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Research Article

**PHYTOCHEMICAL INVESTIGATION AND WOUND
HEALING ACTIVITY OF *Aphanamixis polystachya* BARK
EXTRACT****Vishnu Kant Gupta*, Dr. Ashish Chourasia, Payal Saiju, Dr. Nishi Prakash Jain**
Lakshmi Narain College of Pharmacy, Kalchuri Nagar, Raisen Road, Bhopal,
Madhya Pradesh, India-462021**Abstract:**

The present study aimed to investigate the phytochemical constituents, antioxidant potential, and wound healing activity of *Aphanamixis polystachya* bark extract. Successive extraction was carried out using ethyl acetate, ethanol, and water as solvents, and the extractive values indicated that the aqueous extract yielded the highest percentage (8.21% w/w), followed by the ethanolic (6.70% w/w) and ethyl acetate extract (1.05% w/w). Preliminary phytochemical screening revealed the presence of alkaloids, glycosides, flavonoids, tannins, phenols, diterpenes, and proteins predominantly in the ethanolic extract. Quantitative estimation showed that the ethanolic extract contained a higher total phenolic (0.783 mg/100 mg) and flavonoid content (0.925 mg/100 mg) compared to the other extracts. The antioxidant activity assessed by the DPPH method demonstrated a concentration-dependent increase in radical scavenging with an IC_{50} value of 92.81 μ g/mL for the ethanolic extract, indicating moderate antioxidant potential. The wound healing activity was evaluated using an excision wound model in rats, where the 2% *Aphanamixis polystachya* extract gel showed significant wound contraction (85.9% by day 11) compared to the control and was comparable to the standard Betadine group (92.3%). These findings suggest that the ethanolic extract of *Aphanamixis polystachya* possesses notable antioxidant and wound healing activities, likely attributed to its rich content of phenolic and flavonoid compounds, which play a vital role in tissue repair and protection against oxidative stress.

Keywords: *Aphanamixis polystachya*, phytochemical screening, antioxidant activity, DPPH assay, total phenolic content, wound healing; ethanolic extract.

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INTRODUCTION:

The tree *Aphanamixis polystachya* (Wall.) R. Parker (family Meliaceae), commonly known in India and Bangladesh as “Rohituka” or “Pithraj”, has a long history of use in traditional medicine for a variety of ailments including ulcers, rheumatism, liver disorders, skin infections and wounds (Apu et al., 2013; Snigdha et al., 2024). Its occurrence across the Indian Sub-continent and Southeast Asia makes it a widely available medicinal resource (Wang et al., 2013).

Phytochemical studies of *A. polystachya* have revealed a rich array of secondary metabolites. These include tetranortriterpenoids, limonoids, diterpenes, flavonoids, phenolic acids, saponins and alkaloids. For example, facmines A–C (diterpenoids) isolated from this species exhibited nitric oxide inhibitory activity in macrophages. The stem bark, roots and seeds have been specifically reported to bear these classes of compounds (Bhowon et al., 2022).

Pharmacological investigations further support the ethnomedicinal uses of *A. polystachya*. Bark extracts have shown significant antioxidant, antimicrobial and anti-inflammatory actions (Aladejana; 2023). These biological effects are mechanistically relevant to the process of wound healing: oxidative stress mitigation, microbial suppression, reduction of inflammatory mediators and promotion of tissue repair. Thus, the plant's bioactivity profile renders it a plausible candidate for the development of topical wound-healing agents.

Despite the traditional usage and the emerging pharmacological evidence, there is a relative paucity of systematic studies that integrate detailed phytochemical profiling of the bark extract with in-vitro and in-vivo wound-healing experiments. In view of that gap, the present study aims to (i) perform a solvent-fractionated phytochemical investigation of *A. polystachya* bark extract, (ii) quantify key bioactive markers (e.g., total phenolics/flavonoids), and (iii) evaluate its wound-healing activity using validated models (e.g., scratch assay, excision/incision-wound models). Such an integrated approach may help link specific phytoconstituents to functional outcomes and thereby validate traditional claims for wound-care applications.

