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EXTRACTION, CHARACTERIZATION AND PHYTOCHEMICAL ANALYSIS OF EXTRACT OF DOLICHANDRONE FALCATA PLANT.

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ABSTRACT

This study conducts a thorough investigation of the plant Dolichandrone falcata using a complex procedure involving extraction, characterisation, and analysis of its phytochemicals. The study thoroughly examines the composition and features of the plant, use a range of procedures to assure precision and dependability. The investigation commences with thorough botanical, macroscopic, and microscopic exams, meticulously recorded with pictures and photographs. Microscopic examination is an essential method for examining contaminants, guaranteeing the cleanliness and genuineness of the plant material being researched. The physicochemical analysis confirms the validity and quality of the plant material through the assessment of various characteristics. These criteria include the presence of foreign organic matter (0.45% w/w), compliance with moisture content, and the determination of ash values (total ash: 2.8%, acid insoluble ash: 0.34%, water soluble ash: 0.11%). The plant's extractive values demonstrate its potential for pharmacological action and extraction efficiency, with a higher ethanol-soluble extractive value of 14% w/w compared to a water-soluble extractive value of 3.8% w/w. The process of continuous hot Soxhlet extraction was used to get extracts in methanol (8.3%), ethanol (18.1%), and chloroform (5.5%). Prior to extraction, a preliminary phytochemical screening was conducted. The phytochemical screening of these extracts indicates the presence of vital components like alkaloids, glycosides, flavonoids, and terpenoids. The ethanol extract is particularly noteworthy due to its substantial metabolites, suggesting its potential for additional investigation. The bioactive ethanol extract is thoroughly characterised using Thin Layer Chromatography (TLC). Specific mobile phases and detection technologies are utilised to visualise and identify various chemical elements, such as alkaloids, glycosides, flavonoids, tannins, and terpenoids. The measured Rf values offer crucial information for the isolation and identification of specific bioactive chemicals.

KEYWORDS Dolichandrone falcata, phytochemical screening, TLC, alkaloids, glycosides, flavonoids.

INTRODUCTION

Phytochemistry is defined as the study of chemical composition of medicinal plants or phyto-drugs. Today we are witnessing a rapid growth of herbal drug industry and this growth story is accompanied by search of new herbal drugs. Standardized herbal extracts and phyto-chemicals are in huge demand for applied research as well as for commercial use.[1,2] The identification of biologically active compounds is an essential requirement for quality control and dose determination of plant based drugs There are many approaches for search of new biologically active principles in higher plants.[3] One such approach is systematic screening which may result in the discovery of novel effective bio-compound. Screening techniques of biologically active medicinal compounds have been conducted on well-known species of plants used in traditional medicines.[4,5]

In recent years, the Dolichandrone falcata is becoming very vital plant in medical and pharmaceutical industry. Dolichandrone falcata is a small deciduous tree in the family Bignoniaceae. It is endemic to India. Tree attains a height of 15–20 feet.[6] Leaves are compound 2-6 inches long with 3-6 obovate or oval shaped leaflets. Flowers are white and fragrant. The flowering of this occurs in April–May. The plant has numerous medicinal uses like antiallergic, anti-inflammatory, antioxidant, antiestrogenic, anxiolytic, anticonvulsant, and anti-parasitic. This plant is also used in curing anemia, bloody diarrhea, and also as anthelmintic, analgesic, antiviral, and antifungal agents.[7] The plant is used to prepare snake venom and also used in the treatment of liver disorder. The bark paste of Dolichandrone falcata is applied in case of fractures. The bark juice is used for menorrhagia and leucorrhoea. The leaves of this plant are used as antioxidant, antiestrogenic and anti-diabetic.[8]

The whole plant and its specific parts like leaves stem and roots are known to have medicinal properties. It has long history of use by indigenous and tribal people of world and India. The medicinal value of leaves and bark of this plant is also mentioned in Ayurveda.[9,10] The decoction of bark is given in the treatment of nodules by the Bhil tribes of the kota district in Rajasthan. Leaves paste is mixed with Neem leaves and applied for curb swelling. Fruit paste and bark paste is applied with water three times to get relief from scorpion bite and snake bite. Leaves juices taken orally with water acts an antidote to snake bite by tribals of Kota region. In medicinal folklore Gnanavendhan, (1995) claimed that this plant have anti- snake venom activity.[11,12]

MATERIAL AND METHODS

Plant Collection and authentication

Plant were collected from local place of Pune region. The plant *dolichandron falcata* was authenticated by Botanist of Botanical Survey of India, Pune by comparing morphological features. The herbarium of the plant specimen was deposited at Botanical Survey of India, Pune; with the Voucher specimen number (Ref. No. BSI/WRC/IDEN. CER. /CRC/2022/3003220017600 Dated 07/04/2022).

