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Research Article

PHARMACOGNOSTIC, PHYTOCHEMICAL AND ANTIMICROBIAL SCREENING OF *APHANAMIXIS POLYSTACHYA*, AN ENDANGERED MEDICINAL TREE

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ABSTRACT

In the present study the bark of *Aphanamixis polystachya* was investigated for its macroscopic, phytochemical and antimicrobial characteristics. The dried stem bark of *A. polystachya* was successive extracted with petroleum ether, chloroform, alcoholic and hydromethanolic solvents for 48 hours in soxhlet apparatus and solvents from extracts was evaporated under vacuum. All four extracts were subjected to phytochemical investigation and *in vitro* antimicrobial screening using different bacterial strains in nutrient agar media. For screening of antimicrobial activity, extracts of dried stem bark of *A. polystachya* was used at the different doses (10, 20, 50 and 100mg/ml) against the kanamycin, which was used as standard antimicrobial agent at the dose of 30mg/ml. The zone of inhibitions indicates that the extracts of dried stem bark of *Aphanamixis polystachya* showed significant antimicrobial activity as comparison to kanamycin. The Preliminary phytochemical investigation revealed the presence of various phytoconstituents in each extracts. It showed the presence of carbohydrates, glycosides and saponins.

Keywords: *Aphanamixis polystachya,* Meliaceae, Antimicrobial, Kanamycin.

INTRODUCTION

Aphanamixis polystachya is the large handsome evergreen tree, with a dense spreading crown and a straight cylindrical bole up to 15m in height and 1.5-1.8m in width. The plant *Aphanamixis polystachya* belonging from Meliaceae family is also listed as *Aphanamixis polystachya* (Wall) Parker Syn. It is distributed in the sub-Himalayan tract from Gonda (Uttar Pradesh) eastwards to Bengal, Sikkim and Assam up to 6000ft and in Western ghats, chota Nagpur, Konkarn, Andaman and adjoining hill ranges from the Poona district southwards to Tinnevelly up to 3500ft[1]. A genus of this tree is also found in the Indo-Malaysian region. The stem barks of *Aphanamixis polystachya* is used traditionally in treatment of tumours, cancer, spleen diseases, rheumatism at Bengal, southeast and north region. The hepatoprotective activity[2], antimicrobial, antiviral and antibacterial activity of isolated limonoid rohitukin[3,4] and cytotoxicity of amoorastatin were also established[5]. *Aphanamixis polystachya* has disclosed the presence of Aphanamixis, a new triterpenes[6], a new tetranortriterpenoid *i.e.* aphanamixinin, sterol, saponins[7], flavanone and anthraquinone glycosides.

MATERIAL AND METHODS

Plant Material

The stem bark of Aphanamixis polystachya was collected from Forest Research Institute, Dehradun (Uttarakhand) and authenticated by Dr. **Arvind Bhardwaj,** who is a Botanist. The stem barks were authenticated by comparison of macroscopic and microscopic characteristics with monograph of Pharmacopoeia[8].

Preparations of Extract

After collection and authentication the air-dried bark of *Aphanamixis polystachya* was shade dried and powdered (40 size mesh) separately at the temperature 40-50^{0}C. 500g of powder was subjected to successive hot continuous extraction (soxhlet) with petroleum ether, chloroform, alcohol and hydromethanolic (50:50) solvents (Each time before extracting with next solvent the powdered material was dried at room temperature) for 48hrs. After the effective extraction, solvents were concentrated using rotary flash evaporator and water was removed by freeze drying to yield residues and the extract obtained with each solvent was weighed. The obtained extracts were subjected to chemical investigation and the plants may be considered as biosynthetic laboratory for a multitude of compounds like alkaloids, glycosides, volatile oils and tannin etc. that exert physiology effects. The percentage yield obtained from the no polar solvents i.e. petroleum ether and chloroform (2.41%, 2.21% respectively) was less as compared to the yield obtained from the polar solvents methanol and aq. methanol (12.51%, 5.82% respectively)[9,10].