MATERIAL AND METHODS:

Material

The present study utilized a variety of analytical grade chemicals and reagents obtained from reputed suppliers to ensure accuracy and reproducibility of results. Potassium mercuric iodide, picric acid, ferric chloride, and copper

acetate were procured from Thomas Baker, Mumbai. Iodine, potassium iodide, sodium nitroprusside, sodium hydroxide, lead acetate, and Folin–Ciocalteu reagent were supplied by Loba Chemie Pvt. Ltd., Mumbai. Potassium bismuth iodide, pyridine, gelatin, nitric acid, and sodium chloride were obtained from S. D. Fine Chem. Ltd., Mumbai. Methanol, ethanol, and chloroform of analytical grade were purchased from Qualigens Fine Chemicals, Mumbai, while Fehling's solution was procured from Central Drug House Ltd., New Delhi. All chemicals and solvents used were of analytical or HPLC grade and were employed without further purification.

Methods

Collection of plant materials

The barks of *Aphanamixis polystachya* were collected from local area of Bhopal in the period of March, 2025.

Extraction by maceration method

Collected barks of *Aphanamixis polystachya* were cleaned properly and washed with distilled water to remove any kind of dust particles. Cleaned and dried plant materials were converted into moderately coarse powder in hand grinder. Powdered plant materials were weighed (50 gm) and packed in (250 ml) air tight glass Bottle. The plant materials were subjected to extraction by different solvents e.g., Ethyl acetate, Ethanol, and Water for about 24 hrs (Kokate, 1994). The liquid extract was collected in a tarred conical flask. The solvent removed from the extract by evaporation method. The extracts obtained with ethanol solvent were weighed to a constant weight and percentage w/w basis was calculated.

Determination of percentage yield

The percentage yields of extract were calculated by using following formula:

$$\text{Percentage yield} = \frac{\text{Weight of Extract}}{\text{Weight of powder drug Taken}} \times 100$$

Phytochemical analysis

Preliminary phytochemical screening means to investigate the plant material in terms of its active constituents. In order to detect the various constituents present in the different extracts of barks of *Aphanamixis polystachya*, were subjected to the phytochemical tests as per standard methods. Phytochemical screening was revealed for the presence of alkaloids, glycosides, carbohydrates, tannins, resins, flavonoids, steroids, proteins and amino acids (Audu et al., 2007).

Quantitative estimation of phenols and flavonoids

Estimation of total phenol content

Estimation of total phenol content Total phenol content of the extracts was evaluated with folin-ciocalteu method (Gaur Mishra *et al.*, 2017). Samples containing polyphenols are reduced by the folin-ciocalteu reagent there by producing blue colored complex. The phenolic concentration of extracts was evaluated from a gallic acid calibration curve. To prepare a calibration curve, 2 ml aliquots of 5, 10, 15, 20, and 25 µg/ml gallic acid solutions were mixed with 1ml folin-ciocalteu reagent (diluted ten-fold) and 1 ml (7.5 g/L) sodium carbonate. After incubation at 25°C for 10 min, the quantitative phenolic estimation was performed at 765 nm against reagent blank by UV Spectrophotometer. The calibration curve was constructed by putting the value of absorbance vs. concentration. A similar procedure was adopted for the extract (1000µg/ml) as above described in the preparation of calibration curve. All determinations were performed in triplicate. Total phenolic content was expressed as milligrams of gallic acid equivalent (GAE) mg per 100mg of extract.

Estimation of total flavonoids content

The aluminum chloride colorimetric method was modified (Gaur Mishra *et al.*, 2017). Quercetin was used to make the calibration curve. Ten milligrams of quercetin was dissolved in 10 ml methanol and then diluted for 5-25 µg/ml. The diluted standard solutions (2 ml) were separately mixed with 1 ml of 2% aluminum chloride. After incubation at room temperature for 10 min, the absorbance of the reaction mixture was measured at 420 nm with a UV spectrophotometer. Similarly, extracts

solutions (1000 µg/ml) were reacted with aluminum chloride for determination of flavonoid content. Total flavonoid content was expressed as milligrams of Quercetin equivalent (QE) mg per 100mg of extract.

