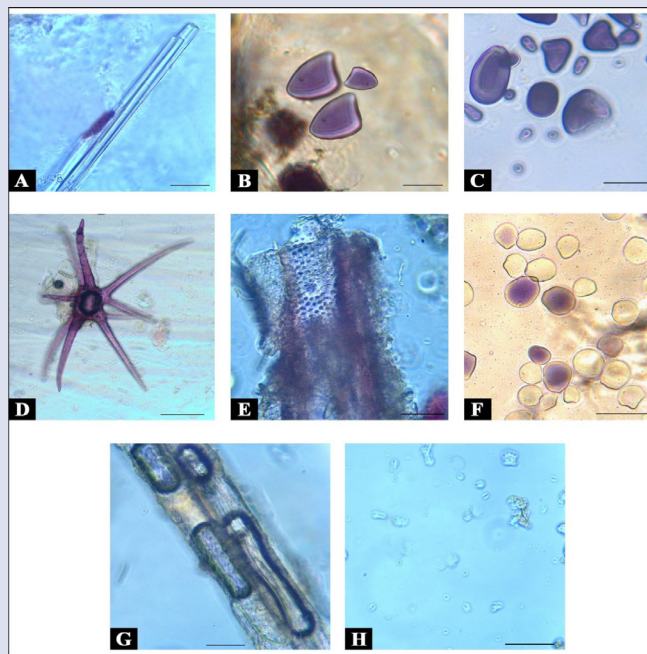


Figure 2. A & B: DYS1: A. Calcium oxalate crystal, B. Starch grains; C & D. DYS2: C. Starch grains; D. Astrosclereid, E & F. DYS3: E. Pitted vessels, F. Starch grains; G & H. DYS4: G. Stone cell, H. Starch grain absent; Scale bar = 330µm (660x)

Table 1: Fluorescence analysis of DYS1, DYS2, DYS3 and DYS4.