Method for Phytochemical Investigation

Tests for Alkaloids-

Extract was evaporated. Residue was shaken with dilute HCL and filtered. To the filtrate tests were performed.

a) Dragendroff"s test:-to the filtrates dragendroff"s reagent (potassium bismuth iodide) was added. A reddish brown precipitate indicated the presences of alkaloid.
b) Mayer's test:-to the filtrated Mayer's reagent (potassium mercuric iodide) was added. A cream precipitate indicates the presence of alkaloid.
c) Wagner's test:- to the filtrates Wagner's reagent (iodine in potassium iodide) was added. A reddish brown precipitate indicated the presence of alkaloid.
d) Hager's test:-to the filter ate Hager's reagent (Saturated picric acid solution) was added. A yellow precipitate indicated the presence of alkaloid.

Test for Glycosides-

a) Keller-Killani test (for deoxy sugar):-1ml of glacial acetic acid containing traces of Ferric chloride and 1ml of concentrate sulphuric acid were added to the extract carefully. A reddish brown colour formed at the junction of two layers and upper layer turned Bluish green indicated the presences of glycosides.
b) Legal test (for cardinolides):-concentrated ethanolic extract was made alkaline with few drops of 10%sodium hydroxide and then freshly prepared sodium nitroprusside Solution was added to the solution. Presence of blue coloration indicated the presence of Glycosides in the extract.
c) Baljet test:-picric acid is added to the extract and made alkaline; give a stable orange colour in presence of glycosides.
d) Borntrager"s test:- to the powdered drug or dried extract 1ml of dilute hydrochloride Acid is added and heated for five minutes, and filtered while hot, to this add an equal Volume of chloroform, add ammonia half of its volume, shake well, lower ammonia Layer produce rose pink colour after few minutes.
e) Modification Borntrager"s test:- add 5ml of 5% ferric chloride and 5ml of dilute Hydrochloric acid to the 5ml of extract and boil for 5min cool and benzene or chloroform shake well, separate the organic layer and add equal volume of dilute ammonia, ammonical layer shows pink colour.

f) Grignard's test:- Strips of sodium picrate filter paper were inserted between split a cork stopper which was fitted in to the neck of the test tube containing a small amount of powdered drug in water. Care was exercised that the paper didn't touch the inner side of the test tube. The content was warmed for half an hour. The red colour of the strips indicated the presence of cyanogenetic glycosides.

Test of Saponins

a) Foam test:- The drug extract or dry powder was shaken vigorously with water. Persistent foam was observed.
b) Foaming index:- Weigh 1g of finely powdered drug accurately and transfer to a 500ml conical flask containing 100ml of boiling water. Maintain at moderate boiling for 30min. Cool and filter into a 100ml volumetric flask and add sufficient water to make the volume to 100ml. Place the above decoction into 10 stopper, graduated test-tubes in a series of successive portions of 1, 2, 3 up to 10ml and adjust the volume of the liquid in each test tube water to 10ml. Stopper the tubes and shake them vertically for 15 seconds, 2 frequencies/ sec. Allow standing for 15min and measuring the height of the foam.

The results assed as follows:

a) If the height of the foam in every tube is less than 1cm, the foaming index is less than 100.
b) If a height of foam of 1cm is measured in any tube, the volume of the plant material decoction in this tube (a) is used to determine the index. If this tube is the first or second tube in the series; prepare an intermediate dilution in a similar manner to obtain a more precise result.

$$\text{Foaming index} = \frac{100}{a}$$

c) If the height of the foam is more than 1cm in every tube, the foaming index is over 1000. In this case repeat the determination using a new series of dilution of the decoction in order to obtain a result.
a = volume in ml of the decoction used for preparing dilution in the tube where foaming to A height of 1cm is observed. Foaming index ranges from 444 to 333.
d) Haemolytic test:- extract or dry powder of drug was poured in one drop of blood. Appearance of haemolytic zone indicated the saponins glycoside in drug.

