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PHARMACOGNOSTICAL, PHYTOCHEMICAL AND ANTI-FUNGAL EVALUATION OF *MURDANNIA NUDIFLORA* (L.) BRENAN EXTRACT-BASED HERBAL SPRAY

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ABSTRACT

The present study was undertaken to formulate and evaluate a herbal spray containing ethanolic extract of *Murdannia nudiflora* (L.) Brenan. The herbal spray was prepared using *Murdannia nudiflora* extract, isopropyl alcohol, acetone, glycerine, and purified water. The prepared formulation was evaluated for physical appearance, colour, odour, pH, specific gravity, sensitivity, irritation, flame test, and spray pattern. Pharmacognostical evaluation of the roots revealed characteristic anatomical features, including parenchymatous cells, endodermal cells, pericycle cells, xylem vessels, fibres, medullary rays, and starch grains. Preliminary phytochemical screening of the ethanolic extract showed the presence of carbohydrates, alkaloids, tannins, flavonoids, and saponins. The formulated herbal spray was a liquid with light-blue colour and aromatic odour. The pH and specific gravity were found to be 7.54 and 1.11, respectively. The sensitivity and irritation tests showed no irritation. Antifungal activity was evaluated against *Candida albicans* by the agar well diffusion method. The formulation showed zones of inhibition of 9, 10, and 12 mm at concentrations of 50, 75, and 100 µg/mL, respectively, while Amphotericin B showed a zone of inhibition of 14 mm. The findings indicate that the prepared *Murdannia nudiflora* herbal spray possesses promising antifungal activity and may have potential for further development as a herbal topical preparation.

KEYWORDS: *Murdannia nudiflora*, Herbal spray, Phytochemical screening, Antifungal activity, *Candida albicans*, Amphotericin B, Agar well diffusion.

1. INTRODUCTION

Pharmacognosy is the branch of pharmaceutical science that deals with the study of crude drugs obtained from natural sources such as plants, animals, minerals, and microorganisms. The word "pharmacognosy" is derived from the Greek words *pharmakon* meaning drug and *gnosis* meaning knowledge. It involves the identification, cultivation, collection, processing, evaluation, and therapeutic uses of natural products. Pharmacognosy plays an important role in drug discovery and development by providing information about the chemical constituents and medicinal properties of natural substances. Many modern medicines, such as morphine, quinine, and digitalis, have originated from natural sources studied under pharmacognosy.

Herbal medicine is one of the oldest systems of healthcare and refers to the use of medicinal plants and plant-derived products for the prevention and treatment of diseases. Herbal medicines contain biologically active compounds such as alkaloids, glycosides, tannins, flavonoids, and essential oils, which produce therapeutic effects. Traditional systems of medicine, including Ayurveda, Siddha, Unani, and Traditional Chinese Medicine, extensively utilize herbal drugs. In recent years, herbal medicines have gained significant importance worldwide because of their efficacy, safety, affordability, and fewer side effects. The integration of traditional knowledge with modern scientific research has contributed to the growing demand for herbal medicines and has strengthened the importance of pharmacognosy in pharmaceutical and healthcare sciences.

Herbal medicines are widely used as source of pharmaceutical drugs. This shows significant effects against pathogens. Globally80% of people uses herbal medicines for their healthcare. The medicinal plants shows significant role in herbal medicine systems like Siddha and Ayurveda. Most of the medicines are produced from natural herbs. The demand for medicinal plants high as become of active constituents in medicinal herbs[1]. As per existing research data, there are number of biodiversity center in the world of these, India is one of the center with 45000 plants and among these 800 herbal plants has been used as therapeutic agents globally. These fundamental phytochemical constituents act as new raw source of new drug and the remaining herbal need to be explored for their pharmaceutical potential.

2. PLANT PROFILE

Plant Profile of *Murdannia nudiflora* (L.) Brenan

2.1 Scientific Classification

- **Kingdom:** Plantae
- **Subkingdom:** Tracheobionta
- **Division:** Magnoliophyta
- **Class:** Liliopsida
- **Order:** Commelinales
- **Family:** Commelinaceae
- **Genus:** *Murdannia*
- **Species:** *Murdannia nudiflora* (L.) Brenan

2.2 Synonyms

- *Commelina nudiflora* L.
- *Aneilema nudiflorum* R. Br.

2.3 Common Names

- **English:** Doveweed
- **Tamil:** Kanangkozhi Keerai
- **Hindi:** Jalghas
- **Malayalam:** Nilappana
- **Telugu:** Adavi Gaddi

2.4 Geographical Distribution

Murdannia nudiflora is widely distributed in tropical and subtropical regions of Asia, Africa, Australia, and America. In India, it is commonly found in Tamil Nadu, Kerala, Karnataka, Andhra Pradesh, and West Bengal. The plant grows abundantly in moist areas, grasslands, agricultural fields, roadsides, and wetlands.

2.5 Habitat

The plant prefers warm and humid climatic conditions. It grows well in fertile, moist soils and is commonly observed during the rainy season. It thrives in open fields, gardens, and marshy places.

2.6 Morphological Characteristics

Murdannia nudiflora is an annual herb with creeping or ascending stems. The leaves are simple, narrow, and lanceolate with parallel venation. The roots are fibrous and well-

developed. The flowers are small, bluish to violet in colour and arranged in terminal or axillary inflorescences. The fruits are capsules containing numerous seeds.