	(DYS1)			(DYS2)			(DYS3)			(DYS4)		
	VISIBLE LIGHT	UV-254	UV-365	VISIBLE LIGHT	UV-254	UV-365	VISIBLE LIGHT	UV-254	UV-365	VISIBLE LIGHT	UV-254	UV-365
50% KOH	Oyster white	Distant blue	Pearl night blue	Sand yellow	Signal blue	Black blue	Saffron yellow	Green blue	Pastel blue	Brown green	Sapphire blue	Green blue
10% FeCl ₃	Maize yellow	Ocean blue	Night blue	Daffodil yellow	Pastel turquoise	Sapphire blue	Daffodil yellow	Pastel turquoise	Pearl night blue	signal yellow	Black green	Grey blue
DISTILLED WATER	Light grey	Signal grey	Light blue	sand yellow	Graphite grey	Gentian blue	Oyster white	Black green	Sky blue	Beige red	Mint turquoise	Signal blue
HCL CONC.	Light ivory	Green blue	Emerald green	lemon yellow	Ocean blue	May green	Lemon yellow	Pearl gentian blue	Night blue	Traffic yellow	Mint turquoise	Grey blue
HCL 50%	Golden yellow	Pearl gentian blue	Leaf green	Ivory	Capri blue	Violet blue	Ivory	Pearl gentian blue	Water blue	Reed green	Pine green	Sapphire blue
H ² SO ₄ CONC.	Traffic black	Dusty grey	Ultramarine blue	Fawn brown	Graphite grey	Ocean blue	Signal orange	Black green	Sky blue	Salmon pink	Mint turquoise	Azure blue
H ² SO ₄ 50%	Signal blue	Cement grey	Graphite black	Fawn brown	Blue green	Distant blue	Steel blue	Azure blue	Sky blue	Melon yellow	Mint turquoise	Gentian blue
HNO ₃ CONC.	Dahlia yellow	Pearl gentian blue	Signal blue	Rape yellow	Blue green	Black blue	Daffodil yellow	Green blue	Ultramarine blue	Dahlia yellow	Signal black	Grey blue
HNO ₃ 50%	Sun yellow	Water blue	Traffic blue	Ivory	Blue green	Traffic blue	Sulfur yellow	Green blue	Traffic blue	Rape yellow	Mint turquoise	Pigeon blue
CH ₃ COOH CONC.	Signal white	Pearl black blue	Sky blue	Transparent	Pearl gentian blue	Pearl night blue	Green beige	Gentian blue	Traffic blue	Papyrus white	Mint turquoise	Signal blue
CH ₃ COOH 50%	Pastel yellow	Pearl beige	Water blue	Sand yellow	Brilliant blue	Violet blue	Golden yellow	Gentian blue	Capri blue	Olive grey	Mint turquoise	Ultramarine blue
CHLOROFORM	Pure white	Sapphire blue	Cobalt blue	Transparent	Brilliant blue	Signal blue	Green beige	Signal blue	Traffic blue	Light grey	Cobalt blue	Signal blue
BENZENE CONC.	Papyrus white	Traffic grey B	Signal blue	Transparent	Pearl gentian blue	Signal blue	Golden yellow	Blue green	Signal blue	Papyrus white	Mint turquoise	Cobalt blue
BENZENE 50%	Silk grey	Traffic grey B	Black blue	Transparent	Pigeon blue	Signal blue	Light ivory	Black green	Traffic blue	Pure white	Grey white	Ultramarine blue
METHANOL CONC.	Pure white	Traffic grey B	Gentian blue	Transparent	Pearl gentian blue	Signal blue	Ochre yellow	Ocean blue	Signal blue	Pure white	Mint turquoise	Signal blue
METHANOL 50%	Green beige	Chrome green	Light blue	Sand yellow	Blue green	Traffic blue	Light ivory	Pearl gentian blue	Light blue	Olive grey	Mint turquoise	Pearl night blue
ETHANOL CONC.	Pure white	Violet blue	Signal blue	Transparent	Sapphire blue	Pearl night blue	Oyster white	Traffic blue	Sky blue	Papyrus white	Night blue	Traffic blue
50% ETHANOL	Beige	Brilliant blue	Sapphire blue	Beige	Ocean blue	Capri blue	Light ivory	Sky blue	Light blue	Yellow grey	Cobalt blue	Traffic blue
ACETONE	Pure white	Sapphire blue	Ultramarine blue	Transparent	Night blue	Brilliant blue	Green beige	Sapphire blue	Signal blue	Pebble grey	Gentian blue	Signal blue
PETROLEUM ETHER	Pure white	Sapphire blue	Ultramarine blue	Transparent	Night blue	Cobalt blue	Oyster white	Steel blue	Cobalt blue	Pure white	Cobalt blue	Violet blue
ALCOHOLIC NaOH	Oyster white	Sapphire blue	Gentian blue	Light Ivory	Night blue	Traffic blue	Honey yellow	Gentian blue	Light blue	Olive grey	Violet blue	Signal blue
AMMONIA	Pastel yellow	Pegion blue	Azure blue	Pearl copper	Night blue	Night blue	Ivory	Traffic blue	Turquoise blue	Signal orange	Signal blue	Sapphire blue

Table 2: Physicochemical analysis of DYS1, DYS2, DYS3 and DYS4

	TOTAL ASH (%)	ACID INSOLUBLE ASH (%)	WATER SOLUBLE ASH (%)	ALCOHOL SOLUBLE EXTRACTIVE (%)	WATER SOLUBLE EXTRACTIVE (%)	LOSS ON DRYING (%)
DYS1	8.18	1.12	4.88	6.17	13.81	11.63
DYS2	14.63	1.37	13.47	3.87	4.64	11.53
DYS3	20.82	8.38	19.41	4.9	4.13	8.22
DYS4	8.11	0.68	5.9	5.71	7.56	6.9

organic reagents. In DYS1 and DYS2, Emerald to leafy green colours were fluoresced in presence of concentrated and 50% hydrochloric acid respectively. Mineral acids like nitric acid and sulphuric acid produced Traffic Blue and Gentian Blue respectively, when interacting with DYS1 and DYS4. Ammonia showed characteristic Turquoise and Sapphire Blue in response with drug formulation DYS3 and DYS4 respectively at UV 365 nm wavelength. DYS 3 also produced unique blue colours in presence of 50% acetic acid and methanol reagent.

