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"Phytochemical Screening and Evaluation of Antibacterial & Antifungal Activity of *Terminalia Bellerica* Bark"

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ABSTRACT:

Terminalia Bellerica Roxb. knows as Bahera , belonging to the family Combretaceae of order Rosales , it is a large deciduous tree found all over in Asia, mostly native in Sri Lanka.it contains various phytoconstituents such as glycosides, flavonoids, gallyl glucose, tannins, gallic acid, ellagic acid, ethyl galate, chebulanic acid which is responsible for the various pharmacological activities like analgesic, antihypertensive, anticancer, antibacterial, antidiarrhoeal activity, antidiabetic activity. The present work conduct to investigate antimicrobial activity and phytochemical analysis of differentextract of bark of *Terminalia bellerica.*The *T. bellerica* bark extract were extracted by Soxhlet apparatus and maceration process by using distilled water, methanol and chloroform as solvent. The antibacterial and antifungal activity was evaluated using agar well diffusion method against the bacteria Staphylococcus aureus and fungi Aspergillus niger. It was observed that methanol extract exhibit significant activity against the tested bacterial and fungal isolates, compared with aqueous and chloroform extract respectively.From this study it can be concluded that bark extract of *Terminalia bellerica* shows effective antibacterial and antifungal activity and used in the search of new drugs and development of some promising formulation.

KEYWORDS: *Terminalia bellerica* , phytoconstituents , antibacterial activity , antifungal activity, zone of inhibition , agar diffusion method.

1.0 INTRODUCTION:

Nature is and will servers as main primary source for the cure of his aliment. However, the potential of higher plants as a source for new drug is not clearly explored [1]. It is estimated that, in many developing countries, two third of the population is dependent on medicinal plants to meet primary healthcare needs [2]. The use of herbal medicine is increasing due to its safety, efficacy and therapeutic potential as

compared to synthetic pharmaceutical products [3]. A report of the world Health Organization revealed that 80% of the world population in developing countriesdepend primarily on herbal medicines [4].

Natural product either in the form of pure compounds or as plant extract, provide ultimately opportunities for development of new drug species. They usually contain biological active substances i.e. secondary metabolites extracted from various parts of plants like roots, leaves, stem, fruits, flowers etc. are used primarily for treating mild or chronic ailments. In India 45,000 plants species have been identified out of which 15-20 thousand plants are good medicinal value [5].

Terminalia bellerica commonly known as Behera, belong to the family Combretaceae. It is abundant in tropical Asia. T.bellerica is found in deciduous forest through in India below the elevation of about 3000ft, except in dry and arid regions of Singh and Rajputhana[6]. The dried ripe fruit of Terminalia Bellerica Roxb (Combretaceae) has traditionally been used in the treatment of diarrhoea, cough, hoarseness of voice, eye diseases and scorpion-sting and as a hair tonic. A decoction of the fruit is used for treating cough and pulp of the fruit is useful in treating dysenteric-diarrhoea, dropsy, piles and leprosy Fruit and fruit extracts of T. bellerica have shown a range of pharmacological activities, including antidiabetic, analgesic,antiulcer, antifungal, antibacterial through in-vitro and in-vivo studies[7] . T.bellerica is one of the three ingredients of the well-known drug triphala, used routinely in Ayurvedic medicine to treat a wide variety of diseases. Triphala powder consist powder of three plant fruits Terminalia chebula, Terminalia Bellerica and Phyllanthusembelica ratio for Triphala is (1:1:1)[8].