2.7 Macroscopic Characters of Root

The roots are fibrous, cylindrical, and light brown in colour. They possess a characteristic odour and slightly bitter taste. The surface is rough and shows branching patterns.

2.8 Phytochemical Constituents

The plant contains several bioactive compounds, including:

- Alkaloids
- Flavonoids
- Tannins
- Glycosides
- Saponins
- Steroids
- Phenolic compounds
- Terpenoids
- Carbohydrates

2.9 Traditional Uses

Traditionally, *Murdannia nudiflora* has been used in folk medicine for the treatment of fever, inflammation, wounds, skin disorders, gastrointestinal problems, and microbial infections. Different parts of the plant are also used as cooling and healing agents.

2.10 Pharmacological Activities

Studies have reported various pharmacological activities of *Murdannia nudiflora*, such as:

- Anti-oxidant activity
- Anti-microbial activity
- Anti-inflammatory activity
- Hepatoprotective activity
- Anti-diabetic activity
- Wound-healing activity
- Anti-cancer activity

2.11 Method of Propagation

The plant propagates mainly through seeds and vegetative methods. Seed germination occurs rapidly under favourable environmental conditions.

2.12 Economic Importance

Murdannia nudiflora possesses significant medicinal and pharmaceutical value because of its phytochemical constituents and therapeutic properties. It has considerable potential for the development of herbal formulations and natural healthcare products.

***Murdannia nudiflora* (L.) Brenan**

Stem & Leaves Seeds Roots & Stem

Fig: 1 Plant Image

3. MATERIALS AND METHODS

3.1 PREPARATION OF ROOT POWDER

The collected roots were thoroughly cleaned to remove adhering soil and foreign materials. The roots were shade-dried at room temperature to avoid degradation of thermolabile constituents. The dried roots were powdered using a mechanical grinder, sieved to obtain uniform particle size, and stored in airtight containers in a cool and dry place until further analysis.

3.2 PHARMACOGNOSTICAL STUDIES

Pharmacognostical studies were carried out to establish the identity, purity, and quality of the plant material. Morphological and microscopic examinations are essential prerequisites in the evaluation of medicinal plants because any error in botanical identification may invalidate the entire research work. Therefore, diagnostic characters of the plant were studied in detail.

Macroscopic Evaluation

Macroscopic evaluation of the roots was carried out by observing the external features such as shape, size, colour, odour, taste, texture, and surface characteristics. The observations obtained were recorded and used as standard parameters for identification.

Collection and Fixation of Specimens

Healthy specimens were collected from the natural habitat. Different parts of the plant were excised and fixed in FAA solution containing formalin (5 mL), acetic acid (5 mL), and 70% ethyl alcohol (90 mL). After fixation for 24 hours, the specimens were dehydrated with graded concentrations of tertiary butyl alcohol according to standard procedures.

Embedding and Sectioning

The dehydrated specimens were infiltrated with molten paraffin wax having a melting point of 58–60°C until complete saturation was achieved. The specimens were then embedded in paraffin blocks and sectioned using a rotary microtome. Sections of 10–12 µm thickness were prepared for microscopic examination.

Staining Procedure

The paraffin sections were deparaffinized and stained with toluidine blue following standard procedures. Toluidine blue acts as a polychromatic stain and imparts different colours to various tissues. Cellulose walls appeared pink, lignified tissues appeared blue, suberin-containing tissues appeared dark green, mucilage appeared violet, and protein bodies appeared blue. Whenever necessary, additional staining was performed using safranin, fast green, and iodine solution for starch detection.

Powder Microscopy and Photomicrography

Powder microscopy was performed to identify characteristic features of the powdered root. Microscopic photographs were taken using a Nikon Labphoto-2 microscope under bright-field and polarized light conditions. Structures such as crystals, starch grains, and lignified tissues exhibited birefringence under polarized light.

Physicochemical Evaluation

The powdered drug was subjected to various physicochemical parameters according to WHO guidelines. These parameters included foreign organic matter, moisture content, extractive values, ash values, and fluorescence analysis.

Determination of Foreign Organic Matter

Ten grams of air-dried powdered drug was spread in a thin layer and inspected visually or with a 6× lens. Foreign materials were separated manually and weighed. The percentage of foreign organic matter was calculated with reference to the weight of the sample taken.

Determination of Moisture Content (Loss on Drying)

Ten grams of powdered drug was placed in a previously weighed evaporating dish and dried at 105°C for five hours. Drying and weighing were continued at one-hour intervals until a constant weight was obtained. The percentage loss on drying was calculated.

Determination of Extractive Values

Five grams of powdered drug was macerated with 100 mL of solvents of increasing polarity, including petroleum ether, benzene, chloroform, ethanol, methanol, and water. The mixture was shaken frequently for six hours and allowed to stand for eighteen hours. The filtrate was evaporated to dryness and weighed.

Alcohol-Soluble Extractive Value

Five grams of powdered material was mixed with 100 mL of 90% alcohol and subjected to continuous shaking for six hours. The mixture was allowed to stand overnight and filtered. The filtrate was evaporated, and the extractive value was calculated.