Physicochemical characteristics

Total ash is mainly essential to check the purity of drugs showing the presence or absence of metallic salts or silica.^{20,21} the results obtained from physicochemical studies were given in Table 2. Total ash of DYS3 showed the highest value among four herbal drugs, which was found to be 20.82% w/w and DYS4 confirmed the lowest value of 8.11% w/w. The percentage of ash was calculated with reference to the air-dried powder. The acid insoluble ash was noted and DYS3 showed the highest value of 8.38% and the lowest value of 0.68% was observed in DYS4. While the presence of material exhausted by water i.e. the water-soluble ash was recorded with high value of 19.41% in DYS3 and the low value of 4.88% in DYS1. Purpose of extractive values is a further way of inspection of the purity of the herbal formulation. The water-soluble extractive value was found to be 13.81% w/w which signifies that more amount of constituents in DYS1 was soluble in water than in alcohol (6.17% w/w). The percentage of loss on drying i.e. the moisture content in the powder was found to be 11.63% w/w, which is highest in DYS1. In case of DYS4 showed the lowest moisture content.

Qualitative phytochemical analysis

The biological activity of herbal formulation mainly depends on the phytoactive secondary metabolites present in it. Thus preliminary screening of phytochemicals which was performed to establish a chemical profile of a crude drug was a part of chemical evaluation.^{22,23} these phytochemical tests (Table 3, 4, 5 and 6) revealed the presence of anthraquinones, cardiac glycosides, coumarine, flavonoids and steroids in different extracts of DYS1, DYS2, DYS3 and DYS4 obtained by using different solvents. Alkaloid and amino acid were found only at DYS1 and DYS3. Tannins were absent in all three formulation, except DYS4. In DYS4, soluble tannins were extracted with partial polar to polar solvents. Triterpenoids were found to be present in all formulations, while in DYS4, the same was found in benzene extract only.

Thin layer chromatography

Initial analysis of active constituents in DYS1, DYS2, DYS3 and DYS4 were carried out by thin layer chromatographic (TLC) method. Following particular spraying reagents for a specific active constituent, the results of TLC showed the presence of arbutin, bitter principle coumarins and saponin in all four formulations. It was found that except DYS2 all formulation showed the presence of anthrone. Anthrone and arbutin bands were most prominent in DYS4. Alkaloid was found only

in DYS1 and a clear orange brown band was visible in case of DYS3 under visible light after Dragendorff reagent application. Five band of cardiac glycosides were found only at DYS4, which was clearly distinguishable under visible light, while flavonoids spots on TLC plate was found only in case of DYS2 and DYS4. R_f values were calculated for all the spot and the analysis were explained at the Table no 7.

DISCUSSION

There has been a remarkable utilization of herbal formulations in traditional folk medicine for the treatment of diseases and disorders, especially as anti-inflammatory and analgesic agent.²⁴ So scientific evaluation is required for each and every formulations mentioned in our traditional indigenous knowledge.²⁵ Thus in this study, the pharmacognostic features of these four herbal formulations for primary dysmenorrhea are being reported for the first time.

Therefore, the powder of DYS1, DYS2, DYS3, and DYS4 were subjected to certain standardization parameters. There are numerous modern tools available for the evaluation technique of plant drugs, but powder microscopy is considered to be the most fundamental, authentic, cheap and reproducible methods to recognize the source materials.²⁶ Presence of starch granules in DYS2 and DYS3 has been confirmed after staining with iodine solution, whereas phloroglucinol solution showed the presence lignified tissues. Oil globules have been found only in powder DYS3 after staining with Sudan III. Fluorescence study is also an important parameter for standardization of crude drug. The different chemical constituents present in plant extract showed characteristic fluorescence when suitably illuminated. Certain chemical substances that not naturally fluoresce themselves are actually treated with different reagents to attain fluorescence.²⁷ the presence of specific amounts of acid, sugar and inorganic matter in the powders are confirmed by water-soluble ash technique, whereas acid insoluble ash methods clearly specify the presence of silica, thus indicating the impurities with earthy materials.²⁸ The extractive values are useful to evaluate the chemical constituents present in crude drug and also help for estimation of specific constituents soluble in a particular solvent. For any drugs and herbal formulation, low moisture content is essential for higher stability of drugs. The general requirement for moisture in a crude drug should not be more than 14%.²⁹ Excess moisture content may support the growth of fungi and may cause contamination by other microorganisms resulting in the degradation of the drugs. But DYS1, DYS2, DYS3 and DYS4 powder showed the presence of moisture content less than the required standard (Table 2) which was not too high to promote the growth of microorganisms. The pharmacological importance of a drug is attributed to the various secondary metabolites present in it and a particular compound might possess a clinical significance. Therefore it is essential to separate the compounds present in the plants with an appropriate chromatographic method. TLC technique has proved its worth as a simple, inexpensive and reproducible method for the chemical and biological screening of plant extracts. It provides a basic idea of the polarity of a particular chemical constituent.²⁹ Development of TLC plates with appropriate group reagents indicate

