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IN VITRO ANTI-BACTERIAL AND ANTI-FUNGAL ACTIVITIES OF ANANTAMOOL ROOT EXTRACT

Abdul Nazeer M.*, Sangami S., Santhosh M., Santhosh Raja G., Silambarasan P. and Sindhuja M.

Sree Abirami College of Pharmacy, Coimbatore-21.

***Corresponding Author: Abdul Nazeer M.**
Sree Abirami College of Pharmacy, Coimbatore-21.

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ABSTRACT
Herbal medicine is the study and application of plant characteristics for medical purpose. Plants are able to manufacture a vast array of chemical compounds that are essential for carrying out vital biological processes and providing protection against pathogens, fungus, insects and other invaders. These have an ethnomedical use connected to existing usage of the plant's active ingredient, demonstrating the effectiveness of ethnobotany as means of discovering new medicines. Anantamool, also known as *Hemidesmus indicus*, is a common ingredient in ayurvedic medicine its medical significance has a long history that extends back toantiquity. Anantamool translate to "the eternal root" because of the plants extensive underground root system. There is a pleasant camphor like scent to the roots. Numerous features of this plant have been studied, including its morphology, Geographical origins, synonyms for the root, biological sources, Taxonomic attributes, Vernacular name, morphology, and therapeutic qualities.

KEYWORDS: Hemidesmus indicus, Phytochemical screening TLC, antibacterial activity, antifungal activity.

INTRODUCTION
The roots of nannari are pounded into a fine powder after being dried. It works well to lower body temperature and has no extra flavor. There are two varieties of Nannari: Siru Nannari and Peru Nannari. Mahali kilangu or Mavali kilangu are other names for Peru Nannari.

In India, the term "nannari" refers to the sarsaparilla, also known as anantmool plant. Given its cooling qualities, it's ideal for this summer. Nannari syrup is the name given to the syrup produced by this root. This hemidesmus indicus root extract is used by the ancients in panam kalkandu, a concoction of lime juice and sugar. During the summer, this drink might help them stay cool. As an alternative to palm sugar, you can instead use white sugar.

Its interior sections are white, while its root is brown in color. The root has a nice scent and an astringent flavor. The sarsaparilla herb has been used for generations to treat skin conditions like dermatitis, eczema, and psoriasis as well as joint issues like arthritis. Its "blood purifying" qualities are also said to aid in the treatment of leprosy. Hemidesmus Indicus, a member of the Apocynaceae family, is the botanical name for the anantmool. This plant is native to all of India's tropical and subtropical areas. This herb can have leaves as long as 2.5 inches. The upper portion of the leaves have a very delicate oval shape. The stem has thick nodes and is cylindrical in shape.Its wood roots give it a wonderfully fragrant flavor.

Benefits
1. Nannari is an amazing herb for losing weight. It has flavanoids and thick antioxidants to reduce body fat and harmful cholesterol.
2. Nannari is a wonderful herb for the skin. Pigmentation-free, age-defying skin can beachieved by applying and eating Nannari paste.
3. Abdominal pain, ulcers, and stomach infections can all be prevented with nannari.
4. Nannari is a diuretic that is excellent for liver and kidney detoxification. It will make theblood pure.
5. Nannari is used by traditional medicine practitioners in Central India to treat internalbleeding and piles.
6. Nannari is the ideal herb for women's health. It might regularize ovulation and lessen menstrual pain.
7. Nannari is a herb that lowers blood sugar and increases energy

Synonyms of anantamool
Hemidesmus indicus, Indian sarsaparilla, Nannari, Tylophora, False sarsaparilla, Pseudosarsa, Nunnari asclepias, Periploca indica, Magarbu, Sariva, Karpoori, Sugandhi

Taxonomic characteristics:
Kingdom: Plantae
Clade-Angiosperms
Order- Gentianales
Family- Apocynaceae
Genes- Hemidesmus
Species- H. Indicus

Biological sources:
It is obtained from the whole plant of anantamool (Hemidesmus indicus) belongs to the family Apocynaceae.