Water-Soluble Extractive Value

Five grams of powdered drug was macerated with chloroform water, shaken for six hours, and kept overnight. The filtrate was evaporated to dryness, and the percentage extractive value was calculated.

Determination of Ash Values

Ash values provide information regarding the purity and quality of crude drugs. Total ash, acid-insoluble ash, and water-soluble ash values were determined.

Total Ash

Three grams of powdered drug was incinerated in a silica crucible at a temperature not exceeding 450°C until it became carbon-free. The crucible was cooled and weighed, and the percentage of total ash was calculated.

Acid-Insoluble Ash

The total ash was boiled with hydrochloric acid, filtered, washed, dried, and ignited. The residue obtained represented the acid-insoluble ash.

Water-Soluble Ash

The total ash was treated with water and boiled. The insoluble residue was separated, dried, and weighed. The difference between total ash and insoluble matter represented water-soluble ash.

3.3 FLUORESCENCE ANALYSIS

The powdered root was treated with different reagents such as water, hydrochloric acid, sulphuric acid, acetic acid, sodium hydroxide, iodine solution, ferric chloride, petroleum ether, and ammonia solution. The treated samples were examined under daylight and ultraviolet light to study their fluorescence characteristics.

3.4 PRELIMINARY PHYTO-CHEMICAL SCREENING

Preliminary phyto-chemical screening was carried out to detect the presence of various secondary metabolites such as carbohydrates, alkaloids, tannins, flavonoids, saponins, proteins, amino acids, glycosides, phenolic compounds, terpenoids, volatile oils, fixed oils, gums, resins, mucilage, quinones, coumarins, sterols, and emodins.

The phytochemical tests included Molisch's test, Fehling's test, Benedict's test, Mayer's test, Dragendorff's test, Wagner's test, Shinoda's test, froth test, Millon's test, ninhydrin test, Biuret test, Keller–Kiliani test, Borntrager's test, Salkowski's test, and Folin–Ciocalteu test for the identification of different classes of phytoconstituents.

3.5 FORMULATION OF SPRAY

Table: 1 Formulation of Spray.

S.NO	INGREDIENTS	QUANTITY
1	*Murdannia nudiflora* (L.) Brenan. Extract	5ml
2	IPA	40ml
3	Acetone	25ml
4	Glycerine	5ml
5	Purified Water	25ml

Procedure

A 500ml conical flask was washed with water and then it was rinsed thoroughly with 90% ethanol. The required chemical was measured accurately using 100ml measuring Cylinder. Isopropyl alcohol (40ml); acetone (25ml); were first accurately measured and transferred into a 500 ml conical flask. Mix uniformly using a glass rod/stirrer. Then add herbal extract 5ml *Murdannia nudiflora* (L.) Brenan. Extract to the conical flask and mix then. Then incorporated water (25ml) to the 500ml conical flask and finally added glycerine (5ml) into the 500 ml conical flask. Mix the contents in the conical flask thoroughly using a stirrer until we get a uniform mixture. Transfer the prepared solution into the sterile spray apparatus.

3.6 EVALUATION OF SPRAY

Colour: Appearance: Examine the Herbal Spray colour, which can range from clear to tinted. The colour should be uniform and stable over time.

pH Level: Acidity/Alkalinity: Measure the serum's pH to ensure it's within a safe range for skin, typically between 4.5 and 6.5. This helps prevent irritation and ensures compatibility with the natural nail environment.

Density: Weight: Determine the density of the Herbal Spray, which influences how much product is needed per application and how it feels on the nails.

Flame Test: Take a small lighter and ignite a steady flame. Hold the spray can about 15cm away from the flame. Press the nozzle so that the spray passes directly through the lighter flame. Observe whether the flame gets extended or if the flame returns back to the nozzle.

Spray Pattern: Take a clean filter paper and place it on a flat surface. Hold the spray bottle 10cm above the centre of the paper. Press once to spray. Allow the liquid to spread for 1 min. Measure the diameter of the stain formed on the filter paper.

Primary Skin Irritation Test: The prepared formulations were assessed for primary skin irritation test. Healthy human volunteer was selected for the study choose a small area of skin, cleanse the area with mild soap and water, let it dry completely. Apply a small amount of the serum to the test area allow to spread on skin. Leave the serum on your skin for 24 to 48 hours. After 24 to 48 hours observe the area for any sign of irritation, redness, swelling, itching or any other reactions.

3.7 EVALUATION OF ANTI-FUNGAL ACTIVITY

The antifungal activity of the prepared sample was evaluated against *Candida albicans* by the agar well diffusion method. Sabouraud Dextrose Agar (SDA) medium was prepared according to the standard procedure, sterilized by autoclaving, and poured into sterile Petri plates. After solidification, a fresh culture of *Candida albicans* was prepared and evenly spread over the surface of the SDA plates using a sterile cotton swab.