1. Chemicals and Reagents

Petroleum ether, Ethanol, Methanol, Dichloromethane, n-hexane, Ethyl acetate, Glacial acetic acid, N-Butanol, Chloroform, Acetone, Formic acid, Benzene, Dimethylsulphoxide (DMSO), conc. Sulphuric acid, Hydrochloric acid, Benzene, pyridine, toluene, anisaldehyde, calcium chloride, copper sulphate, Ferric chloride, Follin's reagent, Iodine, Lead acetate, Magnesium chloride, Mercuric chloride, Ninhydrin, Nitric acid, Phloroglucinol, Potassium iodide, Potassium Dichromate, Potassium sodium Tartarate, Ruthenium red, Sodium acetate, Sodium iodide, Sodium hydroxide, Sodium nitroprusside, Hide powder, Folin Ciocalteu reagent, Sodium bicarbonate, Gallic acid and all the chemicals and reagents are analytical grade (Research lab Fine Chemicals Pvt. Ltd Mumbai, SD Fine Chem Mumbai, and Merck, India) were purchased from local suppliers.

2. Pharmacognostic investigations of selected plant material [184]

Organoleptic, Morphological and Microscopic evaluation

The organoleptic characteristics like colour, odour, taste and macro morphological characteristics like shape, size, surface characteristics (texture and fracture) were studied for selected plant materials.

3. Preparation of plant extracts

The plant material was collected and dried in the shade. Then the dried material is pulverized in grinder. The powdered material was passed through 120 mesh sieves to remove fine powder and course powder was used for extraction.

4. Preliminary phytochemical investigations

Determination of foreign organic matter

5 gm dry air was spread onto a thin layer of coarsely powdered drug. With the unwanted eye or using 6X glasses, the sample was examined. As fully as practicable, the international organic matter was manually isolated. The sample was measured and the weight of the drug was taken as a proportion of foreign organic matter

Determination of moisture content

The glass-stopper was properly measured, and the measuring bottle was shallow. The weight was taken, 2 gm of the sample was moved and coated and dispersed randomly and the sample was scattered over distances of up to a limit of 10 mm. The bottle was then kept primed and removed in an oven. The sample was continually drying up. The desiccator was extracted after drying at room temperature. Weighed and drying losses is measured as a percentage w/w

Determination of Ash value

Ash content in the investigated plant species *dolichandron falcata* was calculated by the methods given below:

a. Determination of Total ash

2gm of aerated crude drug was correctly measured in a tared silica dish and incinerated at not more than 450 livres, before carbon-free, cooled and weight free was taken. The ash percentage was measured using the air-dried medication.

b. Determination of Water- soluble ash

Ash was collected according to the process mentioned above and boiled with 25 ml of water over 5 minutes, washed in hot water and ignited for not more than 350 livres C for 15 minutes on an ash with less filter paper, and then purified and collected the insoluble content on an ash. Difference of weight is water-soluble ash; it separates the weight of the insoluble substance from the weight of the ash. The water-soluble ash percentage was measured for the air-dried medication.

c. Determination of Acid -insoluble ash

According to the mentioned process, the ash was collected, boiled 5 minutes in 25 mL hydrochloric acid, washed in warm water and ignited cooled in a desiccator, and weighted in solutions on ash less filtering paper. The acid-insoluble ash proportion with the air-dried medication was measured..

Extractive values

Different extractive values like alcohol soluble extractive, water soluble extractive values were performed by standard method

Determination of water-soluble extractive value

Five gms of air dried in a gross powdered drug have been macerated in a closed flask for 24 hours with 100 ml of chloroform spray, which has constantly been shook in the first 6 hours and can stand 18 hours. It was then filtered, 25 ml of the filter evaporated and dried at 1050c and weighed in a shallow, flat dish. With relation to air-dried drugs, the water-soluble extractive benefit percentage has been determined.

Determination of Alcohol-soluble extractive value

Five gm of coarsely-powdered air-dried medicinal substance has been macerated for a period of 24 hours in a closed flask with 100 ml of ethanol with indicated strength and has also been shook for the initial 6 hours. It was then purified, and ethanol loss was stopped during filtering, 25 ml of the filtrate was evaporated onto a flat low layer, dried at 105^0C and measured. The proportion of ethanol-soluble extractive value referred to air-dried drugs was determined.