Vernacular Name
Tamil: Suganthi Paalaa, Suganthipaalaa, Nannari
English: Hemidesmus indicus, Indian sarsaparilla, Tylophora, False sarsaparilla, Pseudosarsa, Periploca indica, Magarbu, etc.
Hindi-Anantamool, Sugandi Pala Malayalam – Nannaari Telugu- Sugandhi Bengali - Anantamool Assamese - Anantamoola Kannada- Anamtamoola Oriya - Suguddimalo in Oriya Marati - Anantavel, Upalsari Sanskrit -Anantamoola, Balyam, and Shariva.
Part Used: Whole plant of anantamool (Root).

Plant profile

Fig. no. 1: Flower.

Fig. no. 2: Dried Root.

Fig. no. 3: Seed.

Fig. no. 4: Fresh Root.

MATERIALS AND METHODS
Extraction of anantamool root powder:
Preparation of ethanolic extract Anantamool root powder

- The extraction was done by maceration using hydroalcoholic as solvent 50 gm root powder of Anantamool was weighted into round bottom flasks.
- Add 250 ml of the solvents (Water & Ethanol) were added to the flask containing thepowder, the mixture was allowed to stand for 7days at room with agitation at regular intervals.
- The extract was filtered through filter paper and the extracts was concentrated to removethe solvents.

Thin layer chromatography
TLC method was used for the identification of the secondary metabolites. The chromatographic sheet was set with 5cm width and 8cm length and spotted the sample 1cm above from the bottom using capillary tubes (about 50 µl). Sample placed and this was run in solvent system of Acetone: n-hexane: Methanol, Chloroform, Acetic acid Ethanol (1:2.1:1:1:0.5). After 1cm from the top of the plate this was taken out from the solvent and dried to visualise the compound, the same method was fused for the TLC study. Rf value was calculated after visualisation of the compound as spot in plate. Rf value calculation formula is;

$$R_f \text{ Value} = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent front}}$$

Anti-bacterial activity:
The well diffusion method was used to evaluate the antimicrobial activity of the root extract. The antimicrobial activity of the plant extract was investigated against four pathogenic bacteria (*K. pneumoniae, E. coli, S. typhi, S. aureus)* 15ml of nutrient Agar (for bacteria) were prepared poured onto the surface of sterile petriplate as a basal layer.

After solidification 80 µl of the indicator strains (10^8 cfu) were swabbed uniformly on the surface of the agar plate and allowed to dry for 5 minutes. Five wells of 6 mm were bored in the inoculated media with the help of sterile cork-borer (6 mm). Each well was filled with 10 µl of plant extract. Standard reference antibiotics were used as the positive controls and Dimethyl Sulfoxide (DMSO) serves as the negative control. It was then incubated at 37°c for 18-24 hours for the bacteria. After incubation, plates were observed for the formation of a clear zone around the well which corresponds to the antimicrobial activity of tested compounds. The zone of inhibition (ZOI) was observed and measured in mm.

Nutrient agar medium:
Distilled water for one plate – 15 ml
Distilled water for 4 plates - 60 ml

$$\text{Nutrient medium} = \frac{\text{suspended amount} \times \text{required volume}}{1000}$$

$$= \frac{28 \times 60}{1000}$$

$$= 1.68 \text{ g}$$

Nutrient agar
Suspend 28.0 grams in 1000 ml purified distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 IBS pressure (121°) for 15 minutes. Cool to 45-50 °C. If desired, the medium can be enriched with 5-10% blood or biological fluids. Mix well and pour into sterile petri plates.

Table no. 1: Ingredient used in nutrient agar medium.

Ingredients	gms/ litre
Peptone	5.00
HM peptone	1.50
Yeast extract	1.50
Sodium chloride	5.00
Agar	15.00
Final pH(25°c)	7.4±0.2

USES
Cultivation of less fastidious microorganism from clinical and non-clinical samples. It can be enriched with blood or biological fluids.