Sterile wells of approximately 6 mm diameter were punched aseptically in the inoculated agar plates. The test sample was prepared at concentrations of 50, 75, and 100 µg/mL using a suitable sterile solvent. An appropriate volume of each concentration was added separately into the respective wells. A solvent control was maintained to confirm that the solvent itself did not produce antifungal activity. Amphotericin B was used as the standard antifungal drug. The plates were allowed to stand for a short period to facilitate diffusion of the samples into the agar medium. The plates were then incubated at approximately 25–30°C for 24–48 hours under suitable conditions. After incubation, the plates were examined for clear zones around the wells. The diameter of the zone of inhibition was measured in millimetres (mm) using a transparent ruler or zone reader. The antifungal activity of the test sample was compared with the standard drug based on the zone of inhibition.

Table: 2 Experimental Groups.

Group	Treatment
Control	Solvent/vehicle
Test 1	Sample – 50 µg/mL
Test 2	Sample – 75 µg/mL
Test 3	Sample – 100 µg/mL
Standard	Amphotericin B

4. RESULTS AND DISCUSSION

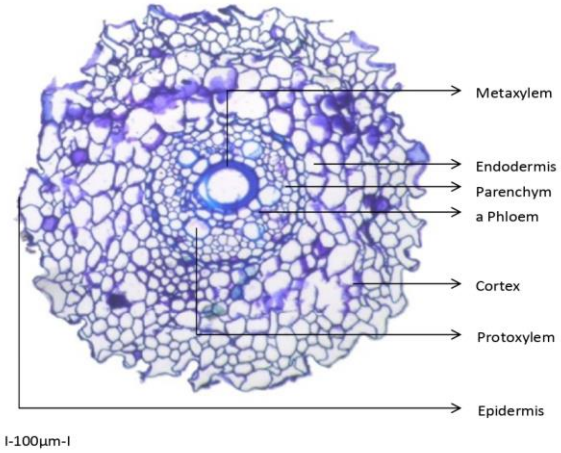
4.1 PHARMACOGNOSTICAL ANALYSIS

Pharmacognostical and Phytochemical Analysis: The pharmacognostical analysis of the roots of *Murdannia nudiflora* (L.) Brenan was carried out using transverse section studies and powder microscopy. The transverse section of the root revealed a thin circular structure measuring approximately 45 µm in diameter. Microscopic examination showed the presence of thin-walled parenchymatous cells, distinct endodermal cells, and angular pericycle cells. The vascular region consisted of a centrally located metaxylem with five radial rows of protoxylem cells.

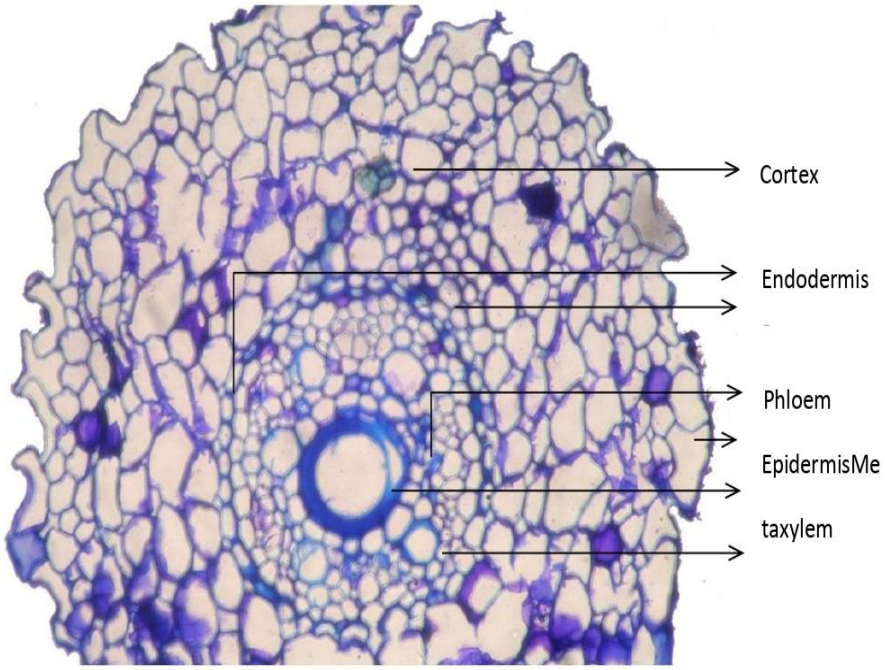
Further microscopic observations demonstrated the presence of secondary xylem cylinders composed of six layers of compressed, thin-walled parenchymatous cells. The medullary rays were narrow in the central region and gradually widened towards the peripheral region of the root. The medullary cells appeared radially elongated and rectangular in shape. The pith was either greatly reduced or completely absent. Powder microscopy of the root powder exhibited the presence of numerous starch grains, which serve as an important diagnostic feature of the plant material. In addition, lignified fibres, vessels, and parenchymatous cells were observed. Microscopic analysis also revealed the occurrence of xylem elements with characteristic reticulate thickening patterns.

Preliminary phytochemical screening of the ethanolic extract of *Murdannia nudiflora* was carried out to identify the major classes of phytoconstituents present in the plant. Carbohydrates were detected by Molisch's test, Fehling's test, and Benedict's test, confirming the presence of reducing sugars. The presence of alkaloids was confirmed by Mayer's test, Dragendorff's test, and Wagner's test, which produced characteristic precipitates indicating positive results. Tannins were identified by the ferric chloride test, which produced a characteristic colour reaction.