Test for Steroids and Triterponids

a) Salkowaski reaction:- extract of residue of each extract was taken in 2ml of chloroform and 2ml of conc. ferric acid was added from the side of test tube. The test tube was shaken for few min. the development of red colour in the chloroforms layer indicated.
b) Liebermann's test:- some few mg. of residues in a test-tube, few ml of acetic anhydrides was added and heated. The contents of the test-tube were cooled. Few drops of concentrated sulphuric acid were added from the side of the test-tube's blue colour gave the evidence presence of sterols.

Test for Tannins and Phenolic compounds

The test residue of each extract was taken separately in water, warmed and filtered. Tests were carried out with the filtrate using following reagents.

a) Ferric chloride reagent:- $feCl_3$ loaded to a little of the above filtrate. If dark green or deep blue colour is obtained, tannins are present.
b) Lead acetic test's 10% w/v solution of basic lead acetate in distilled water was added to test filtrate. If precipitate is obtained, tannins are present.
c) Potassium dichromate test:- if on addition of a solution of potassium dichromate in a Test filtrate. Dark colour is developed, tannins are present.
d) Gelatin solution test:- 1%w/v solution of gelatin in water, containing 10% sodium chloride was prepared. A little of this solution was added to the filtrate. If white precipitates are obtained, tannins are present.
e) Bromine water test:- bromine solution was added to test filtrate. If decolonization of bromine water occurs, tannins are present.
f) Test for catechin:- dip a matchstick in plant extract, dry, moistened it with conc. Hydrochloride acid, warm near a flame, phoroglucinol produced pink or red colour.
g) Mitchell's test for tannins (pulock-326):- when iron and ammonium citrate or iron and sodium citrate is added to the crude extract of the drug a water soluble iron-tannin complex is formed in case of tannins. This complex is insoluble in a solution of ammonium acetate.
h) Precipitation test:- 2-3ml of aqueous or alcoholic extract is subject to potion test by adding few drops of the following reagents.

I. Lead acetate solution, white ppt formed.

II. Acetic acid solution, give red colour.

III. Dilute nitric acid solution, reddish to yellow colour appeared.

IV. Potassium dichromate solution, red ppt formed.

V. Ammonium hydroxide and potassium ferricyanide give red colour.

VI. One drop of ammonium hydroxide and excess of 10% silver nitrite.

VII. Potassium permagnate solution, depolarization of solution.

Test for Flavonoids

a) Shinoda tests:- a small quantity to test residue was dissolved in 5ml ethanol (95%v/v) and reacted with few drop of concentrated hydrochloric acid and 0.5g of magnesium Metal. The pink, crimson or magenta colour is developed within a minute or two, if flavonoids are present.
b) Ammonia test:- filter paper strip were dripped in the alcoholic solution of the extract and ammoniated. The paper strip turned yellow due to the presence of flavonoids.
c) Zinc chloride test:- extract is subjected to reaction with zinc metal and few drops of con. HCl brick red colour gives the presences of flavonoids.
d) Lead acetate when added to small quantity of residue gives yellow colour in case of flavonoids.
e) Addition of increasing amount of sodium hydroxide to residue shows yellow coloration, if flavonoids are present. This yellow colour decolorize after addition of acid.

Test of Carbohydrates

(a) **Molish's Test:-** 2-3 drops of naphthol was added in test solution. Then few drops of conc. H_2SO_4 were added from the side of test tube. Violets co lour ring was formed at the junction of two Layers indicated the presence of carbohydrates.
(b) **Bar ford' Test:-**This reagent was prepared by dissolving 13.3g of crystalline neutral copper acetate in 200ml of 1% acetic acid solution. The test residue dissolved in water and heated with a little of the reagent. If a red precipitate of cuprous oxide is formed within two minutes, monosaccharide is present.
(c) **Benedict test:-** To the test extract, when added benedicts reagent, depending upon the amount of sugar Present, green, yellow or red is formed.
(d) **Fehling' solution:-** Fehling a Solution and Fehling B Solution were mixed in equal amount and boils for one Minute. Equal amount of test solutions was added. It was heated for 5-10 minutes. First Yellow and then a brick red colour appearance indicated the presences of reducing Sugars.
(e) **Toll en's Phoroglucinol test for Galactose:-** 2.5ml of conc. Hydrochloride acid and 4ml of 0.5% Phoroglucinol and to this is added 1-2ml of test solution. Mixture is heated. Yellow to red colour appear.
(f) **Seliwanoff's test (for keto hexose's):-** To the test solution add crystals of resorcinol and an equal volume of concentrated Hydrochloric acid. Rose co lour is produced for ketones after a gentle heating.