In-vitro antioxidant activity of Ethanolic extract using DPPH method

Total free radical scavenging capacity of extract estimated according to the previously reported method with slight modification (Parkhe and Jain, 2018). Solution of DPPH (6 mg in 100ml methanol) was prepared and stored in dark place. Different concentration of standard and test (10-100 µg/ml) was prepared. 1.5 ml of DPPH and 1.5 ml of each standard and test was taken in separate test tube; absorbance of this solution was taken immediately at 517nm. 1.5 ml of DPPH and 1.5 ml of the methanol was taken as control absorbance at 517nm. The percentage inhibition of free radical DPPH was calculated from the following equation:

$$\% \text{ inhibition} = \frac{[(\text{absorbance of control} - \text{absorbance of sample}) / \text{absorbance of control}] \times 100\%}{1}$$

Formulation of gel

Method of Preparation

In order to prepare the gel formulation, first dissolve carbopol 940 in distilled water. Next, add methyl paraben, propyl paraben, and glycerine, and let the mixture sit overnight. Consider the leaf extracts in propylene glycol, to which polymer dispersion was added later. After that, the remaining water was added, then triethanolamine was added while stirring constantly to get the pH down to 7 (Rathore and Nema, 2012).

Table 1: Formulation of Gel

S. No.	Ingredients	F1	F2
1	Ethanolic extract (%)	1	2
2	Carbopol 934 (%)	1	1
3	Propylene Glycol (ml)	8	8
4	Methyl paraben (%)	0.4	0.4
5	Propyl paraben (%)	0.2	0.2
6	Triethanolamine (ml)	qs	qs
7	Glycerin (ml)	1	1
8	Distilled water (ml)	Upto 100 ml	Upto 100 ml

In vivo wound healing activity of gel prepared from ethanolic extract

Animals

Wistar rats weighing between 180-200 g with no prior treatment were used for the study. Animals were housed in polypropylene cages maintained at 22 ± 2°C temperature with 12 h light and 12 h dark cycle. The animals were fed on a standard pelleted diet and had free access to water throughout the experiment. Animals that are described as fasting

were deprived of food for at least 16 h but allowed free access to drinking water. The animal study was approved by the Institutional Animal Ethics Committee, as stated by prescribed guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India.

Acute dermal toxicity

Acute dermal toxicity was determined, according to OECD 404, in rats. Approximately 24 hours before

the test, fur was removed by closely clipping the dorsal area of the trunk on both flanks using a suitable depilatory material. Special attention was given not to abrade the skin. Skin was washed with sterile water a day before the toxicity test procedure. A limit test dose of 2,000 mg/Kg of the 10% (w/w) gel was applied on the shaved back of the rats within a range that was approximately 10% of the body surface area and held in contact to the skin with a gauze patch to facilitate good contact and uniform distribution of the test chemical on the skin for the duration of the exposure period (24 hours). The test was first conducted in a single rat for initial testing, and then after observing the response of the first rat for 24 hours a confirmatory test was done on another two rats simultaneously.

At the end of the exposure period, the gel was removed and the animals were observed for 14 days for the development of any adverse skin reactions like inflammation, irritation, or redness. Behavior, general condition, posture and reflexes, attitude towards food, and water were also evaluated.

Experimental model

Male Sprague-Dawley rats were divided into four groups of six rats each and housed separately in individual cages. Before the creation of wounds, the animals were anaesthetised with ketamine (30 mg/kg, i.m.) and xylazine (3 mg/kg, i.m.). To create the wound, an electrical shaver was employed to shave the dorsal neck of each rat. A mark was made in the dorsal thoracic region, one centimetre away from the vertebral column and five centimetres from the ear. An excision wound was created by cutting out the full thickness of skin using a circular stamp with a diameter of two centimetres (Latif *et al.*, 2015).

Experimental designs

Wounds in the control group, designated as

Group 1, were treated with normal saline (0.2 ml) twice daily. The reference standard control, labelled as

Group 2, involved rats that were given 0.2 ml of Betadine twice daily.

Groups 3 and 4 received *Aphanamixis polystachya* extract gel (1% and 2%, respectively) twice daily, and no dressing was applied to the wound site.