T.bellerica contains different chemical constituents in different parts of plant such as bark contains arjungenin and its glycosides, belleric acid, bellericosides. Fruits contain hexahydroxydiphenic acid, methyl ester, β - sitosterol, gallic acid, ellagic acid, ethyl gallate, galloyl glucose, chebulagic acid, mannitol, glucose, galactose, and rhamnose. T. bellerica bark is mildly diuretic and useful in anaemia and leucoderma. Fruits are anti-inflammatory, antihelmintic, expectorant, antipyretic, antiemetic and useful in asthma and bronchitis, dropsy, dyspepsia, cardiac disorders, skin diseases, leprosy, ulcer.[9,10]Glucosides, tannins, galliacid, ellagic acid, ethyl gallate, gallylglucose, chebulanic acid are believed to be mainly responsible for its wide therapeutic actions

Fig 1: fruits and powder of *Terminilia bellerica*Fig 2: whole plant of *T.bellerica*[11]

It has anti-HIV-1, antimalarial and antifungal activity. It is used as antioxidant, antimicrobial, antidiarrheal, anticancer, antidiabetic, antihypertensive and hepatoprotective agent. It also possesses analgesic, antipyretic and anti- ulcerogenic effect and antimicrobial activity[9,12].

Many microbial diseases can be cured by medicinal plants without any side effects and economical issues[13]. Herbal medicines can be used as supplements with conventional medicines. Many medicinal plants are considered to be potential antimicrobial and antifungal crude drugs as well as source of novel compounds with antimicrobial and antifungal activity.

S.aureus a gram positive bacterium is a common cause of blood stream infection, skin and soft tissue infection and post influenza pneumonia. The efficacy of antibiotics in the control of this bacterium is fading because of the rapid emergence of MDR strains . The aqueous and methanol extract of T.bellerica fruit have shown antimicrobial activity against S.aureus , salmonella typhimurium, E.coli [14]. The crude extract of bark of behara have antibacterial activity against Streptococcus mutans[15].

Aspergillosis is an infection caused by Aspergillus , a common mold that lives indoors and outdoors. Most people with weakened immune system or lungs disease are at higher risk of developing health problems due to Aspergillus [16].

The present study has been undertaken the three extract viz. Aqueous, methanolic, chloroform extract of bark of *T.bellerica*evaluated it'sphytochemistry, ethnopharmacological effect and antibacterial and antifungal activity by agar well diffusion assay.

Why we use?

In India bahera mostly found in common locality and are easily available in market. They have fewer side effects and easily affordable. *T. bellerica* contains active constituent with antimicrobial and antifungal activity that can be used in novel drugs for the treatment of microbial diseases.

2.0 Distribution and habitat [17]:

It grows wild at an elevation of upto 2000m in wide variety of ecologies. It is native to Sri Lanka, India, Bangladesh, Bhutan, Thailand, China, Indonesia, Pakistan, Malaysia, Nepal, Cambodia and Vietnam. In India, it is commonly found in Madhya Pradesh, Uttar Pradesh, Punjab and Maharashtra. Ecology: It is mostly found in monsoon forests, mixed

2.1 Ecology: [18] : It is mostly found in monsoon forests, mixed deciduous forests or dry deciduous dipterocarp forests, associated with teak.

2.2 Biology [18] : It flowers in the month of October-November and fruits in November- December. The tree sheds leaves in November with young ones appearing together with flowers.

2.3 Biophysical limits Altitude [18]:0-2000m Mean annual rainfall: 900-3000 mm Mean annual temperature: 22-28°C Soil type. It grows well loamy fertile soil with good drainage.

2.4 Plant description [19]:

It is large deciduous tree with the height of 50m and diameter of 30m with a rounded crown. It is branchless upto 20m. It is perennial and requires cold climate.

2.4.1Bark: The outer bark is bluish or ashy-grey whereas inner bark is yellow in colour. The bark contains number of longitudinal cracks.

2.4.2 Leaves: Young leaves are copper red in colour which turn into parrot green and later they become dark green. Leaves are large, alternate, glabrous, with the dimension of 4-24cm x 2- 11cm, mostly clustered at the twig ends. Leaf tip is narrowly pointed.

2.4.3Flowers: They are greenish white in colour usually appear along with new leaves having an offensive odour or strong honey like smell. Inflorescence is axillary spikes generally 3-15cm long. Upper flowers of the spike are male. Lower flowers are bisexual. Calyx tube is sericeous or tomentulose.