(g) **Cobalt chloride test:-** To 3ml of test solution 2ml of cobalt chloride is added boil and cool, to this add few drops of sodium hydroxide solution. Solution appears greenish blue (glucose) or purplish (Fructose) or upper layer greenish blue and lower layer purplish (mixture of glucose and fructose).

Tests for Amino Acids:

(a) **Ninhydrine test:-** The Ninhydrine reagent is 0.1% w/v solution of ninhydrine in n-butanol. A little of this reagent was added to the test extract. A violet or purple colour is developed if amino acids are present.
(b) **Cysteine test (Sulphur containing amino acid):-** To the 5ml of test extracts is added, 2ml of 40% w/v sodium hydroxide and 2 drops of 10% w/v lead acetate solution, resulting solution is boiled for few minutes black precipitate occurs if cysteine amino acid is present.

Test for Proteins

(a) Biuret test's few mg of the residue was taken in water and 1ml of 40% sodium hydroxide solution was added to it. A drop of 1% solution of copper sulphate followed by this violet or pink colour is formed if proteins are present.
(b) **Xanthoprotein test (for tyrosine and tryptophan):-** to the extract add 1ml of conc. sulphuric acid, white precipitate is observed, boil the solution, yellow precipitate is observed, to this add ammonium hydroxide and precipitate turns yellow.
(c) **Mellon's test (Mercuric nitrate solution):-** aqueous solution of the 3ml of residue was taken and to it, 5ml of million's reagent was added. The white precipitate slowly turns to pink or red when warmed gently, if proteins are present.
(d) **Proteins containing sulphur:-** To 5ml of test extract add 2ml of 40% sodium hydroxide and 2 drop of 10% lead acetate solution, solution turns black or brown when heated gently. The test extract when added trichloroacetic acid, precipitate is formed.

Tests for Vitamins

(a) **Test for Vitamin A:-** Dissolve a quantity equivalent to 10-15 units in 1ml chloroform and add 5ml of Antimony dichloride solution, a transient blue co lour is produced immediately.
(b) **Test for vitamin C (Ascorbic acid):-** Dilute 1ml of 2% w/v solution with 5 ml of water and added 1 drop of freshly prepared 5% w/v solution of sodium nitroprusside and 2ml dilute NaOH solution. Added 0.6ml of hydrochloric acid drop wise and stir, the yellow colour turns blue.
(c) **Test for Vitamin D:-** Dissolved a quantity equivalent to about 100 units of Vitamin D, activating in chloroform **a**nd added 10ml of antimony trichloride solution, a pinkish-red colour appeared at once.
(d) **For vitamin:-**

(1) 2% W/V solution was diluted with 5ml of water. One drop of 5%w/v solution of sodium nitroprusside and 2ml of dil. sodium hydroxide solution was added to the diluted solution. 0.6ml of HCL was added drop wise and stirred. Yellow colour was appeared which turned blue after sometimes, indicated the presence of vitamin was appeared which turned blue after sometimes, indicated the presence of vitamin C.

(2) 2%w/v solution was diluted with 2ml of water 0.1g of sodium bicarbonates and about 20mg of ferrous sulphate were added. It was shaken well allowed to stand for some time; a deep violet colour was produced. Then 5ml of 1M sulphuric acid was added, colour was disappeared, indicate the presence of vitamin C.

