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Antimicrobial potential of *Nyctanthes arbor-tristis* and isolation of *Colletotrichum gleosporioides* –an endophyte.

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ABSTRACT

A comparative study has been carried out to assess antibacterial potential of different plant parts of *Nyctanthes arbor-tristis***.** Leaf was experienced as the most efficient plant part having antibacterial activity against pathogen tested. Antibacterial activity of aqueous and methanolic extract of root, shoot and leaf were tested by well diffusion method. Leaf was taken for further activity by preparing four fractions (ethyl acetate, n- butanol, chloroform and aqueous) of its' methanolic extract. Antibacterial activity of these fractions were analysed for more accuracy. Sections of leaf of *N. arbor-tristis* were inoculated on PDA media to isolate and identify endophytic fungi. Aqueous extract of leaf possess greater activity against pathogenic bacteria than methanolic extract. Ethyl acetate, n- butanol and aqueous fraction of methanolic leaf extract is the effective fraction having antibacterial activity against *Klebsiella pneumoniae, Staphylococcus epidermidis, Listeria monocytogens, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus.* On the basis of morphological features, biomass production and enzymatic studies the isolated endophytic fungus was identified as *Colletotrichum gleosporioides.*

KEYWORDS: Medicinal plant, *Nyctanthes arbor-tristis,* phytochemical test, antimicrobial activity, pathogenic bacteria, endophytic fungus, *Colletotrichum gleosporioides*

INTRODUCTION

Medicinal plants represent a rich source of antimicrobial agents. Wide range of different parts of medicinal plants was used for extract as raw drugs and they posses varied medicinal properties. Some of these raw drugs are collected in larger quantities and traded in market as raw material for many herbal industries[1]. The increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents has led to the screening of several medicinal plants for their potential antimicrobial activity[2]. *Nyctanthes arbor-tristis* is commonly known as Harshinghar or Night Jasmine. It belongs to the family Oleaceae.[3] It has also been reported to possess hepatoprotective, anti-leishmanial, anti-viral and anti-fungal activities and analgesic, antipyretic and ulcerogenic activities. The plant also possess anti-allergic anti-malarial[4] anti-helminthic,[5] activities and recently reported heapatoprotective[6], anti-spermatogenic and anti-oxidant activities[7]. In present work comparative analysis of different plant parts and then different fractions of methanolic extract of leaf was carried out. An endophytic fungus was also isolated from leaf of this tree and identified as *Colletotrichum gleosporioides.*

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1.MATERIALS AND METHODS

2.1Plant material

The healthy leaves shoot, and root of the 6 years old *Nyctanthes arbor-tristis* was collected from the reserve forest area of Jabalpur district, Madhya Pradesh. Leaves root and stem first washed thoroughly with tap water and were then dipped in sterile distilled water to remove the fine soil particles. These parts of plant were oven dried and finally powdered by grinding. The homogenized powder is stored in airtight polythene.

2.2 Extraction of secondary metabolites from the N. arbor-tristis parts (leaves, stem and root)

One gram of dried powder of *N. arbor-tristis* plant parts (leaves, stem and root) was dissolved in 50 ml of methanol and distilled water separately and were incubated in an orbital shaker for 24 hour. After 24 hours of incubation period, crude methanol and aqueous extract of taken plant materials were filtered and evaporated to dryness. Residue was dissolved in approximately 1ml of solvent. Different concentrations (25%, 50%, 75% and 100 %) of each extract was prepared and applied to assay their antimicrobial activity against seven pathogenic bacterial cultures.

2.3 Preparation of fractions (chloroform, ethyl acetate, n-butanol and aqueous) from the methanolic leaf extract of N. arbor-tristis

The dried leaves were grinded into fine powder form. The leaves powder (5gm) was dissolved in 70% methanol and was incubated in an orbital shaker for 24 hours. After incubation period 70% methanolic crude leaf extract was filtered and was evaporated to dryness. Residue was dissolved in distilled water which was then acidified, neutralized and was again filtered. The filtrate was fractionized in separating funnel using chloroform, ethyl acetate, n-butanol and aqueous solvents. The fractions were collected separately and the solvent was evaporated. Residue was dissolved in approximately 1ml of solvent. Different concentrations (25%, 50%, 75% and 100 %) of each fraction was prepared and applied to assay their antimicrobial activity against seven pathogenic bacteria cultures.

2.4 Phytochemical analysis

Phytochemical analysis of different parts of *N. arbor-tristis* was carried out applying various phytochemical tests [8,9].

a. Test for anthraquinone : 0.5 ml of the extract was boiled with 1 ml of sulphuric acid (H_2SO_4) and filtered while hot. The filtrate was shaken with 1 ml of chloroform. The chloroform layer was pipetted out another test tube and 1 ml of dilute ammonia was added. The resulting solution was observed for color change.

b. Test for terpenoids : To 0.5 ml each of the extract was added 2 ml of chloroform. Concentrated H_2S0_4 (3 ml) was carefully added to form a layer. A reddish brown coloration of the interface indicates presence of terpenoids.

c. Test for flavonoids: Diluted ammonia (2 ml) was added to a portion of an aqueous filtrate of the extract. Concentrated sulphuric acid (1 ml) was added. Yellow colorations that disappear on standing indicate the presence of flavonoids.

d. Test for saponins: To 0.5 ml of extract was added 2 ml of distilled water in a test tube. The solution was shaken vigorously and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously after which it was observed for the formation of an emulsion.