Assessment of degree of wound healing

Progressions of the wound healing were assessed on days 0, 3, 7, and 11 days post-wound creation. Wound area was traced by a transparent tracing paper placing over the wound, which was then placed on a sheet of graph paper (2 mm²) to count the number of squares within the wound area

(Nayak *et al.*, 2006). The percentage of wound closure was measured using the following formula:

$$\text{Wound Closure (\%)} = \left[\frac{(\text{Wound Area at Day 0} - \text{Wound Area at Later Day})}{\text{Wound Area at Day 0}} \right] \times 100$$

Data Analysis

The data is expressed as mean $\pm$ Standard Deviation (SD). Results were analysed using one-way ANOVA followed by Dunnet's test. Differences were considered statistically significant at $P < 0.05$ when compared with control.

RESULTS AND DISCUSSION:

The present study was undertaken to evaluate the phytochemical constituents, antioxidant potential, and wound healing activity of *Aphanamixis polystachya* bark extracts. The extractive value (Table 2) revealed that the aqueous extract had the highest yield (8.21% w/w), followed by the ethanolic (6.70% w/w) and ethyl acetate extract (1.05% w/w), indicating that a higher number of polar constituents were extracted using polar solvents such as ethanol and water.

The preliminary phytochemical screening (Table 3) demonstrated the presence of a variety of secondary metabolites. The ethanolic extract tested positive for alkaloids (Wagner's test), glycosides (Legal test), flavonoids, tannins, phenols, proteins, amino acids, and diterpenes, suggesting that ethanol efficiently extracted a broad range of phytochemicals of moderate polarity. The aqueous extract showed the presence of carbohydrates, saponins, and flavonoids, while the ethyl acetate extract was rich in resins, steroids, and diterpenes. These findings indicate that the phytochemical composition of *Aphanamixis polystachya* is solvent-dependent and that ethanol serves as an effective solvent for extracting bioactive compounds responsible for pharmacological activity.

The quantitative estimation of total phenolic and flavonoid contents (Tables 4 and 5) further confirmed that the ethanolic extract possessed the highest phenolic (0.783 mg/100 mg) and flavonoid (0.925 mg/100 mg) concentrations. Phenolic and flavonoid compounds are known for their strong antioxidant and free radical scavenging properties, which contribute to tissue protection and wound repair mechanisms.

Antioxidant activity assessed using the DPPH radical scavenging assay (Table 6) showed that the ethanolic extract exhibited significant free radical scavenging ability in a dose-dependent manner, with a maximum inhibition of 53.12% at 100 $\mu\text{g/mL}$ and an IC_{50} value of 92.81 $\mu\text{g/mL}$ compared

to the standard ascorbic acid ($IC_{50} = 18.02 \mu\text{g/mL}$). Although the extract showed lower potency than ascorbic acid, the notable activity indicates the presence of antioxidant constituents, primarily phenolics and flavonoids, which can neutralize reactive oxygen species and promote wound healing by reducing oxidative stress at the wound site.

The wound healing activity (Table 7) revealed that topical application of *Aphanamixis polystachya* extract gel significantly enhanced the wound contraction rate in rats compared to the control group. The 2% extract gel showed superior wound healing activity, achieving 85.9% contraction by

day 11, which was comparable to the standard Betadine-treated group (92.3%). The improved healing rate may be attributed to the synergistic action of flavonoids, phenols, and diterpenes, which possess antimicrobial, anti-inflammatory, and collagen-stimulating properties that facilitate re-epithelialization and tissue regeneration.

The findings suggest that the ethanolic extract of *Aphanamixis polystachya* possesses potent antioxidant and wound healing properties. These effects are likely due to its rich phytochemical profile, particularly phenolic and flavonoid constituents, which play a crucial role in combating oxidative damage and enhancing tissue repair mechanisms.