5. Preliminary phytochemical investigations

1) Test for Carbohydrates

a) Molisch test (General test)

Two ml of extract solution was added with few drops of 15 % ethanolic alpha naphthol solution in a test tube and 2ml of concentrated sulphuric acid was added carefully along the side of the test tube. The formation of reddish violet ring at the junction of two layers indicates the presence of carbohydrates.

b) Test for reducing sugar

i) Benedict's test

Mix equal volume of Benedict's regent and extract solution in test tube. Heat in a boiling water bath for 5 min. Solution appears green, yellow or red depending on amount of reducing sugar present.

ii) Fehling's test

Five ml of extract solution was mixed with 5 ml Fehling's solution (equal mixture of Fehling's solution A and B) and boiled. Development of brick red precipitate indicates the presence of reducing sugars.

c) Test for monosaccharides

Barford's test

Mix equal volume of barford's reagent and extract solution. Heat for 1-2 min. in boiling water bath and cool. Development of red precipitate indicates presence of monosaccharides.

2) Test for Proteins

i) Biuret test

The extract was treated with 1 ml of 10 percent sodium hydroxide solution and heated. A drop of 0.7 percent copper sulphate solution was added to the above mixture. The formation of purple violet color indicates the presence of proteins.

ii) Millon's test

The extract was treated with 2 ml of Millon's reagent. Formation of white precipitate indicates the presence of proteins and amino acids.

3) Test for Amino acids

Ninhydrin test

The extract was treated with Ninhydrin reagent at pH range of 4-8 and boiled. Formation of purple color indicates the presence of amino acids.

4) Test for Steroids

i) Salkowski test

One ml of concentrated sulphuric acid was added to 10 mg of extract dissolved in 1 ml of chloroform. A reddish-brown color exhibited by chloroform layer and green fluorescence by the acid layer suggests the presence of steroids.

ii) Liebermann-burchard test

10 mg extract was dissolved in 1 ml of chloroform and 1 ml of acetic anhydride was added following the addition of 2 ml of concentrated sulphuric acid from the side of the test tube. Formation of reddish violet color at the junction indicates the presence of steroids.

iii) Liebermann's test

To 2 ml of the residue a few ml of acetic anhydride was added and gentle heated. The content of the test tube was cooled and 2 ml of concentrated sulphuric acid was added from the side of the test tube. Development of blue color gave the evidence for presence of steroids.

5) Test for Terpenoids

One ml of extract added with one ml of Vanillin sulfuric acid. Development of violet color gave the evidence for presence of Terpenoids.

6) Test for Glycosides

i) Cardiac glycoside

Keller-Killiani test (Test for deoxysugars)

To 2 ml of extract, glacial acetic acid, one drop 5 % Ferric chloride and conc. Sulphuric acid was added. Presence of cardiac glycosides is indicated by formation of reddish brown color at junction of the two liquid layers and upper layer appeared bluish green.

ii) Anthraquinone glycosides

Borntrager's test

To 3 ml extract add dilute sulphuric acid, boil and filter. To the cold filtrate, add equal volume benzene or chloroform. Shake well. Separate organic solvent. Add ammonia, the ammonia layer turns pink or red color.

7) Test for Saponins

Foam formation test

One ml solution of the extract was diluted with distilled water to 20 ml and shaken in a graduated cylinder for 15 minutes. The development of stable foam indicates the presence of Saponins.

8) Test for Alkaloids

Evaporate all extracts separately. To residue, add dilute HCL. Shake well and filter. Use filtered solution for test.

i) Dragendroff's test

2-3 ml test solution and 0.1 ml Dragendroff's reagent was added in test tube. Formation of orange brown precipitate indicates the presence of alkaloids.

ii) Mayer's test

2-3 ml test solution and 0.1 ml of Mayer's reagent were added. Formation of yellowish buff precipitate indicates the presence of alkaloids.

iii) Hager's test

2-3 ml test solution and 0.1 ml of Hager's reagent. Formation of yellowish precipitate indicates the presence of alkaloids.

iv) Wagner's test

2-3 ml test solution and 0.1 ml of Wagner's reagent. Formation of reddish brown precipitate indicates the presence of alkaloids.

9) Test for Tannins and Phenolic compounds

i) 5 %Ferric chloride

Five ml of extract solution was allowed to react with 1 ml of 5 percent ferric chloride solution. Deep blue-black coloration indicates the presence of tannins.

ii) Dilute nitric acid test

Two ml of extract solution was allowed to react with few drops of dilute HNO_3 solution. Formation of reddish to yellow color indicates the presence of tannins.

iii) Bromine water test

Two ml of extract solution mix with 2 ml of bromine water. Discoloration of bromine water indicates presence of tannins.

iv) Potassium dichromate test

2-3 ml of extract solution and mix with 2 ml of Potassium dichromate. The formation of red precipitate indicates presence of tannins.