5. Culture of pathogenic bacterial strains:

Pure cultures of *Klebsiella pneumoniae* (KP) [Causes pneumonia] (MTCC 7407), *Staphylococcs epidermidis* (SE) [Leading pathogens of nosocomial infections] (MTCC 3086), *Listeria monocytogens* (LM) [Causes destruction of red blood cells and causes Listeriosis] (MTCC 657), *Bacillus subtilis* [Causes food poisoning] (BS) (MTCC 121), *Escherichia coli* (EC) (MTCC 40), [Causes bacterial infections, including cholecystitis, bacteremia, cholangitis, urinary tract infection (UTI), and traveler's diarrhoea, and other clinical infections such as neonatal meningitis and pneumonia and causes severe food poisoning and contamination], *Pseudomonas aeruginosa* [Opportunistic human pathogen causing bacteremia meningitis and brain abscesses] (PA) (MTCC 424) and *Staphylococcus aureus* [Leading cause of skin and soft tissue infections such as abscesses (boils), furuncles, and cellulites] (SA) (MTCC 3160) were procured from Microbial Type Culture Collection (MTCC), Chandigarh**.** The bacterial suspension of the above pure cultures was prepared by inoculating the powdered form of the above strains into their respective nutritional broth and then into their respective media.

6. Antibacterial Activity

The antimicrobial activity of methanolic and aqueous extracts of different parts (leaves, stem, and root) and fractions of methanolic leaf extract of plant *Nyctanthes arbor-tristis* performed separately by agar well diffusion method.

7. Minimum inhibitory concentration (MIC) or zone of inhibition

After incubation, antibacterial activity of various extracts and fractions was observed by measuring zone of inhibition surrounding each well. The zone of inhibition was measured by measuring the diameter (mm) of zone of inhibition against pathogenic bacteria by various concentrations of fractions. One set was kept as control.

a. Identification of endophytic fungus

Cultures were grown in darkness at28°C. Colony diameter was recorded daily (three replicates, two measurements per replicate) for 1week. Growth rate was calculated as the 7-day average of mean daily growth (mm per day). Size and shape of conidia were recorded from the colonies grown on PDA plates at room temperature (28-30°C). Conidia were taken from actively growing colonies mounted in lactic acid, and examined for size and shape. Colony morphology was noted after 7 days.

The isolates of endophytic fungi was purified and identified to various genera by biomass assay, morphological and biochemical tests using established procedures (lacto phenol cotton blue staining technique). The isolated species were described according to their macroscopic features (i.e. color, shape and growth of cultured colonies) as well as microscopic characteristics (i.e., the structure and type of hyphae and spores). Obtained data were then compared with the descriptions of endophytic fungi species in the literature and similar characters matches were recorded.

b. Biomass reading

Fungus isolated from leaves of *N. arbor-tristis* was inoculated in to the P.D. (Potato Dextrose) broth and incubated for 5 days on 28^0 C. After 5 days, the mycelial culture was separated from rest of the broth through filtration. After drying, weight of mycelia mat was taken which a measure of biomass.

c. Enzymatic activity

The endophytic fungal isolates were checked for the production of various hydrolyzing enzymes like amylase, lipase, protease and laccase.

Amylolytic Activity: Amylase activity was assessed growing the fungi on Glucose Yeast Extract Peptone Agar (GYP) medium with 0.2% soluble starch, pH 6.0. After incubation, the plates were flooded with 1% iodine in 2% potassium iodide.

Lipolytic Activity: For lipase activity, the fungi were grown on Peptone Agar medium supplemented with Tween 20 separately sterilized and added 1% to the medium. At the end of the incubation period, a visible precipitate around the colony due to the formation of calcium salts of the lauric acid liberated by the enzyme indicated positive lipase activity.

Proteolytic Activity: Glucose Yeast Extract Peptone Agar medium with 0.4% gelatin (pH 6.0) was used. 8% of gelatin solution in water was sterilized separately and added to GYP medium at the rate of 5 mL per 100 mL of medium. After incubation, degradation of the gelatin was seen as clear zone around the colonies. The plate was then flooded with saturated aqueous ammonium sulphate, which resulted in formation of a precipitate. This made the agar opaque and enhanced the clear zone around the fungal colony.

Laccase Activity: Glucose Yeast Extract Peptone Agar medium pH 6.0 was used. As the fungus grows the colorless medium turns blue due to oxidation of 1-napthol by laccase around the colony due to the formation of calcium salts of the lauric acid liberated by the enzyme indicated positive lipase activity.

2. RESULTS

3.1 Phytochemical analysis

It is clear that anthraquinones were not present in all samples. A reddish brown coloration of the interface in all samples indicates presence of terpenoids. Maximum color intensity indicating presence of terpenoids was observed in methanol extract of root and minimum in aqueous extract of shoot and leaf. It was moderately present in all extracts. Flavonoids were present in all extracts except acetone extract of shoot. It was found maximum in methanol extract of leaf and root. Saponins were present in all aqueous as well as organic extracts of leaf, shoot and root (Table- 1).