2.4.4Fruit: It is light yellow in colour. It is drupe, globose or ovoid, densely velutinous or sericeous, 2-4 x 1.8-2.2 cm. It is slightly 5 ridged, 3cm across. It is one seeded and covered with minute pale pubescence.

Fig.3: Flowers of *T.Bellerica*[20]

Fig 4: leaves of *T.Bellerica*[21]

Fig 5: Bark of *T.Bellerica* [22]

Fig 6: Fruits of *T.Bellerca* [23]

Microscopy:[3]

The various microscopic al details that can be observed are hairs, stone cells, fibres, starch grains, xylem and calcium oxalate crystals.The details of which are as follows

Microscopic characters of powderof ***T.bellerica*:**

- Hairs :Length-168µm -91µm, Breadth -14-7µm
- Stonecells:Singlestone cellsLength -378-42µmBreadth-70-28µm

Group of stone cells Length–462µm-112µm Breadth-308-70µm.

- Fibres : Length-630µm-105µm ,Breadth-1.16µm
- Starch grains:Simple starch grains

Spherical with diameterran gingfrom35µm-14µm

Oval with the length of 31.5 - 28 µm and breadth of 28-21µm.Compound starch grains Diameter-85.9µm to 112µm

- Xylem:Spiral vessels with an average diameter of 17.5µmto70µm Pitted vessels with an average diameter of 27.13 µm to 92.16 µm Pitted trachieds.

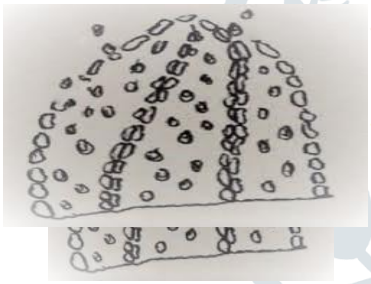


Fig7: Pitted xylem vessels present in powder *bellerica*[24]

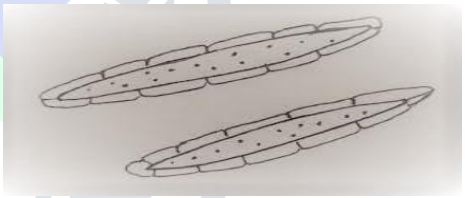


Fig8: Stone cells present in powder of of *T. T.bellerica*[24]

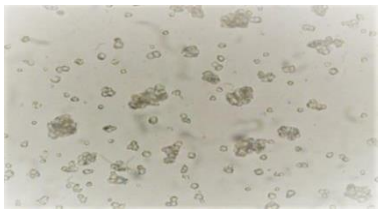


Fig9: Starch grain of *T.Bellerica*[24]

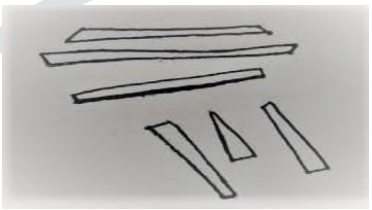


Fig 10: Needle shaped calcium oxalate

Crystal present in powder of *T. Bellerica*[24]

3.0 METHODS AND EXPERIMENTAL WORK METHODS:

Standardization of raw material:

3.1 Collection and Authentication: The bark of *Terminalia Bellerica* was collected during march-april 2021 from areas in an around Gondia district, Maharashatra. The bark was authenticated by Dr. V.I. Rane from Jagat Arts, Commerce and I.H.P. Science college Goregoan, Dist. Gondia. Department of Botany. The bark were washed thoroughly under running tab water for 2-3 times to remove dirt and then shade dried ,then grind into coarse powder with the help of mechanical grinder and store in air tight container.

Kingdom: Plantae

Division: Magnoliophyta

Class: Mangoliopsida

Order: Myrtales

Family : Combretaceae

Genus : Terminalia

Species: Terminalia Bellerica

4.0 Preparation of plant extracts:

4.1 Preparation by aqueous extraction of *T.Bellerica*[25]

T. Bellerica bark were washed, dried and then weighed. The bark is reduced to finely divided size by the process of grinding. For the preparation of aqueous extract, 10gm of the sample was added with 100ml of distilled water and kept in a shaker at 90-120 rpm for 24 hr. the extract were concentrated under the vaccum and dried.