10) Test for Flavonoids

i) Shinoda test

To the extract 5 ml (95%) ethanol and few drops of con. HCl and 0.5 g of magnesium turnings was added gives pink color.

ii) Lead acetate test

Few drops of 10 percent lead acetate are added to the extract. Development of yellow colored precipitate confirms the presence of flavonoids.

iii) Sodium hydroxide test

Increasing amount of sodium hydroxide add in an extract solution which shows yellow coloration, which disappears after addition of acid.

6. Extraction Procedure

The extraction was carried out with selected solvent Methanol, ethanol and chloroform. The extraction was carried out in Soxhlet extractor till all the constituents were extracted. The completion of extraction was indicated by taking sample of siphon tube on TLC plate and placing it in iodine chamber. Absence of colored spot on plate indicated complete extraction. After completion of extraction, solvent was distilled off and concentrated extract was air-dried. The extract was stored in airtight container. The same procedure was followed during extraction with other solvents. After Methanol, Ethanol (95%) extraction the exhausted marc was kept in oven to remove the solvent completely. Finally, the same dried powdered material was extracted with chloroform.

RESULTS AND DISCUSSION

Each monograph contains detailed botanical, macroscopic and microscopic descriptions with detailed illustrations and photographic images which provide visual documentation of accurately identified material. A microscopic analysis assures the identity of the material and as an initial screening test for impurities.

1. Determination of foreign organic matter:

Foreign organic matter in *Dolichandrone falcata leaves* powder was found to be 0.45% w/w when observed under 6X lens.

2. Determination of moisture content:

Moisture content was measured and it was observed that, the results were complying as per standard guidelines.

Table 2. Observation of loss on drying.

Time (Min.)	Loss of moisture (%w/w)
0	0.00
30	0.300
60	0.220
90	0.180
120	0.180
Values are mean ± SEM, (n=3)	

3. Determination of Ash value:

Ash value is useful in determining authenticity and purity of sample and also these values are important qualitative standards.

Table 2. Ash value of plant material

Sr. No.	Evaluation Parameters	Content (% by weight)
1.	Total ash value	2.8
2	Acid insoluble ash value	0.34
3	Water soluble ash value	0.11
Values are mean ± SEM, (n=3)		

4. Determination of Extractive values: -

Ethanol-soluble extractive value was found to be greater than other extractive value; it indicates that compounds present in the leaves are soluble in alcohol in high amount. This might guide us for the isolation of maximum active components form plant.

Table 3. Extractive Values (%w/w) plants

Sr. No.	Extractive values	Extractive value (%w/w)
1	Ethanol soluble extractive values	14
2	Water soluble extractive values	3.8
Values are mean ± SEM, (n=3)		

The total ash value, acid insoluble ash, water soluble ash was observed for both plant crude drug materials. Acid value percentage clearly indicates that the plant may be best for drug action and effects. The Water-soluble extractive value plays an important role in evaluation of crude drugs. Less extractive value indicates addition of exhausted material, adulteration or incorrect processing during drying or storage. The water-soluble extractive value proved to be lesser than

alcohol soluble extractive value. This shows that the constituents of the drug are more extracted and soluble in alcohol as compared to water.

5. Extraction:

The plant material was collected and dried in the shade. Then the dried material is pulverized in grinder. The powdered material was passed through 120 mesh sieves to remove fine powder and course powder was used for extraction.

Method

Continuous hot Soxhlet extraction

6. Extraction Yield

Percent yield Reported and extract was evaluated for Preliminary Phytochemical Screening.

Table 4: Characteristics of Extracts

Sr. No.	Extract/ Solvent	Yield (%)	Colour
1.	Methanol	08.3	Brown
2.	Ethanol	18.1	Green, Dark brown
3.	Chloroform	05.5	Black

7. Preliminary Phytochemical Screening:

Extracts were tested for the presence of active principles such as Terpenoid, Steroids, Glycosides, Saponins, Alkaloids, Flavonoids, Tannins, Proteins, Free Amino Acids, Carbohydrate and Vitamin C. Qualitative chemical test used to identify drug quality and purity. The identification, isolation and purification of active chemical constituents are depending chemical methods of evaluation.