Table-1. Phytochemical screening of different extracts of different plant parts of *N. arbor-tristis*

Plant parts	Fractions	Anthraquinones	Flavonoids	Saponins	Trepenoids
Leaf	Water	-	++	+	+
	Methanol	-	+++	++	+++
	Acetone	-	+	-	++
Root	Water	-	+	+++	+++
	Methanol	-	+++	+	+++
	Acetone	-	++	+++	++
shoot	Water	-	+	+	+
	Methanol	-	++	++	+++
	Acetone	-	-	+++	+++

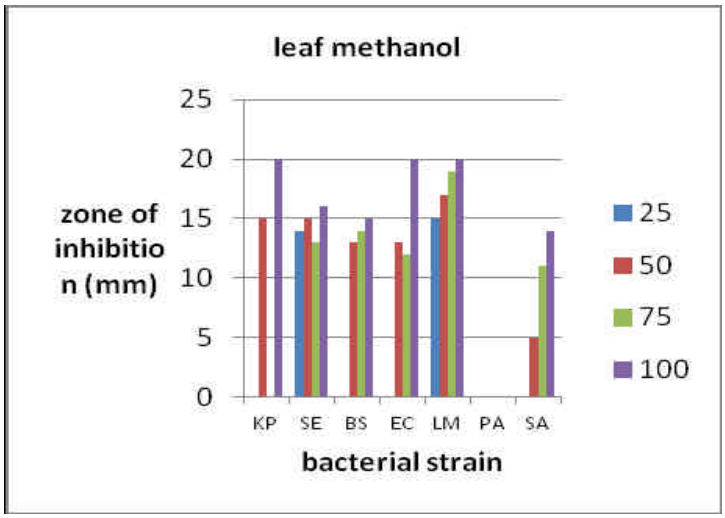
3.2 *Antibacterial activity of different parts (leaf, shoot and root) of N. arbor-tristis*

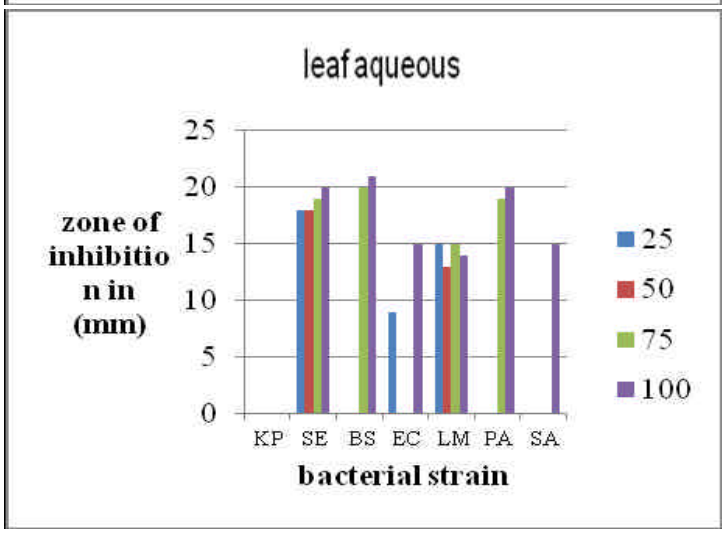
i) Leaf

Methanolic and aqueous extracts of leaf of *N. arbor-tristis* showed strong antibacterial activity against pathogenic bacterial strains tested. A clear, prominent zone of inhibition was obtained against *Klebsiella pneumoniae, Staphylococcus epidermidis, Bacillus subtilis, Escherichia coli, Listeria monocytogens* and *Staphylococcus aureus* was obtained. When tested with methanolic extract of leaf (maximum zone of inhibition against *Klebesiella pneumoniae, Escherichia coli* and *Listeria monocytogens*, 20 mm) (Fig-1.a)**.** Methanolic extract showed minimum activity against *Pseudomonas aeruginosa* showed minimum antibacterial activity.

Fig.1 a): Antibacterial activity of methanolic extract of leaf of *N. arbor-tristis* against SE and EC

Aqueous extracts of leaf showed zone of inhibition against *Listeria monocytogens, Escherichia coli, Pseudomonas aeruginosa, Bacillis subtilis, Staphylococcus epidermidis, Staphylococcus aureus* (maximum zone of inhibition against *Bacillis subtilis,* 21mm) whereas minimum zone of inhibition was observed against *Klebsiella pneumoniae* (Graph-1), (Fig-1.b)





Graph1: Antibacterial activity of leaf extracts *N. arbor-tristis* against pathogenic bacterial strains