Tests for Organic acids

Aqueous drug extract was neutralized with dil. ammonium hydroxide solution. Then following tests were performed. Calcium chloride test: few drop of 5% calcium chloride solution were added to the test solution.

(A) White precipitate was observed-presence of oxalic acid.

(B) Precipitate was observed on shaking or scratching with glass rod-presence of tartaric acid.

(C) Precipitate was observed on boiling and then cooling-presence of citric acid.

(D) Precipitate was observed on adding absolute alcohol-presence of malic acid.

Confirmatory test for oxalic acids:-

1) Few drops of 5% lead acetate solution were added to 2ml of test solution. White precipitate was observed, indicated the presence of oxalic acid.
2) Few drops of 1% $KMnO_4$ was dil. H_2SO_4 was added to 2ml of test solution. Decolouration of $KMnO_4$, Confirmed the presences of oxalic acid.

Confirmatory test for tartaric acid:- 3 drops each of saturated $FeSO_4$ solution, water and NaOH were added to 2-3ml of test solution. Violet colour was appeared.

Confirmatory test for citric acid:- one drop of dil. NH_4OH and excess of calcium chloride solution were added to 2ml of test solution. It was boiled for 15min. in boiling water bath. White gelatinous precipitates were observed.

Confirmatory test for malic acid:- 2-3 drops of 5% $FeCl_3$ were added to 3ml of test solution. Solution was turned yellow.

Tests for Inorganic elements

Prepare ash of drug material. 50%v/v nitric acid was added. It is kept for one hour or longer, and then filtered. With the filtrate following tests were performed.

(a) Test for calcium:- to 10ml filtered, 1 drop of dil. ammonium hydroxide and saturated ammonium oxalate solution added. White ppt of calcium oxalate forms, which is soluble in hydrochloride acid insoluble in acetic acid.

(b) Test for magnesium:- calcium oxalate ppt. obtained above is filtered and separated. Heated and cooled filtrate, with a solution of sodium phosphate in dilute ammonia solution gives white crystalline precipitate.

(c) Test for sodium:- to 10ml of ash, 2ml of potassium pyroanthllolate added, gives white ppt.

(d) Test for potassium:- to 3ml test solution cobalt nitrite solution when added, gives yellow ppt of potassium cobalt nitrite.

(e) Test for iron:- to 5ml of test solution added few drops of potassium ferricyanide. Dark blue colour is observed in case, iron is present.

(f) Test for sulphate:- to 5ml filtrate when added few drops of 5% barium chloride solution, white crystalline ppt. of barium sulphate appears, which is insoluble in hydrochloride acid, confirms the presence of sulphate.

Test for Phosphate

To the 5ml test solution prepared in nitric acid, added few drops of ammonium molybedate solution, resulting solution is heated for 10min, cooled. Yellow crystalline ppt. of ammonium phosphor molybedate is observed if phosphate is present.

Test for Chloride

To 3ml of test solution prepared in nitric acid, was added few drops of 10% silver nitrate. White ppt. of silver chloride is observed in presences of chlorides. This ppt. is soluble in dilute ammonia solution.

Test for Carbonate

Test solution with mercuric chloride solution produces a brownish red ppt. in presence of carbonate.

Pharmacognostic Investigation

Organoleptic / Macroscopic evaluation

In the present study the bark of *Aphanamixis polystachya* was investigated for its macroscopic characteristics.[11]

Macroscopic characters, which were observed, are given follow

Colour: Brownish red

Odour: Aromatic, Pleasant

Taste: Astringent

Shape: Straight

Texture: Rough

Fracture: Short

Standardization of bark of *Aphanamixis polystachya* Wall (Parker)[12,13]

The evaluation of a crude drug involves the determination of identity, purity and quality. Purity depends upon the absence of foreign matter whether organic or inorganic, while quality refers essentially to the concentration of the active constituents in the drug that makes it valuable to medicine. The following standardization parameters were evaluated to obtain the qualitative information about the purity and quality of *Aphanamixis polystachya* Wall (Parker).The results are shown in Table No. 5.