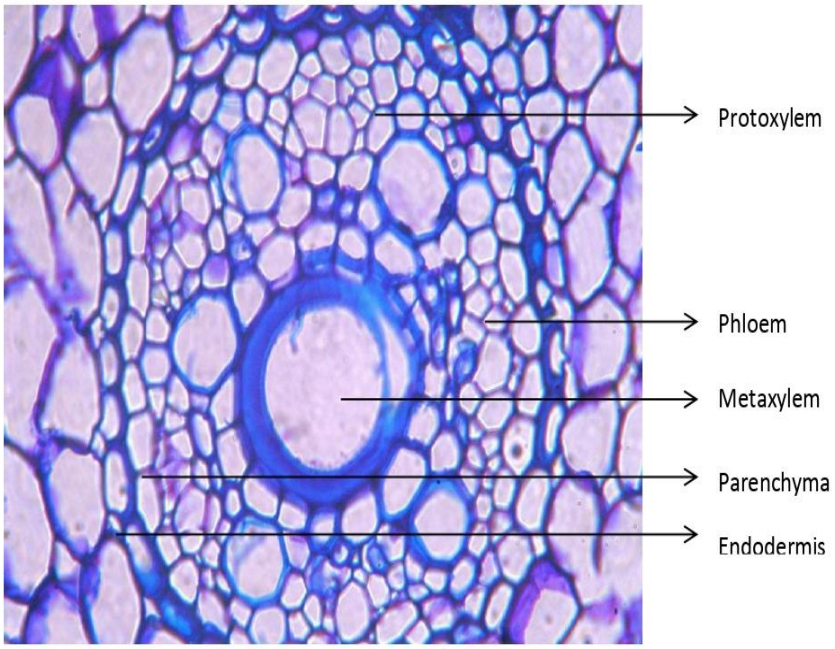
Flavonoids were detected using Shinoda's test, alkali test, lead acetate test, and acid test. Positive results in these tests confirmed the presence of flavonoid compounds in the extract. The presence of saponins was established by the froth test, which produced stable foam on vigorous shaking. The results of the pharmacognostical and phytochemical investigations confirmed the presence of various anatomical and chemical constituents in *Murdannia nudiflora* roots, thereby supporting its medicinal significance and potential use in herbal formulations.