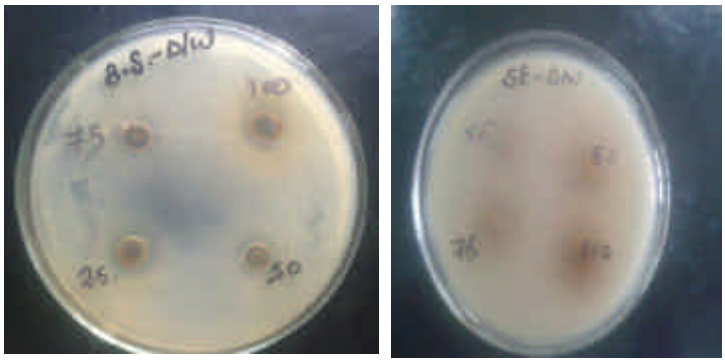


Fig. 1.b) Antibacterial activity of aqueous extract of leaf of *N. arbor-tristis* against BS and SE

ii) Root

Inhibition of *Staphylococcus epidermidis, Bacillus subtilis, Escherichia coli, Listeria monocytogens* and *Pseudomonas aeruginosa* growth was observed by methanol root extract of *N. arbor-tristis* was observed. Methanolic extracts of *root* showed clear and prominent zone of inhibition against *Staphylococcus epidermidis, Listeria monocytogens, Pseudomonas aeruginosa, Staphylococcus aureus and Bacillus subtilis*, (maximum zone of inhibition against *Staphylococcus epidermidis*, 23 mm) (Fig-2.a), whereas comparatively small zone of inhibition was observed against *Bacillus subtilis* and *Staphylococcus aureus* (Graph-2).

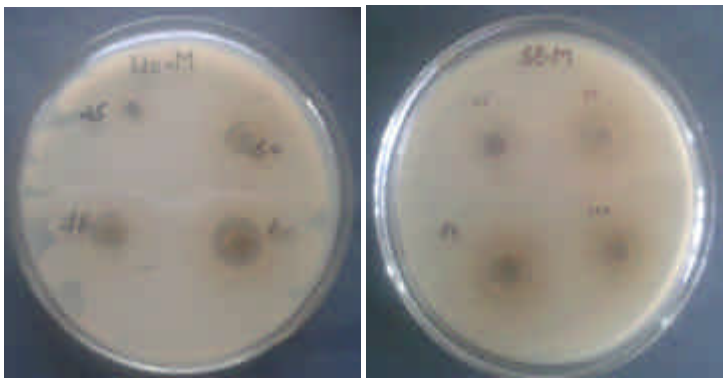
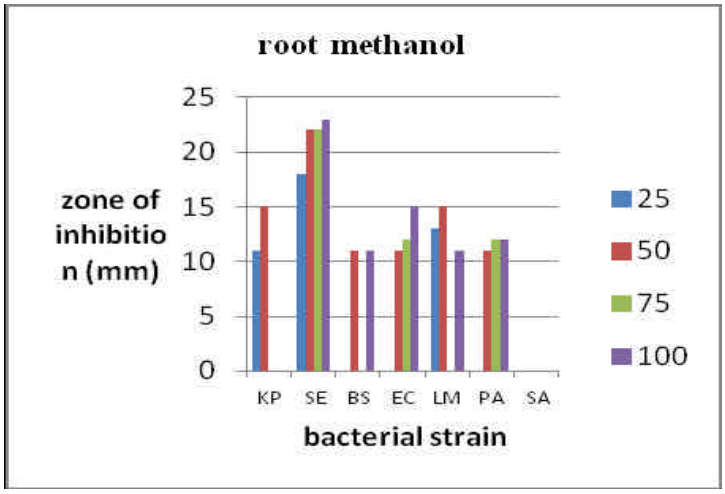
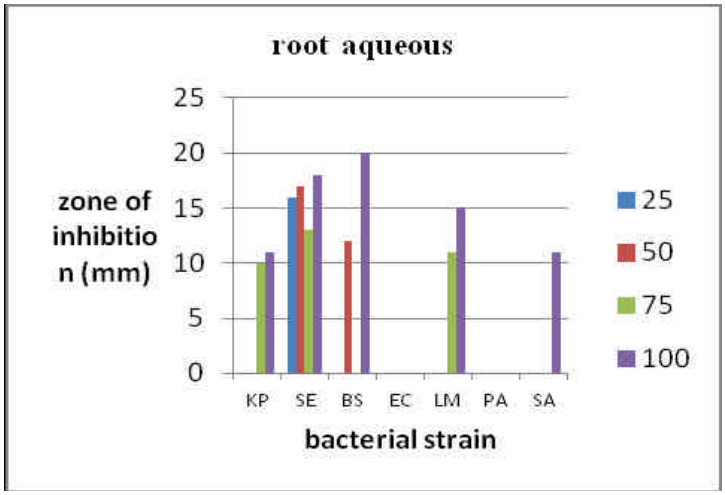


Fig. 2.a) Antibacterial activity of methanolic extract of root of *N. arbor-tristis* against EC and SE





Graph 2: Antibacterial activity of root extracts *N. arbor-tristis* against pathogenic bacterial strains

Aqueous extract of root showed zone of inhibition against *Staphylococcus epidermidis , Listeria monocytogens, Klebsiella pneumoniae, Bacillus subtilis and Staphylococcus aureus* (maximum zone of inhibition against *Bacillus subtilis*, 20 mm) (Fig-2.b), whereas minimum zone of inhibition was observe against, *Pseudomonas aeruoginosa* and *Escherichia coli.*

Fig. 2.b): Antibacterial activity of aqueous extract of root of *N. arbor-tristis* against SE and EC