Anti-fungal activity
The well diffusion method was used to evaluate the antimicrobial activity of the root extract. The antimicrobial activity of the plant extract was investigated against two pathogenic fungi (*A. niger* and *F. oxysporum*) 15 ml malt agar (fungi) were prepared poured onto the surface of sterile petriplate as a basal layer. After solidification 80 µl of the indicator strains (10^8 cfu) were swabbed uniformly on the surface of the agar plate and allowed to dry for 5 minutes. Five wells of 6 mm were bored in the inoculated media with the help of sterile cork-borer (6 mm).

Each well was filled with 10 µl of plant extract. Standard reference antibiotics were used as the positive controls and Dimethyl Sulfoxide (DMSO) serves as the negative control. It was then incubated at 300°C for 3 to 5 days for the fungi. After incubation, plates were observed for the formation of a clear zone around the well which corresponds to the antimicrobial activity of tested compounds. The zone of inhibition (ZOI) was observed and measured in mm.

Malt agar medium
Distilled water for one plate – 15 ml
Distilled water for two plates - 30 ml

$$\text{Malt agar medium} = \frac{\text{suspended amount} \times \text{Required volume}}{1000}$$

$$= \frac{45 \times 30}{1000}$$

$$= 1.35 \text{ g}$$

Malt AGAR
Suspend 45.0 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 118° C for 15 mins. Mix well and pour into sterile petri plates. Δ corresponds to 12 IBS pressure.

Table no. 2: Ingredients used in malt agar medium.

Ingredient	gms/ litre
Malt extract	30.00
Agar	15.00
Final pH. (25° C)	5.5+0.2

Uses
Detection and isolation of yeasts and moulds from dairy products, food and other materials also used for carrying stock culture of yeast and moulds.

RESULT AND DISCUSSION

Fig. no. 5: Maceration.

Fig. no. 6: Filtra.

Fig. no. 7: Filtrate.

Fig. no. 8: Extract.

Table no. 3: Phytochemical screening.

S. No	Plant constituents	Test/Reagents used	Ethanol extract
1	Alkaloids	Mayers Reagent Dragendorff 's reagent Wagner'sreagent	+ + +
2	Carbohydrates	Molisch's reagent Fehling's solution Benedict'sreagent	+ + +
3	Glycoside	Libermann test Keller killani test	+ +
4	Protein	Millon test Biuret test Ninhydrin test	+ + +
5	Tannins & Phenol	$FeCl_3$ solution Lead tetra acetic acid	+ +
6	Terpenoid	Salkowski's test	+

Tlc for aqueous extract
Solvent: Acetone: n-hexane: Methanol, Chloroform, Acetic acid Ethanol (1:2.1:1:1:0.5).

Fig. no. 9: TLC for aqueous extract.

TLC for aqueous extract

$$R_f \text{ Value} = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent front}}$$

$$= \frac{4}{6.5}$$

$$= 0.615$$

The R_f value for aqueous extract is 0.615.

TLC for ethanol extract
Solvent: Acetone: n-hexane: Methanol, Chloroform, Acetic acid Ethanol (1:2.1:1:1:0.5).

Fig. no. 10: TLC for ethanol extract.

$$Rf \text{ Value} = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent front}}$$

$$= \frac{4.2}{6.5}$$

$$= 0.64$$

R_f value for ethanol extract is 0.64.

Anti- bacterial activity

Fig. no. 11: Nutrient agarmedium.

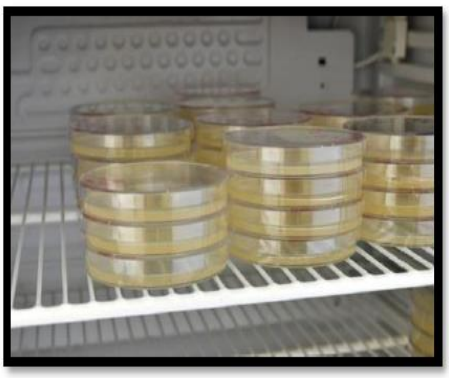

Fig. no. 12

Fig. no. 13: *K. Pneumoniae.*

Fig. no. 14: *Esch.*

Fig. No. 15: *Staphylococcus aureus.*

Fig. No. 16: *ty.*

Table no. 4: Antibacterial activity zone of the inhibition.