Table 3 : Preliminary phytochemical analysis of different solvent extracts of DYS1

Phytochemical	HP	BZ	EA	AC	BT	MT	AQ
Amino acid	-	-	-	-	-	+	-
Alkaloids	-	-	-	-	-	-	+
Anthraquinones	-	-	-	-	-	-	-
Cardiac glycosides	+++	+++	++	++	+	+	+
Coumarin	-	-	+	+	+	+	++
Flavonoids	-	-	-	-	-	-	-
Saponins	-	+	++	+	+	-	-
Steroids	++	+	+	++	++	+++	++
Tannin	-	-	-	-	-	-	-
Tri-terpenoids	-	-	+	+	-	-	-

Table 4 : Preliminary phytochemical analysis of different solvent extracts of DYS2

Phytochemical	HP	BZ	EA	AC	BT	MT	AQ
Amino acid	-	-	-	-	-	-	-
Alkaloids	-	-	-	-	-	-	-
Anthraquinones	-	-	-	+++	++	+	-
Cardiac glycosides	+++	++	++	+	+	++	++
Coumarin	-	-	++	++	+	++	++
Flavonoids	-	-	++	++	+	+	+
Saponins	++	+	+	+	+	-	-
Steroids	+	+	+	++	++	+++	+
Tannin	-	-	-	-	-	-	-
Tri-terpenoids	++	+	+	+	+	-	-

Table 5 : Preliminary phytochemical analysis of different solvent extracts of DYS3

Phytochemical	HP	BZ	EA	AC	BT	MT	AQ
Amino acid	-	-	-	+	-	+++	++
Alkaloids	++	+	-	-	-	-	-
Anthraquinones	-	+++	++	+	-	-	-
Cardiac glycosides	+++	+++	+++	++	++	+	+
Coumarin	++	+++	++	+	+	++	++
Flavonoids	-	+	-	-	-	-	-
Saponins	-	+	+	+	-	-	-
Steroids	+++	+++	++	++	+	++	+++
Tannin	-	-	-	-	-	-	-
Tri-terpenoids	-	-	+	+	+	+	-

Table 6 : Preliminary phytochemical analysis of different solvent extracts of DYS4

Phytochemical	HP	BZ	EA	AC	BT	MT	AQ
Amino acid	-	-	-	-	-	-	-
Alkaloids	-	-	-	-	-	-	-
Anthraquinones	-	+	+	++	+	++	++
Cardiac glycosides	++	++	++	++	++	++	++
Coumarin	-	+	++	++	++	+++	+++
Flavonoids	-	-	++	++	++	++	++
Saponins	+	+	+	+	-	-	-
Steroids	+	+	++	+++	+++	+++	++
Tannin	-	-	+	+	+	+	+
Tri-terpenoids	+	+	-	-	-	-	-

Table 7: Detection of active phytoconstituents in powder formulation DYS1, DYS2, DYS3 and DYS4 by TLC method

Phytoconstituents	Solvent system	Detection reagents	(DYS1)			(DYS2)			(DYS3)			(DYS4)		
			No. Of Spot	Rf values	No. Of Spot	Rf values	No. Of Spot	Rf values	No. Of Spot	Rf values	No. Of Spot	Rf values	No. Of Spot	Rf values
ANTHRONE	Ethyl acetate: methanol: water (100:13.5:10)	KOH reagent	1	0.04	0	0	1	0.22	1	0.22	1	0.47		
			2	0.29			2	0.33						
			3	0.55			3	0.4						
ALKALOIDS	Ethyl acetate: methanol: water (100:13.5:10)	Dragendorff reagent	1	0.69	0	0	1	0.71	0	0.71	0	0		
ARBUTIN	Ethyl acetate: methanol: water (100:13.5:10)	Berlin blue	1	0.74	1	0.54	1	0.66	1	0.66	1	0.16		
											2	0.24		
											3	0.43		
FLAVONOIDS	Ethyl acetate: Formic acid: glacial acetic acid: water (100:11:1:26)	NP/PEG reagent	0	0	1	0.97	0	0	0	0	1	0.93		
BITTER PRINCIPLES	Ethyl acetate: methanol: water (100:13.5:10)	Vanillin-sulphuric acid reagent	1	0.04	1	0.06	1	0.12	1	0.12	1	0.16		
			2	0.72	2	0.57	2	0.71	2	0.71	2	0.24		
					3	0.67					3	0.31		
CARDIAC GLYCOSIDES	Ethyl acetate: methanol: water (100:13.5:10)	Antimony-III chloride reagent	0	0	0	0	0	0	0	0	1	0.43		
											2	0.56		
											3	0.6		
											4	0.66		
											5	0.72		