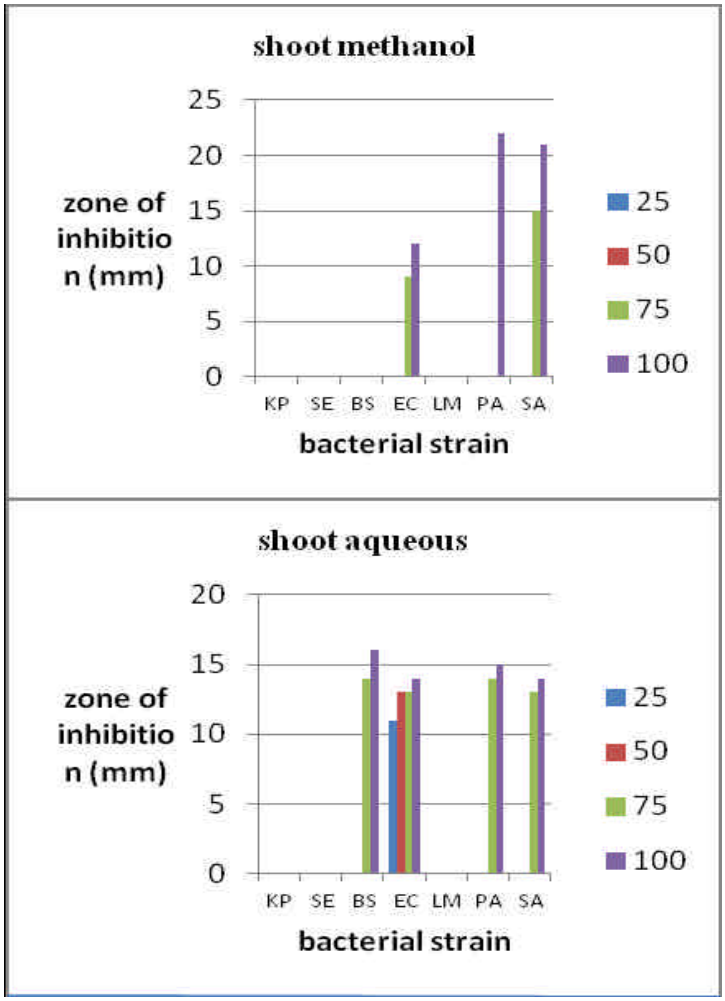
Table-2. Antimicrobial activity of different extracts and different parts of *N. arbor-tristis*

Plant parts	Bacterial Strain	Zone of inhibition (mm) Methanol				Aqueous			
		25	50	75	100	25	50	75	100
Leaf	KP	0	15	0	20	0	0	0	0
	SE	14	15	13	16	18	18	19	20
	BS	0	13	14	15	0	0	20	21
	EC	0	13	12	20	9	0	0	15
	LM	15	17	19	20	15	13	15	14
	PA	0	0	0	0	0	0	19	20
	SA	0	5	11	14	0	0	0	15
Root	KP	11	15	0	0	0	0	10	11
	SE	18	22	22	23	16	17	13	18
	BS	0	11	0	11	0	12	0	20
	EC	0	11	12	15	0	0	0	0
	LM	13	15	0	11	0	0	11	15
	PA	0	11	12	12	0	0	0	0
	SA	0	0	0	0	0	0	0	11
Shoot	KP	0	0	0	0	0	0	0	0
	SE	0	0	0	0	0	0	0	0
	BS	0	0	0	0	0	0	14	16
	EC	0	0	9	12	11	13	13	14
	LM	0	0	0	0	0	0	0	0
	PA	0	0	0	22	0	0	14	15
	SA	0	0	15	21	0	0	13	14

iii) Shoot

This part of plant *N. arbor-tristis* gave least antibacterial activity. Methanolic extracts of shoot showed zone of inhibition against *Staphylococcus aureus, Pseudomonas aeruginosa, and Escherichia coli* more in comparison with *Listeria monocytogens, Bacillus subtilis, Staphylococcus epidermidis* and *Klebsiella pneumoniae* (maximum zone of inhibition against *Pseudomonas aeruginosa* (22 mm).

iv) Aqueous extracts of shoot showed better antibacterial activity than methanolic extract against *Escherichia Coli, Bacillus subtilis, Pseudomonas aeruginosa and Staphylococcus aureus* (maximum zone of inhibition against *Bacillus subtilis*, 16 mm) whereas minimum zone of inhibition was observe against *Listeria monocytogens, Staphylococcus epidermidis* and *Klebsiella pneumoniae* (Graph-3). Among all the plant parts tested leaf responded best for its antimicrobial activity. So for further study different fractions of methanolic leaf extract have been taken.