Table 2: Extractive values obtained from *Aphanamixis polystachya*

S. No.	Extracts	Colour	% Yield (w/w)
1.	Ethyl acetate	Dark black solid	1.05
2.	Ethanolic	Dark black solid	6.70
3.	Aqueous	Dark black solid	8.21

Table 3: Preliminary phytochemical screening of Ethyl acetate extract of *Aphanamixis polystachya*

Phytoconstituents	Test Name	Ethyl acetate extract	Ethanolic extract	Aqueous extract
Alkaloids	Mayer's Test	-ve	-ve	-ve
	Dragendorff's Test	-ve	-ve	-ve
	Wagner's Test	-ve	+ve	-ve
	Hager's Test	-ve	-ve	-ve
Glycosides	Raymond's Test	-ve	-ve	-ve
	Killer Killani Test	-ve	-ve	-ve
	Legal Test	-ve	+ve	-ve
Carbohydrates	Molisch's Test	-ve	-ve	-ve
	Fehling's Test	-ve	-ve	+ve
	Benedict's Test	-ve	-ve	-ve
Tannins	Vanillin- HCl Test	-ve	-ve	-ve
	Gelatin Test	-ve	+ve	-ve
Flavonoids	Lead acetate Test	-ve	+ve	-ve
	Alkaline Reagent Test	-ve	+ve	+ve
Resins	Turbidity Test	+ve	-ve	-ve
Steroids	Liebermann- Bur chard Test	-ve	-ve	-ve
	Salkowski Reaction	+ve	-ve	-ve
Proteins & Amino acids	Biuret Test	-ve	+ve	-ve
	Precipitation test	-ve	-ve	-ve
Phenols	Ferric chloride test	-ve	+ve	-ve
Saponins	Froth Test	-ve	-ve	+ve
Diterpenes	Copper acetate Test	+ve	+ve	-ve

[+ve = positive; -ve = negative]

Table 4: Total phenol content of extract of *Aphanamixis polystachya*

S. No.	Extracts	Total phenol content (mg/100mg)
1.	Ethyl acetate	-
2.	Ethanolic	0.783
3.	Aqueous	-

Table 5: Total flavonoid content of extract of *Aphanamixis polystachya*

S. No.	Extracts	Total Flavonoid content (mg/100mg)
1.	Ethyl acetate	-
2.	Ethanollic	0.925
3.	Aqueous	0.632

Table 6: % Inhibition of ascorbic acid and Hydroalcoholic extract of *Aphanamixis polystachya* using DPPH method

S. No.	Concentration (µg/ml)	% Inhibition	
		Ascorbic acid	Ethanollic extract
1	10	45.77	28.15
2	20	52.98	34.69
3	40	66.02	37.08
4	60	70.73	42.51
5	80	84.69	46.98
6	100	91.24	53.12
IC ₅₀ value		18.02	92.81

Table 7: Evaluation of wound healing activity of *Aphanamixis polystachya* extract gel on rats

Groups	Day 0	Day 3	Day 7	Day 11
I (Normal saline)	0.0 ± 0.0	17.8 ± 2.4	41.2 ± 3.0	60.1 ± 3.5
II (Betadine)	0.0 ± 0.0	33.5 ± 2.6	71.2 ± 2.7	92.3 ± 2.3***
III (<i>Aphanamixis polystachya</i> extract gel 1%)	0.0 ± 0.0	24.3 ± 2.8	56.0 ± 3.1	78.4 ± 2.9*
IV (<i>Aphanamixis polystachya</i> extract gel 2%)	0.0 ± 0.0	29.1 ± 2.6	63.4 ± 3.4	85.9 ± 2.8**

A one-way ANOVA was used for testing comparison with controls, and significance was assessed. Significance was defined as * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$. All values are expressed as mean ± SD.

CONCLUSION:

The findings of the present study demonstrate that *Aphanamixis polystachya* bark possesses significant phytochemical, antioxidant, and wound healing potential. Among the various extracts, the ethanollic extract exhibited the highest yield of bioactive compounds such as phenolics, flavonoids, glycosides, and diterpenes. The strong antioxidant activity observed through the DPPH assay indicates its capacity to scavenge free radicals and protect against oxidative stress, which is crucial for tissue regeneration. The topical application of ethanollic extract gel, particularly at 2% concentration, significantly enhanced wound contraction and accelerated healing compared to the control group, showing results comparable to the standard Betadine treatment. These outcomes suggest that the ethanollic extract of *Aphanamixis polystachya* can serve as a promising natural source for developing wound healing formulations. Further studies involving isolation, characterization, and mechanistic evaluation of the active constituents are warranted to substantiate its therapeutic potential and pharmacological applications.

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