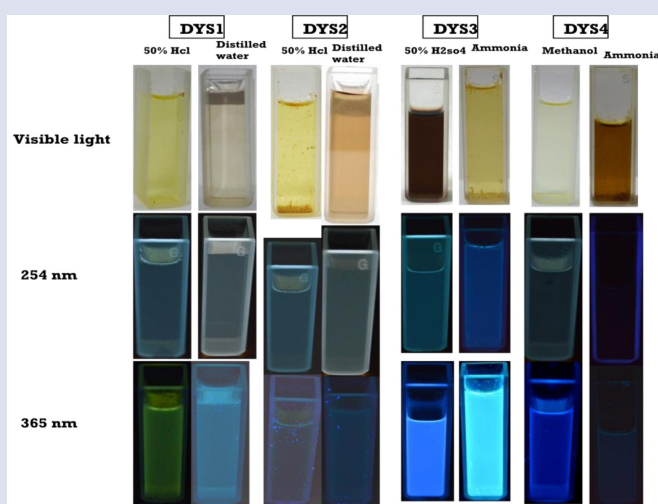


Figure 3: Different colours exhibited after treating the powder of DYS1, DYS2, DYS3 and DYS4 with different chemical reagents.

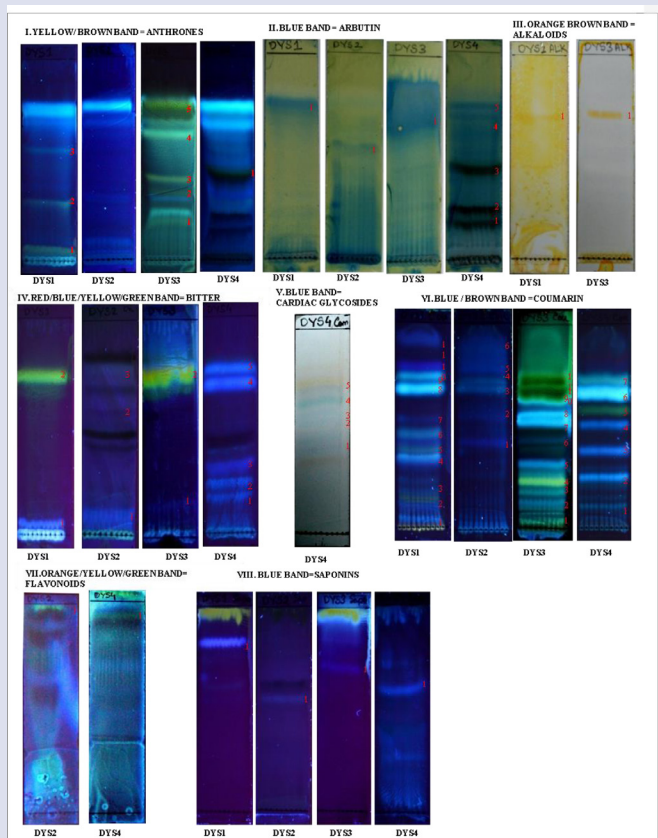


Figure 4: Preliminary investigation by Thin layer chromatography I. Anthrone II. Arbutin III. Alkaloid IV. Bitter Principle V. Cardiac Glycosides VI. Coumarin VII. Flavonoids VIII. Saponins.

the presence of anthrone, arbutin, bitter principle, cardiac glycosides, coumarins, flavonoids and saponins in DYS1, DYS2, DYS3 and DYS4 powder. The pattern of bands on TLC plates provides primary data and is used to show the uniformity and stability of herbal components. It is an effective and speedy way to differentiate between chemical classes which may not be fulfilled by macroscopic and microscopic analysis.³⁰

TLC is commonly used for generating fingerprints of herbal medicines as it is simple, versatile, sensitive to specific compounds.³¹ Thus, TLC is a convenient method of determining the quality and possible adulteration of herbal drugs.