Graph 3: Antibacterial activity of shoot extracts *N. arbor-tristis* against pathogenic bacterial strains

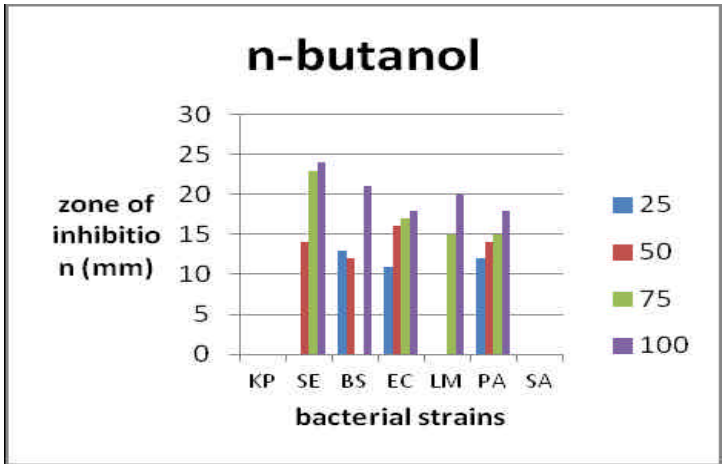
***3.3Antibacterial activity of different fractions of methanolic leaf extract of N. arbor-tristis* (Table-3) (Graph-4).**

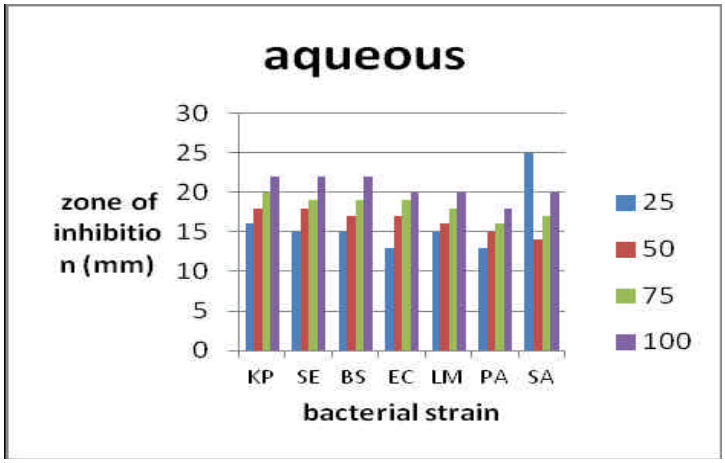
i) *n-Butanol*

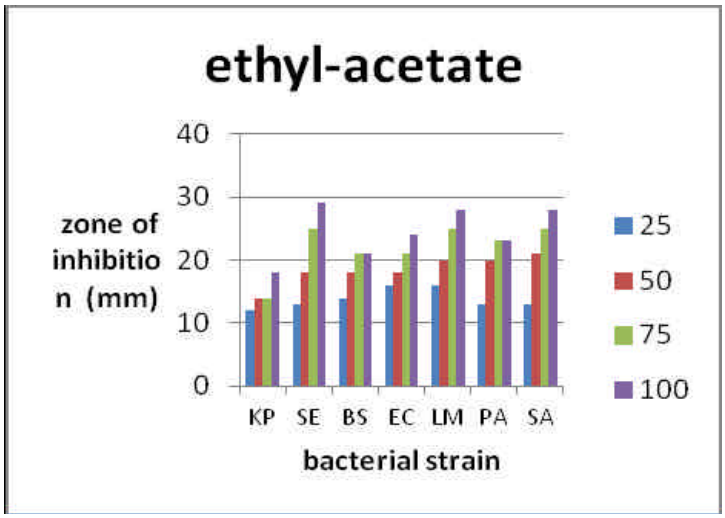
n- butanol fraction of methanolic leaf extract was showing high antimicrobial activity against 5 pathogenic bacteria (*Staphylococcus epidermidis, Bacillus subtilis, Escherichia coli, Listeria monocytogens, Pseudomonas aeruoginosa* and *Staphylococcus aureus*) out of seven tested pathogens. Maximum inhibition of growth

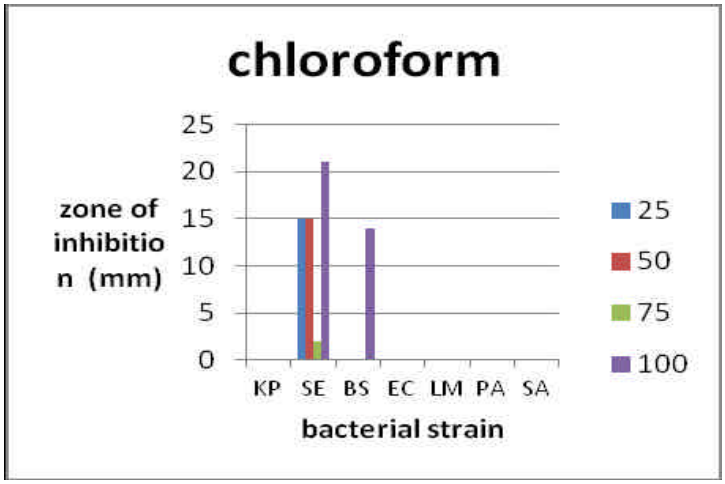
Table-3. Antimicrobial activity of different fractions of leaf extract *N. arbor-tristis*