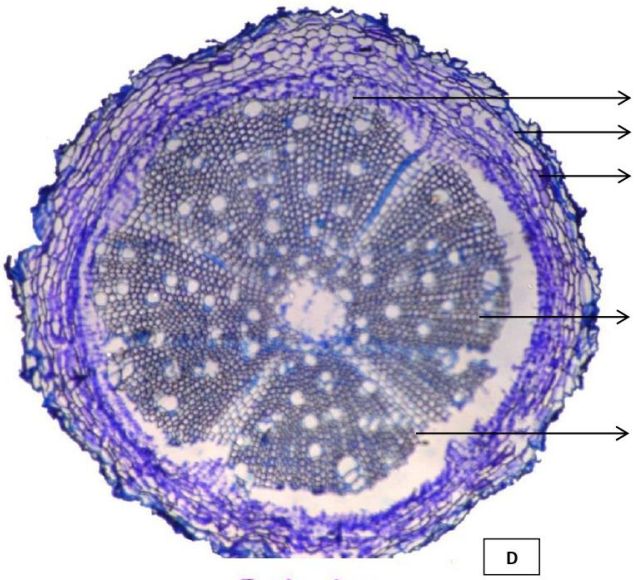
A


Cortex
Endodermis
Phloem
EpidermisMe
taxylem

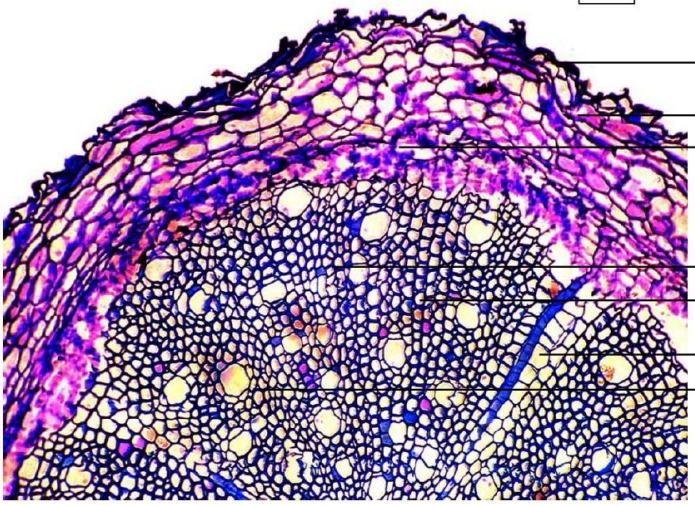
B


Protoxylem
Phloem
Metaxylem
Parenchyma
Endodermis

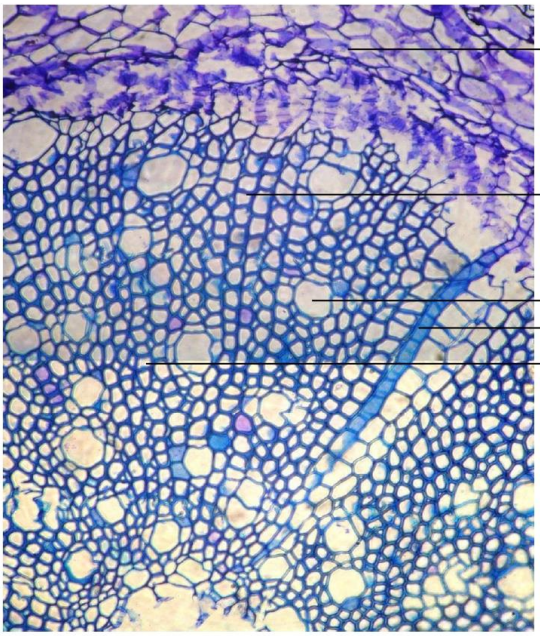
C


Secondary Phloem
Epidermis
Cortex
Secondary xylem
Xylemrays

D


Epidermis
Cortex
Secondary Phloem
Xylem fibers
Secondary xylem
Xylemray Vessel

E


Secondary Phloem
Secondary
Vessels
Xylem Rays
Xylem Fibers

F

A. TS OF YOUNG ROOT (THIN)-ENTIREVIEW (10X)
B. TS OF YOUNG ROOT(THIN)-ENLARGED (20X)
C. TS OF YOUNG ROOT(THIN)-VASCULAR CYLINDER (40X)
D. TS OF THICK ROOT-ENTIREVIEW (4X)
E. TS OF THICK ROOT –A SECONDARY ENLARGED (10X)
F. TS OF THICK ROOT-SECONDARY PHLOEMAND XYLEM (10X)

Fig: 2 TS.

4.2 POWDER MICROSCOPY

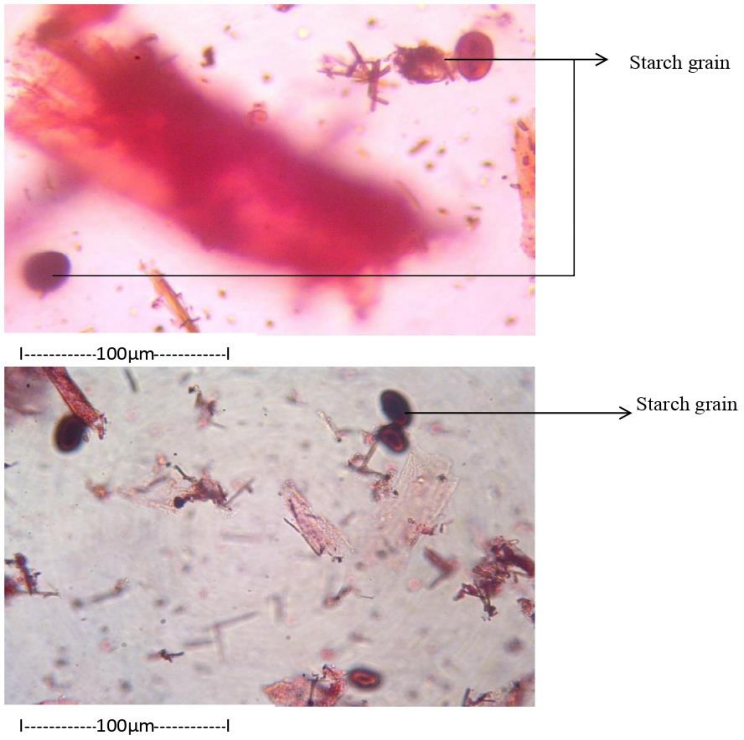


Fig: 3 Starch Grains. (40X)

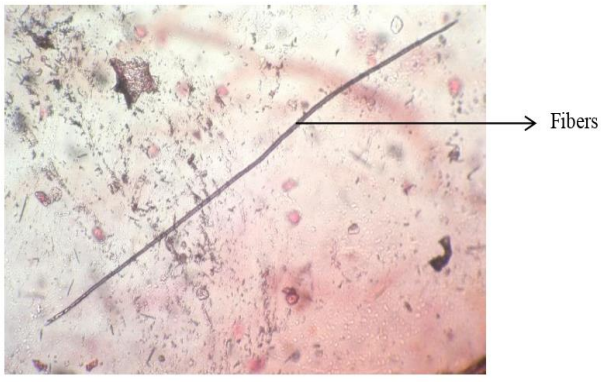


Fig: 4 Libriform Fibres. (10X)

Fig: 5 Vessels. (20X)

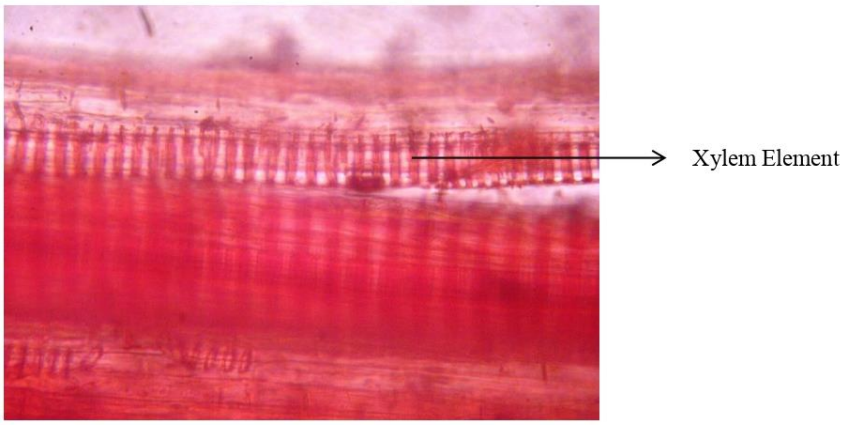


Fig: 6 Xylem. (40X)