Fig 11: Powder form of *T. Bellerica*

4.2 Preparation by Methanol of *T. Bellerica* [14]:

The powdered plant materials 25gm was packed in a cellulose thimble placed in the extraction tube of a soxhlet apparatus and extracted with methanol 250ml for 6hr.the extract were concentrated under the vaccum and dried.

Fig no 12: Soxhlet apparatus with sample

4.3Preparation by chloroform of *T. Bellerica* : [25]

The powdered plant materials 25 gm was packed in cellulose thimble placed in the extraction tube of a soxhlet apparatus and extracted with chloroform 250ml for 6hr. the extract were concentrated under the vaccum and dried.

Fig 13: Electrical water bath

5.0 Phytochemical Test: [25,26]

It involves various chemicals tests to identify different Phytochemical for example; alkaloids can be detected by Mayers reagent etc. observation and results of qualitative chemical constituents of *T. Bellerica* a combined knowledge of 175 Phytochemical screening.

5.1Chemical test for *T. Bellerica* extract are as follows:

1. Test for Alkaloid:

a. Dragandroff's test :

To 2.0 ml of filtrate plant drug extract, 2.0 ml of dragandrofff's reagent was mixed formation of orange brown precipitate was formed the presence of alkaloid.

b. Hager's Test :

To 2.0 ml of filtrate drug extract 2.0 ml of hangers reagent was mixed formation of yellow colour indicated the presence of alkaloid .

C. Mayer's Test;

To 2.0 ml of filtrate drug extract 2.0 ml of mayer's reagent was mixed. Formation of reddish brown colour indicated the presence of alkaloid.

d. Wagner's Test:

To 2.0 ml of filtrate drug extract 2.0 ml of wagner's reagent was mixed. Formation of reddish brown ppt indicated the presence of alkaloid.

2. Test for Flavonoid:

a. Schinoda Test:

To drug powder on extract add 5 ml 95% ethanol few drop conc. HCl and 0.5 gm magnesium turning . pinkcolour indicates present of flavonoid.

b. Lead acetate Test:

2 ml of extract is added to 2ml of of 10% lead acetate. Yellowish green colour indicates presence of flavonoid.

3. Test for Saponins:

2ml of extract is dissolved with 2 ml of Benedict's reagent. Blue black ppt indicates presence of saponins.

4. Tannins :

a. Iodine Test :

To extract and 2 ml dilute Iodine solution red colour indicates presence of Tannins.

b. Ferric chloride Test:

To 2ml of extract is treated with 0.1% ferric chloride brownish green layer indicates the presence of Tannins.

5. Phenols:

Extract treated with $fecl_3$ to give violet colour ppt indicates the presence of phenol

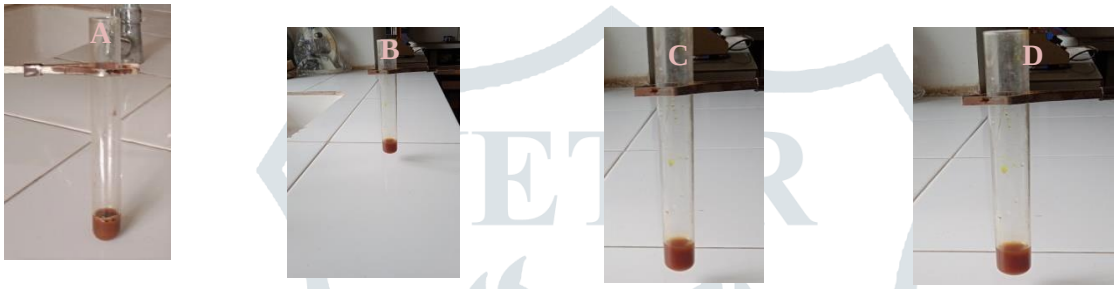


Fig.14: Dragandroff's Test **Fig.15:Mayer's Test** **Fig 16: Hagers Test** **Fig 17: Wagners Test**