Fractions	Bacterial strain	Zone of inhibition (mm) 25%	50%	75%	100%
n- Butanol	KP	0	0	0	0
	SE	0	14	23	24
	BS	13	12	0	21
	EC	11	16	17	18
	LM	0	0	15	20
	PA	12	14	15	18
	SA	0	0	0	0
Aqueous	KP	16	18	20	22
	SE	15	18	19	22
	BS	15	17	19	22
	EC	13	17	19	20
	LM	15	16	18	20
	PA	13	15	16	18
	SA	25	14	17	20
Ethyl acetate	KP	12	14	14	18
	SE	13	18	25	29
	BS	14	18	21	21
	EC	16	18	21	24
	LM	16	20	25	28
	PA	13	20	23	23
	SA	13	21	25	28
Chloroform	KP	0	0	0	0
	SE	15	15	2	21
	BS	0	0	0	14
	EC	0	0	0	0
	LM	0	0	0	0
	PA	0	0	0	0
	SA	0	0	0	0









Graph 4: Antibacterial activity of different fraction of methanolic leaf extract against pathogenic bacterial strains

was observed for *Staphylococcus epidermidis* (24 mm) with 75% & 100% concentrations. Growth of *Klebsiella pneumoniae* and *Staphylococcus aureus* was not affected by n-butanol fraction. (Fig-3)

Fig-3. Antibacterial activity of n-butanol fraction of methanolic leaf extract of *N. arbor-tristis* against LM and SE

ii) *Aqueous*

Aqueous fraction of methanolic leaf extract of *N. arbor-tristis* was showing strong activity against all pathogenic bacteria tested. It inhibits growth of pathogenic bacteria even on lower concentration (25%) applied. Maximum zone of inhibition was observed against *Klebsiella pneumoniae, Staphylococcus epidermidis, Bacillus Subtilis, Escherichia coli, Listeria monocytogens, Pseudomonas aeruginosa Staphylococcus aureus,* (maximum zone of inhibition against *Klebsiella pneumoniae, Staphylococcus epidermidis*, and *Bacillus subtilis* 22 mm). (Fig-4)

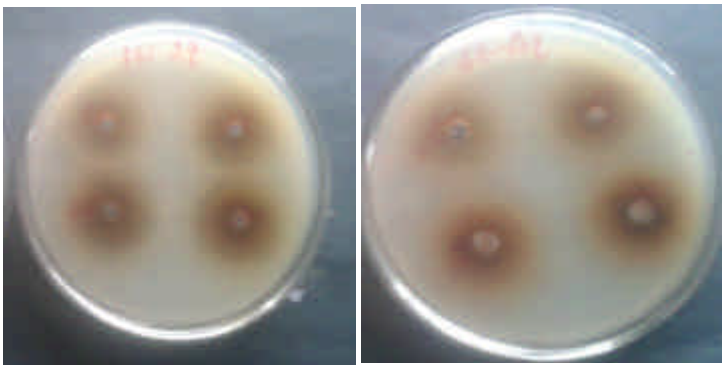


Fig-4. Antibacterial activity of aqueous fraction of methanolic leaf extract of *N. arbor-tristis* against SE and BS

iii) *Ethyl acetate*

Ethyl acetate fraction of methanolic leaf extract was also showing strong antibacterial activity against all the bacteria tested. It was observed that growth of *Staphylococcus epidermidis* (29 mm) affected highly by this fraction, whereas minimum zone of inhibition against *Klebsiella pneumoniae* was observed (Fig-5).

Fig-5. Antibacterial activity of ethyl acetate fraction of methanolic leaf extract of *N. arbor-tristis*

v) Chloroform

This fraction was showing least antibacterial activity. Growth of only two bacteria (*Staphylococcus epidermidis* and *Bacillus subtilis*) affected by this fraction. Maximum antibacterial activity was observed against *Staphylococcus epidermidis* (21 mm).

3.4 Identification of endophytic fungi

a) **Morphological characteristics of isolated fungi (Table-4)**

The cultures had sparse, cottony, white to creamy mycelium with abundant hypha furnished with pink background in the Petriplates (Figs. 6.1-6.3). Mycelium was consist of branched, septate hypha bearing 1-2 conidia at the aerial end. Hypha containing brown conidial masses produced in concentric rings on the colonies. Conidia (Fig. 6.4) borne on elongated phialides in acervular conidiomata, or, in early stages of development, on solitary fertile hyphae and appresoria. Conidia straight, cylindrical, obtuse at the apex, 9-24 x 3.0-4.5 µm. Appresoria 6-20 x 4-12 µm, irregular in shape.

Fig-6. Isolation of endophytic fungus from leaf of *N. arbor-tristis*

Fig- 6.1 Leaf pieces of *N. arbor-tristis* inoculated on PDA

Fig 6.2 Endophytic fungus colony

Fig- 6.3 Slide culture of endophytic fungus

Fig-6.4 Microscopic view of isolated fungus

b)Biomass production

The growth rate of isolated fungus was 0.1708g/day.

c) Enzymatic activity:

i) Amylolytic Activity: A fungus isolated from the leaves of *N. arbor-tristis* was showing strong amylolytic activity. Clear zone was 15 mm formed around the fungal colony.

i**i) Lipolytic Activity:** At the end of the incubation period, a visible precipitate around the colony was observed which was formed due