Fig: 7 Vessel Element. (40X)

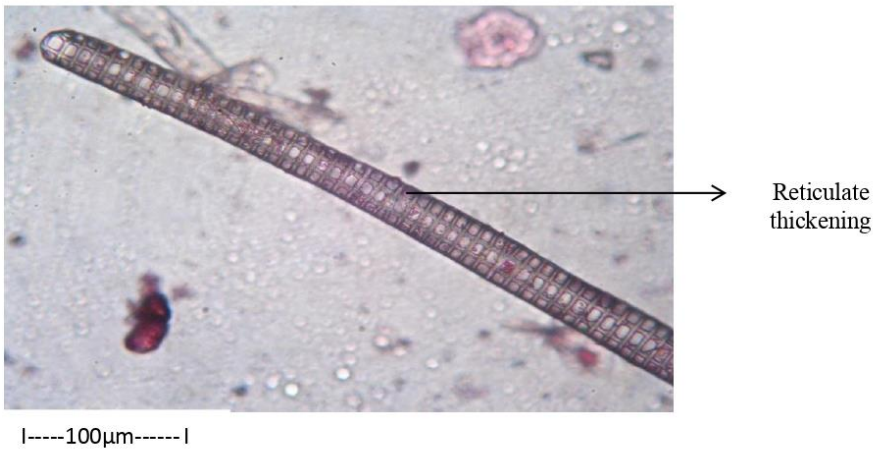


Fig: 8 Reticulate Thickening. (40X)

4.3 PHYSIO-CHEMICAL PARAMETERS

Table: 3 Physio-Chemical Parameters.

S.No	Parameters	*Murdannia nudiflora* (%w/w)
1.	Foreign organic matter	9%
2	Moisture content	10.7%
3.	Extractive value	26%
4.	Alcohol soluble extractive	44%
5	Water soluble extractive	46%
6.	Total ash	27.4%
7.	Acid insoluble ash	9.2%
8.	Water soluble ash	16.4%

4.4 FLUORESCENCE ANALYSIS

Table: 4 Fluorescence Analysis.

S. No	Reagents	Colour Observed	Fluorescence Observed
1.	Powder as such	Red	Light Green
2.	Powder + 1N HCl	Light Red	Light Green
3.	Powder + Conc. HNO_3	Dark Brown	Dark Green
4.	Powder + 1N H_2SO_4	Light Red	Pale Green
5.	Powder + Acetic acid	Light Red	Light Green
6.	Powder + 1N NaOH solution	Red	Green
7.	Powder +5% Iodine solution	Dark Brown	Black
8.	Powder +5% Ferric chloride	Light Red	Dark Green
9.	Powder + Petroleum ether	Light Red	Pale Green
10.	Powder +Dil. Ammonia	Red	Dark Green

4.5 PRELIMINARY PHYTOCHEMICAL ANALYSIS'

Table: 5 Preliminary Phyto-Chemical Analysis.

S. No.	Phytochemical Constituents	Test Performed	Ethanolic Extract
1	Carbohydrates	Molisch's Test	+
		Fehling's Test	+
		Benedict's Test	+
2	Alkaloids	Mayer's Test	+
		Dragendorff's Test	+
		Wagner's Test	+
3	Tannins	Ferric Chloride Test	+
		Goldbeater's Skin Test	+
4	Flavonoids	Shinoda's Test	+
		Alkali Test	+
		Lead Acetate Test	+
		Test for Acid	+
5	Saponins	Froth Test	+
6	Proteins and Amino Acids	Millon's Test	−
		Ninhydrin Test	−
		Biuret Test	−
7	Cardiac Glycosides	Keller–Killiani Test	−
		Raymond Test	−
		Legal's Test	−
8	Anthraquinone Glycosides	Borntrager's Test	−
		Modified Borntrager's Test	−
9	Phenolic Compounds	Ferric Chloride Test	−
		Folin–Ciocalteu Reagent Test	−
10	Terpenoids	Test for Terpenoids	−
11	Volatile Oil	Test for Volatile Oil	−
12	Fixed Oil	Test for Fixed Oil	−
13	Gum	Test for Gum	−
14	Resins	Test for Resins	−
15	Mucilage	Test for Mucilage	−
16	Quinones	Test for Quinones	−
17	Emodins	Test for Emodins	−
18	Coumarins	Test for Coumarins	−
19	Sterols	Salkowski's Test	−

4.6 EVALUATION OF HERBAL SPRAY

Table: 6 Evaluation of Herbal Spray.

S.NO	PARAMETERS	RESULTS
1	State	Liquid
2	Color	Light Blue
3	Odour	Aromatic
4	pH	7.54
5	Specific gravity	1.11
6	Sensitivity Test	No Irritation
7	Irritation Test	No Irritation

Fig: 9 Herbal Spray.

Fig: 10 Flame Test.