Determination of foreign matter

Foreign matter in herbal drugs consists of either parts of the medicinal plant or it may be any organism, part or product of an organism. It may also include mineral admixtures not adhering to the medicinal plant materials e.g. soil, stones, dust etc. The specified quantity of plant material is spread on a thin layer of paper. By visual inspection or by using a magnifying lens (5X or 10X), the foreign matters are picked out and the percentage is recorded.

Determination of physical constants

Loss on drying at 110^{0}C

Loss on drying is the loss of mass expressed as per cent w/w. The test for loss on drying determines both water and volatile matter in the crude drug. Moisture is an inevitable component of crude drug, which must be eliminated as far as possible. An accurately weighed quantity of about 5g of powdered drug was taken in a tared porcelain dish. The powder was distributed evenly. The porcelain dish kept open in vacuum oven and the sample was dried at a temperature 110°C for 2h until a constant weight was recorded. Then it was cooled in a desiccator to room temperature, weighed and recorded. % Loss on drying was calculated using the following formula.

$$\% \text{ Loss on drying} = \frac{\text{Loss in weight of the sample}}{\text{Weight of the sample}} \text{ X } 100$$

Ash values

Ash values are helpful in determining the quality and purity of a crude drug, especially in the powdered form. The objective of ashing vegetable drugs is to remove all traces of organic matter, which may otherwise interfere in an analytical determination. On incineration, crude drugs normally leave an ash usually consisting of carbonates, phosphates and silicates of sodium, potassium, calcium and magnesium. The total ash of a crude drug reflects the care taken in its preparation. A higher limit of acid-insoluble ash is imposed, especially in cases where silica may be present or when the calcium oxalate content of the drug is very high.

i. Total ash value

Weighed accurately about 2 to 3g of the powdered drug in a tared silica crucible. Incinerated at a temperature not exceeding 450^{0}C for 4h, until free from carbon, cooled and weighed. Calculated the percentage of ash with reference to air-dried drug using following formula,

$$\% \text{ Total ash value} = \frac{\text{Wt. of total ash}}{\text{Wt. of crude drug taken}} \text{ X } 100$$

ii. Water soluble ash value

Boiled the ash with 25ml of water. Filtered and collected the insoluble matter on an ashless filter paper, washed with hot water and ignited in a tared crucible at a temperature not exceeding 450^{0}C for 4h. Cooled in a desiccator and weighed. Subtract the weight of insoluble matter from the total weight of ash. The difference in weight represented weight of water soluble ash. Calculated the percentage of water soluble ash with reference to the air-dried drug using the following formula:

$$\% \text{ Water soluble ash value} = \frac{\text{Wt. of total ash - Wt. of water insoluble ash}}{\text{Wt. of crude drug taken}} \text{ X } 100$$

iii. Acid insoluble ash value

Boiled the ash for 5min with 25ml of 2M HCl. Filtered and collected the insoluble matter on an ashless filter paper, washed with hot water and ignited in a tared crucible at a temperature not exceeding 450^{0}C for 4h. Cooled in a desiccator and weighed. Calculated the percentage of acid insoluble ash with reference to the air-dried drug using following formula:

$$\% \text{ Acid insoluble ash value} = \frac{\text{Wt. of acid insoluble ash}}{\text{Wt. of crude drug taken}} \text{ X } 100$$

Extractive values

i. Alcohol soluble extractive value

Macerated 5g accurately weighed coarse powdered drug with 100ml of alcohol (90%v/v) in a stoppered flask for 24h, shaking frequently during first 6h. Filtered rapidly through filter paper taking precaution against excessive loss of alcohol. Evaporated 25ml of alcoholic extract to dryness in a tared dish and weighed it. Calculated the percentage w/w of alcohol soluble extractive with reference to the air-dried drug.

ii. Water soluble extractive value

Followed the procedure as above using methanol, water (50:50) instead of alcohol.