Phytochemicals are important for the establishment of pharmaceutical companies; the constituents are playing a significant role in the identification of crude drugs. The medicinal value of these plants lies in some chemical substances that produces a definite physiological action on the human body.³³ Qualitative phytochemical studies on the different solvent extracts of four herbal formulations revealed that some solvents are good for extraction of phytoconstituents while some others are not suitable. Table no. 3, 4, 5 and 6 showed that the alkaloids and amino acids were found in DYS1 and 3 only. Presence of alkaloid is evidenced by the presence of Dragendorff's band in TLC Plate (Figure 3). All the extracts indicated the presence of cardiac glycoside and steroids in large amount (Table no. 3, 4, 5 and 6). TLC fingerprinting and preliminary phytochemical screening, both proved the presence of coumarin. Coumarins act as competitive inhibitors of vitamin K, which is a blood clotting factor. Coumarins are potent anti-inflammatory drugs and it directs against cell-adhesion molecules, thus highly significant in inflammatory responses.³⁴

CONCLUSION

All the scientific investigations support the traditional use of those powdered polyherbal formulation for the treatment of primary dysmenorrhea. The present study focused on establishing pharmacognostic standards for the identification and authentication of DYS1, 2, 3 and 4, as well as detection of adulterants. The important diagnostic features of all four polyherbal formulations established in this study may be useful for the further studies.

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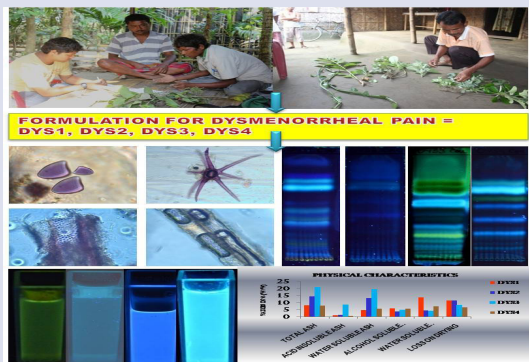
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GRAPHICAL ABSTRACT



SUMMARY

Dysmenorrhea is a sort of painful menstrual disorder. By semi-structured questionnaires in the course of scheduled interviews with the local herbal practitioners, four herbal formulations were recorded. Several plants like *Allium sativum* L., *Areca catechu* L., *Zingiber officinale* Roscoe, *Crinum amoenum* Ker Gawl. ex Roxb, *Cuscuta reflexa* Roxb., *Nymphaea rubra* Roxb. ex Andrews, *Piper nigrum* L., *Citrus limon* (L.) Osbeck were used in appropriate ratio to make herbal formulation to cure dysmenorrheal pain by the traditional healers of Rajbanshi community. The present research article focuses on the evaluation of pharmacognostic identity and quality parameters to be used as herbal drug and thus validating its ethno-medicinal use to treat Dysmenorrhea.

ABOUT AUTHORS



Mr. Priyankar Roy: Is a senior research fellow at the Department of Botany, University of North Bengal under the Basic Scientific Research scheme, UGC. He has 5 publications and his doctoral research is focused on the Pharmacognostic evaluation of herbal medicines used by traditional healers of Rajbanshi community in Terai & Duars region of West Bengal, India.



Dr. Subhasis Panda: Is an Associate Professor at Department of Botany, Maulana Azad College, University of Calcutta. Earlier in PG Deptt. of Botany, Barasat and Darjeeling Govt. Colleges. Also KS Manilal National Awardee in Angiosperm Taxonomy. Discovered 12 new plant species. Earlier he was Research Fellow in Central National Herbarium (1999-2003).



Dr. Mrs. Sonia Mitra Roy: Is an Assistant teacher of Biology at Himalayan International Residential School, Rajganj, Jalpaiguri, West Bengal, India. She has 7 publications and completed her doctoral thesis on "Foliar Architecture of Indian members of the Family Sterculiaceae and its Systematic Relevance".



Dr. Palash Mandal: Is an Assistant Professor at the Department of Botany, University of North Bengal, West Bengal, India. His research interest is in the area of Plant Physiology and Pharmacognosy. He has 70 nos. of research publications; 15 nos. of abstracts and 4 nos. of book chapters. He is having 17 years of teaching experience at North Bengal University and guiding many post graduate and doctoral students. Currently he is working on bioactivities of plant peptides; therapeutic potential of various ethnomedicine.

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