Table- 4. Morphological characteristics of isolated fungus

Features	Characters	Fungus features
Macroscopic	Color of cultured colonies	Creamy-pinkish
	Shapeof cultured colonies	Round
	growth of cultured colonies	Regular
	Growth rate	0.5 cm/day
	Growth media	PDA
	Back color	Pink
	Sporulation pattern	Conidia on conidiophores
	Conidiophores	2-4 conidia present terminally on each conidiophore
Microscopic	Structure of hypha	Septate
	Type of hypha	Branched
	Conidia	Straight cylindrical up to 2-4 apices
	Size	5.8µm
	Color	Medium to dark brown
	Conidial ends	Smooth round
	Appersoria shape	Regular in shape often with deep lobes

Table-5. Response for different enzymatic tests from isolated fungus

Enzymatic tests	Response
Amylolytic	+ (15mm, clear zone around fungal growth)
Lipolytic	+ (38mm, area of colony formed)
Pectinolytic	-
Laccase	-

to the formation of calcium Salts of the lauric acid liberated by the enzyme. Size of the fungal colony formed for this test was 38 mm, which is an indication of strong Lipolytic activity of isolated fungus. (Table-5)

iii) Proteolytic Activity: The isolated fungus was showing negative response for this test.

iv) Laccase Activity: The isolated fungus was showing negative response for this test.

On the basis of above morphological (macroscopic & microscopic) features and enzymatic assay of the isolated fungi, from the leaves of *N. arbor-tristis* is identified as a *Colletotrichum gleosporioides (Penz) Sacc.*

3. DISCUSSION

Plant revealed some differences in the constituents of the various parts of plant tested. From present study it is clear that flavonoid, saponins and terpenoids present in all extracts (methanol, water and acetone) of different plant parts i.e. leaf, shoot and root of *N. arbor-tristis*. Whereas anthraquinones was totally absent in all parts. Presence of these secondary metabolites have also been reported in hot water, ethanol, benzene, petroleum ether and chloroform extract of plant parts of *N. arbortristis*[10].

In present study a comparison was made for antibacterial activity of different plant parts of *N. arbor-tristis*. Different plant parts responded differently against various microbes according to the presence of types of secondary metabolites they possess. Leaf of *N. arbor-tristis* has been proved as a strong growth inhibitor of almost all pathogenic bacteria tested. So, further research towards the secondary metabolites advance present in the leaf of *N. arbor-tristis* may open new vistas in the field of microbiology. Antibacterial activity of different leaf extract was also reported against *Staphylococcus aureus, Escherichia Coli, Pseudomonas aeruginosa and Staphylococcus aureus* [11]. Antibacterial activity of root extract was lower in comparison with leaf extract. Whereas shoot extract showed least antibacterial activity. Pathogenic bacteria *Staphylococcus aureus* (Leading cause of skin and soft tissue infections such as abscesses (boils), furuncles, and cellulites), *Klebsiella pneumoniae* (Causes Pneumonia), *Escherichia coli* (Causes bacterial infections, including cholecystitis, bacteremia, cholangitis, urinary tract infection (UTI), and traveler's diarrhoea, and other clinical infections such as neonatal meningitis and pneumonia and causes severe food poisoning and contamination) and *Listeria monocytogens* (causes destruction of red blood cells and causes Listeriosis) were most susceptible for leaf extract.

The successful prediction of botanical compounds from plant material is largely dependent on the type of solvent used in the extraction procedure[12]. In the present study methanol, water and acetone extract of leaf, root and shoot exhibited varying degree of inhibitory effect against all pathogenic strains tested. Methanol and aqueous extract were more effective to inhibit the growth of pathogens. Whereas aqueous extract has been responded better than methanolic extract [13].

Among all plant parts leaf is the most valuable part of *N. arbor-tristis* .The decoction of leaves is widely used in ayurveda medicine for

treatment of various diseases [14]. Similarly in present study also leaf has been proved as most effective part against bacteria. It is a reservoir of different metabolites. For further study different fractions of methanolic crude extract have been taken to observe response of different soluble flavonoids separately. In this study it was observed that metabolites soluble in aqueous fraction as well as in organic solvents i.e. n-butanol and ethyl acetate were strongly effective against pathogenic bacterial strains. *Staphylococcus epidermidis* was most susceptible pathogen for these fractions.

Colletotrichum gloeosporioides obtained especially as symptomatic pathogens but can be found as asymptomatic endophytes. The genus has wide geographic distribution, being more important in the tropics. In the present study, we isolated endophytic fungi from leaves of medicinal tree called *N. arbor-tristis.* These endophytes were identified by morphological and enzymatic assay and identified as *Colletotrichum gloeosporioides* [15,16] Previously *Colletotrichum dematium* was also isolated from leaves of *N. arbortristis*[17]. *Colletotrichum* has also been isolated from leaves of *Ficus benghalensis* L. [18]

4. CONCLUSION

Leaf part of the plant *Nyctanthes arbor-tristis***.** has potential to suppress the growth of six pathogenic bacteria. Maximum antibacterial activity has been observed for ethyl acetate fraction of methanolic extract of leaf. Endophytic fungus isolated from the leaf of *N. arbortristis* was demonstrated as *Colletotrichum gleosporioides.* On the basis of data received from present work, it may be suggested that leaf extract of *Nyctanthes arbor-tristis* may become an alternative source of medicine against many pathogens in the world of pharmacy. A further study is required to observe the effect of metabolites of endophytic fungi present in the leaf, against pathogens.

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