Fluorescence analysis of drug[14]

Many crude drugs show the fluorescence when the sample is exposed to UV radiation. Evaluation of crude drugs based on fluorescence in day-light is not much used, as it is usually unreliable due to the weakness of the fluorescent effect. Fluorescence lamps are fitted with suitable filters, which eliminate visible radiation from the lamp and transmit UV radiation of definite wavelength. Several crude drugs show characteristic fluorescence useful for their evaluation.

Antimicrobial Screening

Bacterial Strains

The various gram positive and gram negative bacterial strains i.e. MTCC 0729 and 0443 Escherichia coli, MTCC 0432 Klebsiella pneumonia, MTCC 0902 Staphylococcus aureus, MTCC 1272 Bacillus cereus, MTCC 0621 Enterobacter gergoviae, MTCC 0098 Salmonella entericatyphim, MTCC 1457 Shigella flexneri, MTCC 0435 Staphylococcus epidermidis, MTCC 1925 Streptococcus pyogenes were obtained from IMTECH (Institute of Microbial Technology, Sector 39-A, Chandigarh 160036, INDIA) and used for in vitro antimicrobial screening.

Preparations of Media

The suitable bacterial growth media was prepared by weighing accurately of all ingredients with continuously stirring and boiling in distilled water (as per the guideline of IMTECH) and sterilized, than performed the "Test for sterility" and completely sterile medium used for in vitro antimicrobial screening.

The Antimicrobial activity was studied by disc diffusion method and determines the zone of inhibitions of various extracts against different microorganisms using ten bacterial strains under aseptic condition. The sterile growth media was poured into sterile

petridishes. Overnight nutrient broth culture of the respective test organism was firmly seeded over the growth media plate surfaces using a sterile cotton swab.

Five discs of 5mm diameter on the inoculated plates were placed by using sterile forceps. The kanamycin was used as the standard antimicrobial agent or positive control at the dose of 30 mg/ml. Petroleum ether, chloroform, alcoholic (95.0%) and hydroalcoholic (alcohol: water, 50:50) extracts of stem bark of *Aphanamixis polystachya* were used as test for screening of antimicrobial activity at the different doses (10, 20, 50 and 100 mg/ml)[15,16]

Observation

The plates were incubated at 37ºC for 18-24 hours. The plates were observed for a clear zone around the holes which is known as "zone of inhibition" and measured by zone reader.

RESULT AND METHODS

Phytochemical Investigation

In the present study, bark of *Aphanamixis polystachya* was investigated for the macroscopic characterization. Physical constants for bark of *Aphanamixis polystachya* (Wall.) Parker was tabulated in [Table: 01].

The Preliminary phytochemical investigation revealed the presence of various phytoconstittuents in each extracts. It showed the presence of carbohydrates, triterpenoids, and saponins [Table: 02].

Antimicrobial Screening

The results of antibacterial activity of bark extract against ten food pathogenic bacterial strains are presented in [Table: 03]. The "zone of inhibition" of kanamycin against *Staphylococcus aureus* was 18.0mm, *Staphylococcus epidermidis* was 20.5mm, *Escherichia coli* (both) was 18.0 and 22.0mm, *Klebsiella pneumonia* was 12.0mm, *Bacillus cereus* was 18.0mm, *Enterobacter gergoviae* was 17.6mm, *Salmonella entericatyphium* was 27.0mm, *Shigella flexneri* was 9.0mm and *Streptococcus pyogenes* was 18.0mm. On the basis of these "zone of inhibitions" when compared with bark extracts, it was concluded that the ethanolic extract of dried stem bark of Aphanamixis polystachya showed significant antimicrobial activity against all ten food pathogenic bacterial strains, in comparison to kanamycin, which is a broad spectrum antimicrobial agent.