Table 5: Preliminary Phytochemical Screening

Extracts	Methanol	Ethanol	Chloroform
Tests for carbohydrates			
Molish Test	-	+	-
Fehling Test	+	-	-
Benedict Test	-	-	-
Test for Monosaccharide			
Barfoed's Test	-	-	-

Test for Non-reducing polysaccharides			
Iodine Test	-	+	-
Test for Proteins			
Biuret test	+	-	-
Millions test	-	-	-
Tests for Steroids			
Salkowaski reaction	+	+	-
Libermann Burchard reaction	+	-	-
Libermann reaction	-	-	-
Tests for Terpenoids	+	+	-
Test for Glycosides			
Borntrager's Test	-	-	-
Killer- Killani Test	-	+	-
Test for Saponin			
Foam test	+	-	-
Tests for Flavonoids			
Shinoda test	+	+	-
Lead acetate Test	-	+	-
Sod-hydroxide Test	-	-	-
Tests for Alkaloids			
Meyers Test	-	+	-
Wagner's Test	-	+	-
Hager's Test	-	-	-
Dragendorff Test	+	+	-
Test for Tannins & Phenolic compounds			

$FeCl_3$	+	+	+
Lead acetate	-	+	+

+ Indicates presence of phytoconstituents, - Indicates absence of phytoconstituents

The all extracts were screened for the presence of various constituents. The result of this preliminary phytochemical examination is shown in Table.

The ethanol extract showed presence of significant metabolites like alkaloids, glycoside and flavonoids. So the ethanol extract will utilize for further plan of research work.

8. **Characterization of bioactive extract**

Table 6. TLC- Characterization of bioactive extract.

Sr. no.	Chemical constituent	Mobile Phase	Visualization / Detection	Observed Rf-value
1.	Alkaloids	n- butanol : Ethyl acetate: Formic acid : Water (30:50:10:10)	UV -365nm	0.78
2.	Glycoside	Ethyl acetate : Methanol : Water (100 : 16.5 : 13.5)	UV -365nm	0.45
3.	Flavonoid	Toluene : Ethyl acetate : Glacial acetic acid :Water (100:11:11:26)	Anisaldehyde – Sulfuric acid. UV -365nm	0.63
4.	Tannins	Ethyl acetate: Formic acid : Acetic acid : Water (100:11:11:26)	5% $FeCl_3$ in 0.1N HCl	--
5.	Steroids	Ethyl acetate : Methanol : Water (70 : 20 : 10)	Vanillin – Sulfuric acid.	--
6.	Terpenoids and Carotenoids	Cyclohexane : Ethyl acetate (75 : 25)	UV- 268nm	--
		Petroleum ether : Benzene (9 : 1)	UV- 254nm	--
7.	Triterpenoids	Chloroform : Glacial acetic acid Methanol :Water (60 : 32 : 12 : 8)	--	--
		Ethyl acetate : Glacial acetic acid : Formic acid : Water (100 : 11: 11 : 26)	Anisaldehyde – Sulfuric acid. UV – 254nm, 365nm	--

CONCLUSION

The present study focused on the comprehensive extraction, characterization, and phytochemical analysis of Dolichandrone falcata leaves. The research employed meticulous methods to ensure the accuracy and reliability of the results. The plant material exhibited minimal foreign organic matter (0.45% w/w), indicating a high level of purity. Ash values, including total ash, acid insoluble ash, and water soluble ash, were within acceptable limits, confirming the authenticity of the sample. The higher alcohol soluble extractive value (14% w/w) compared to water soluble extractive value (3.8% w/w) suggested superior extraction potential of active compounds using alcohol. The continuous hot Soxhlet extraction method yielded extracts in methanol (8.3%), ethanol (18.1%), and chloroform (5.5%). The ethanol extract was particularly rich in alkaloids, glycosides, and flavonoids, indicating its potential for further research. The extracts underwent rigorous phytochemical screening, revealing the presence of essential compounds like alkaloids, glycosides, flavonoids, and tannins. These compounds are often associated with various pharmacological activities. TLC analysis further confirmed the presence of specific chemical constituents in the ethanol extract, providing valuable insights for the isolation and identification of individual bioactive compounds.

The findings of this study provide a strong foundation for further exploration of Dolichandrone falcata in the field of pharmacology. The ethanol extract, rich in alkaloids, glycosides, and flavonoids, holds promise for the development of novel drugs or natural remedies. Subsequent studies could focus on isolating and characterizing these bioactive compounds to understand their individual therapeutic potential. Moreover, detailed pharmacological investigations, including in vivo studies, can shed light on the specific health benefits associated with these compounds. In conclusion, this research contributes valuable data to the understanding of Dolichandrone falcata's phytochemical composition and underscores its potential applications in the pharmaceutical industry. These findings not only enhance our knowledge of this plant species but also pave the way for the development of new and effective medicinal products.

CONFLICT OF INTEREST

None declared by authors.

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