S. no.	Name of the	Zone of the inhibition			
	Organism	Dmso	Aqueous	Ethanol	Disc (cefazolin)
1	*Klebsiella pneumoniae*	0	10mm	0	15mm
2	*Escherichia coli*	0	0	12mm	0
3	*Staphylococcus aureus*	0	17mm	12mm	14mm
4	*Salmonella typhi*	0	25mm	12mm	14mm

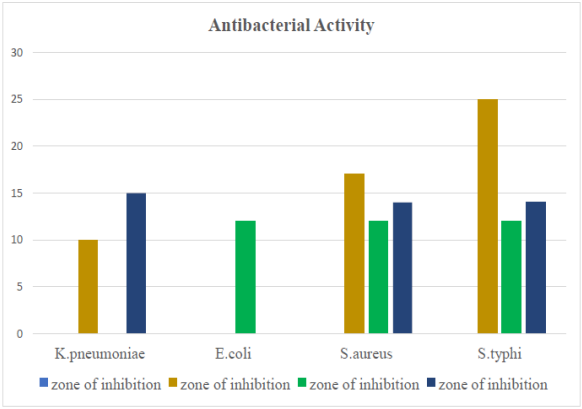

Fig. no. 17: Antibacterial activity (Zone of the injibition).

Anti -fungal activity

Fig. no. 18: *Fusarium oxysporum.*

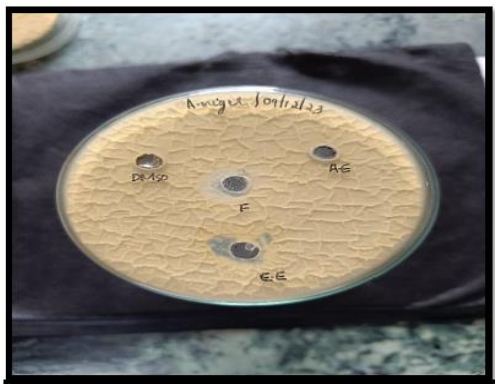

Fig. no. 19: *Aspergillus niger.*

Table no. 5: Anti-fungal (Zone of the inhibition).

S. no.	Name of theorganism	Zone of the inhibition			
		Dmso	Aqueous	Ethanol	Fluconazole
1	*Aspergillus niger*	0	0	13mm	10mm
2	*Fusarium oxysporum*	0	0	13mm	0

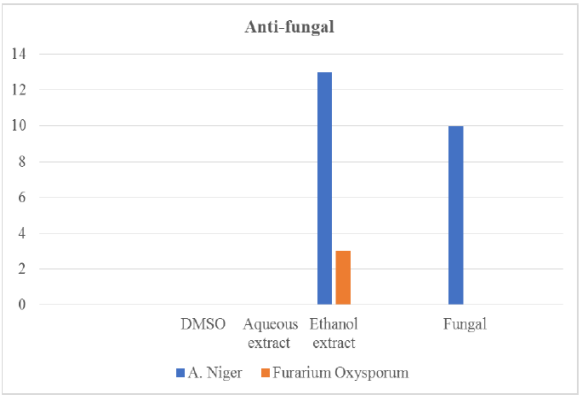

Fig. no. 20: Anti-fungal activity (Zone of the inhibition).

Table no. 6: Parameters and Results.

S. No.	Parameters	Results	
1	**Powder microscopy**	Xylem vessels, tyrecheids, cork cells, fibres, starch grains.	
2	**Microbial test**	**Aqueous extract**	**Ethanolic extract**
	A) anti- bacterial Escherichia coli salmonella typhi streptococcus aureus klebsiella pneumoniae	Absent present present present	Present present present absent
	B) anti- fungal Aspergillus niger fusarium oxysporum	Absentabsent	Presentpresent

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