Table 1: Physical constants for barks of *Aphanamixis polystachya* (Wall.) *Parker*

S. No.	Physico-chemical parameter	Stem bark
1.	Foreign matter	Nil
2.	**ash values**	
	(a) total ash value	6.5 %
	(b) acid insouble ash	2.5 %
	(c) water souble ash	1.5 %
3.	**extractive value**	
	(a) alcohol souble extractive	2.8 %
	(b) water souble extractive	2.2 %
4.	Loss in drying(105^0c)	33.3 %
5.	Fluorescence	Nil

Table 2: Preliminary qualitative tests of various extracts of *Aphanamixis polystachya* (Wall) Parker

Phytoconctituntes	Pee	Chle	Ale	Aqmh
Carbohydrates	–	–	+	–
Proteins	–	–	–	–
Steroids	–	–	–	–
Amino acid	–	–	–	–
Glycosides	–	–	–	–
Alkaloids	–	–	–	–
Flavonides	–	–	–	–
Vitamins	–	–	–	–
Saponins	–	–	–	+
Tannis and phenol	–	–	–	–

Table 3: Antimicrobial screening of different extracts of *Aphanamixis polystachya (Wall) parker* against ten food pathogenic bacteria's

S. No.	Pathogenic bacteria	Sample sol. (mg/ml)	Pt. Ether (mm)	Chloroform (mm)	Ethanol (mm)	Aq. Methanol (mm)
01.	MTCC1925	05	----	----	05	----
	(STREPTOCOCUS)	10	----	----	12	----
	(species-progenies)	20	----	----	05	----
		50	----	08	05	05
		100	----	10	08	05
02.	MTCC 435	05	----	05	----	----
	(STAPHYLOCOCUS)	10	07	10	----	----
	(species-epidermidis)	20	----	----	----	----
		50	----	----	----	08
		100	----	----	----	10
03.	MTCC 432 (KIEBSIELLA)	05	----	02	----	----
	(species-pneumoniae subs)	10	----	09	----	----
		20	----	05	----	----
		50	----	06	08	----
		100	----	06	08	----
04.	MTCC 443 (ESCHERICHIA)	05	05	----	05	----
	(species-coli-UTI)	10	08	----	08	----

		20	----	06	08	----
		50	06	05	08	----
		100	07	05	09	----
05.	MTCC 729 (ESCHERICHIA)	05	05	----	----	----
	(Species-coli-food poison)	10	10	----	----	----
		20	----	04	07	----
		50	----	08	06	----
		100	----	10	08	----
06.	MTCC 98 (SALMONELLA)	05	----	----	05	----
	(species-enterica typhim)	10	----	----	07	----
		20	----	----	07	----
		50	----	----	06	06
		100	----	----	07	07
07.	MTCC 1457	05	----	----	----	----
	(SHIGELLA)	10	----	----	----	----
	(species-flexneri)	20	----	----	----	----
		50	----	----	----	----
		100	----	----	----	----
08.	MTCC 1272	05	----	----	----	----
	(BACILLUS)	10	----	----	----	----
	(species-cereus)	20	----	----	----	----
		50	----	----	10	07
		100	----	----	08	05
09.	MTCC621	05	----	----	----	----
	(ENTEROBACTER)	10	----	----	----	----
	(species-gingival)	20	----	----	----	----
		50	----	----	06	----
		100	----	----	07	----
10.	MTCC 902	05	----	----	----	----
	(STAPHYLOCOCOU)	10	----	----	----	----
	(species-aureus snap.au)	20	----	----	----	----
		50	----	05	07	----
		100	----	09	12	08

(----) NIL

CONCLUSION

Investigations on the phytochemical screening of *A. polystachya* stem bark extracts revealed the presence of carbohydrates and saponins. These compounds are known to be biologically active and therefore aid the antimicrobial activities of *A. polystachya*. These secondary metabolites exert antimicrobial activity through different mechanisms. The *in vitro* antimicrobial studies present *A. polystachya* to have considerable efficacy against various food pathogenic bacteria. The study provides a scientific basis for the use of the plant as folk medicine. However, more advanced pharmacological and clinical studies would be required to investigate in vivo mechanism of nutraceuticals effects of this important wild plant. Hence, it is concluded that *A. polystachya* stem bark could be a potential source of active antimicrobial agents and a detailed assessment of its *in vivo* potencies and toxicological profile is ongoing.

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