Fig. 18:Aqueous extract: i)phenol test ii)flavonoid iii)saponin test iv)Iodinetest v)$fecl_3$ test **Fig 19: Methanolic extract i) Phenol ii) flavonoid iii) saponin iv)iodine v) Fecl3**

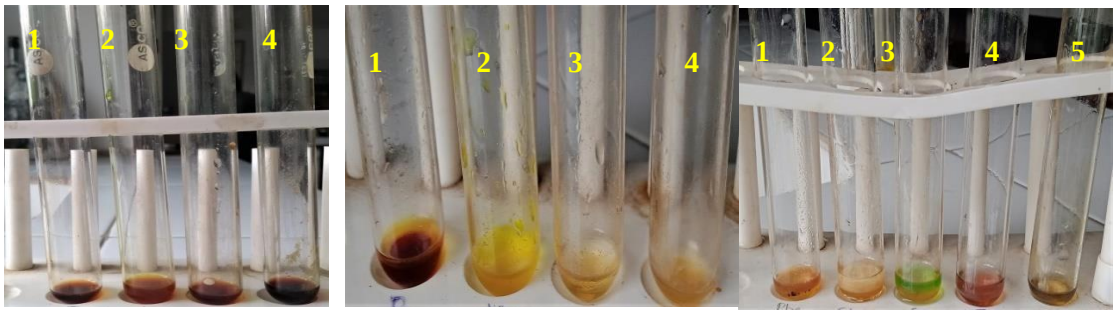


Fig. 20: Methanol extract i)Dragandroff's test **Fig. 21: Aqueous extract i)Dragandroff's test** **Fig. 22: Chloroform extract i)pheol test**

ii)Hager'stest	**ii) Hagers test**	**ii) flavonoid test**
iii)Mayer'stest	**iii)Mayer'stest**	**iii)saponin test**
iv)Wagner'stest	**iv)Wagner'stest**	**iv)iodine test v)$FeCl_3$test**

Table No 1: Preliminary Phytochemical screening of *T.Bellerica*:

Sr. no.	PlantConstituents	Test/Reagent	Aqueous extract	Methanol extract	Chloroformextract
1	Alkaloid	Dragendroff'sreagent	+	+	+
		Hager'stest	-	-	-
		Mayer'stest	+	-	-
		Wagner'stest	–	+	-
2	Flavonoids	Schinoda'stest	+	+	+
		Leadacetatetest	+	-	+
3	Saponin	2mlextract+2mlBenedictsreagent	-	+	-
4	Tannins	Iodinetest	+	+	+
		Ferricchloridetest	+	-	-
5	Phenols	Extract+$fecl_3$	+	+	+

Note:(+)indicatespresentand(-)indicates absence.

6.0 Thin layer chromatography: [26,27]

TLC is the method used for phytochemical screening of plant extract.it is the important analytical method for qualitative and quantitative analysis of number of phytochemical.

6.1. Test solution: Reflux the 0.4 gm powdered drug with 50ml methanol for 30 min cool and filter. Evaporate the filtrate to dryness. Dissolve the residue in 50 ml methanol.

6.2. Stationary phase: Silica gel G

6.3. Mobile phase: Ethyl: acetic acid [9:1]

6.4. Visualization of spots: Spray the plate alcoholic 5% ferric chloride reagent. Rf value was found to be 0.65.

Fig no 23: TLC plate

7.0 Antibacterial Activity:[14,25,26,28]

Antibacterial sensitivity testing of *T.bellerica* bark extract was performed by agar well diffusion method

7.1 Test organism: The strain Staphylococcus aureus (bacteria), Aspergillus niger (fungi) was obtained from D.B. Science College, Gondia.

7.2 Preparation of Inoculums:

Bacterial suspensions were prepared from overnight cultures by the direct colony method. Colonies were taken directly from the plate and suspended broth. These pre culture broth were allowed to stand overnight in a rotary shaker at 37°C, after which these cultures were maintained on broth in freeze for further use.