Fig: 11 Spray Pattern.

Flame Test: Take a small lighter and ignite a steady flame. Hold the spray can about 15cm away from the flame. Press the nozzle so that the spray passes directly through the lighter flame. Observe whether the flame gets extended or if the flame returns back to the nozzle.

Spray Pattern: Take a clean filter paper and place it on a flat surface. Hold the spray bottle 10cm above the centre of the paper. Press once to spray. Allow the liquid to spread for 1 mins. Measure the diameter of the stain formed on the filter paper.

4.7 ANTI-FUNGAL ACTIVITY

Table: 7 Anti-Fungal Activity.

S.No.	Microorganisms	Control	50	75	100	Amphotericin B
		Zone of inhibition in mm				
1.	*Candida albicans*	-	09	10	12	14

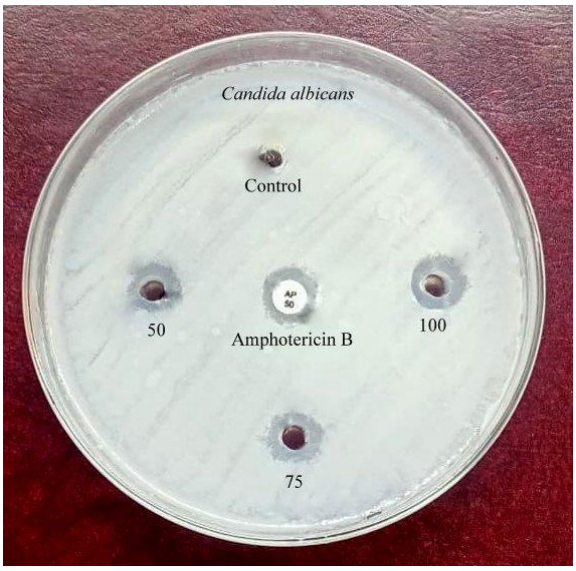


Fig: 12 Anti-Fungal Activity.

DISCUSSION

The present investigation focused on the formulation and evaluation of a herbal spray containing *Murdannia nudiflora* (L.) Brenan extract. Pharmacognostical examination of the root provided characteristic microscopic features that can be useful for identification and authentication of the plant material. The presence of starch grains, lignified fibres, vessels, parenchymatous cells, and reticulate-thickened xylem elements supported the identity of the powdered root material.

The physicochemical evaluation showed a foreign organic matter content of 9%, moisture content of 10.7%, extractive value of 26%, alcohol-soluble extractive of 44%, water-soluble extractive of 46%, total ash of 27.4%, acid-insoluble ash of 9.2%, and water-soluble ash of

16.4%. These parameters provide useful information regarding the quality and purity of the crude drug.

Preliminary phytochemical investigation of the ethanolic extract revealed positive reactions for carbohydrates, alkaloids, tannins, flavonoids, and saponins, whereas proteins and amino acids, cardiac glycosides, anthraquinone glycosides, phenolic compounds, terpenoids, volatile oils, fixed oils, gums, resins, mucilage, quinones, emodins, coumarins, and sterols were reported negative in the respective tests.

The herbal spray was prepared by mixing isopropyl alcohol, acetone, *Murdannia nudiflora* extract, purified water, and glycerine to obtain a uniform preparation. The final formulation showed a liquid state, light-blue colour, aromatic odour, pH of 7.54, and specific gravity of 1.11. The sensitivity and irritation tests showed no irritation, indicating acceptable observations under the reported test conditions.

The antifungal activity was assessed against *Candida albicans* using the agar well diffusion method. The formulation produced inhibition zones of 9 mm at 50 µg/mL, 10 mm at 75 µg/mL, and 12 mm at 100 µg/mL. Amphotericin B produced a 14 mm zone of inhibition. The progressive increase in inhibition from 50 to 100 µg/mL indicates increased antifungal activity with increasing test concentration under the experimental conditions. However, the standard drug produced a greater inhibition zone than the tested formulation.

Overall, the findings demonstrate that the *Murdannia nudiflora* extract-containing herbal spray showed measurable antifungal activity against *Candida albicans*. The observed activity, together with the positive phytochemical screening results, supports further investigation of the formulation. However, additional studies would be required to establish its efficacy, safety, stability, and mechanism of antifungal action.

5. CONCLUSION

The present study demonstrated the successful formulation and preliminary evaluation of a herbal spray containing *Murdannia nudiflora* (L.) Brenan extract. The pharmacognostical and phytochemical investigations confirmed characteristic microscopic features and the presence of several phytochemical constituents. The prepared spray showed satisfactory physical characteristics, including light-blue colour, aromatic odour, pH 7.54, and specific gravity 1.11, with no irritation observed in the reported evaluation.

The formulation exhibited antifungal activity against *Candida albicans*, producing inhibition zones of 9, 10, and 12 mm at concentrations of 50, 75, and 100 µg/mL, respectively. Although the activity was lower than that of Amphotericin B (14 mm), the increasing zone of

inhibition with increasing concentration indicates promising antifungal potential. Therefore, *Murdannia nudiflora* herbal spray may be considered a potential candidate for further investigation and development as a herbal antifungal preparation. Further studies are required to confirm its efficacy, safety, stability, and therapeutic potential.

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