7.3Nutrient Medium:

Culture of the test organisms was maintained on sabouraud's dextrose agar medium and was subculture in petri plate to testing.

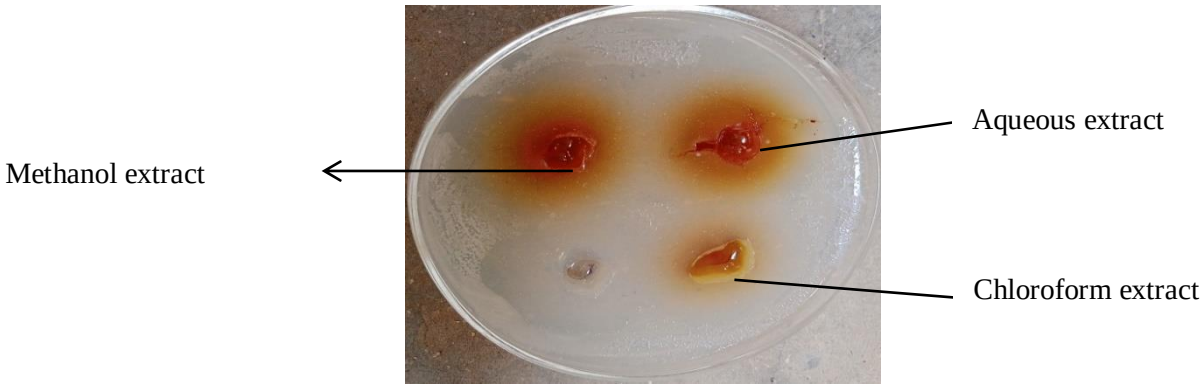
Composition of Sabouraud's Dextrose Agar Medium:

Table No 2: Composition of sabouraud's dextrose agar medium for antibacterial activity

Dextrose	10gm
Peptone	5gm
Agar	10gm
Distilledwaterto make	500ml

7.4 Disc Diffusion Method For Antimicrobial Activity:

The bark extract of *T.bellerica* was tested by well diffusion method for the detection of its antimicrobial activity. The sterilization of media was done by autoclaving at 121^0c under 15 lbs pressure for 15 min and the media was poured in petri plate under laminar flow with suitable sterile condition. 1ml of culture is placed in petri plate and sabouraud's agar was poured mix thoroughly and plates were allowed to solidify. Prepare well in plates by using cork borer. All extract were separately dissolved in 100% Dimethylsulfoxide (DMSO) solution with respect to 10 mg/ml. Plant extract of different solvent i.e. aqueous, methanol and chloroform added in labeled well and incubated at 370 c for 24 hours. The test material having antimicrobial activity inhibit the growth of microorganism and clear distinct zone of inhibition was seen around the well. The diameter of inhibited zone around each disc was measured.



Figno24:Antibacterial activity against S.aureus

Table No3: Antibacterial activity of the bark extract of *T.Bellerica*

	Zone of inhibition		
Extractofbarkof *T.bellerica*	Methanol	Chloroform	Aqueous
Staphylococcusaureus	20	16	19

8.0 Antifungal activity:[14,25,26,28]

Antifungal sensitivity testing of *T. bellerica* bark extract was performed by agar well diffusion method.

Test organism: The strain Aspergillus niger (fungi) was obtained from D.B. Science College, Gondia.

8.1 Preparation of Inoculums :

Fungal suspensions were prepared from overnight cultures by the direct colony method. Colonies were taken directly from the plate and suspended broth. These pre culture broth were allowed to stand overnight in a rotary shaker at 37°C, after which these cultures were maintained on broth in freeze for further use.

8.2: Composition of Sabouraud Dextrose Agar Medium :

Table No 4: Composition of sabouraud dextrose agar medium for antifungal activity

Dextrose	-10gm
Peptone	-5gm
Agar	-10gm
Distilledwatertomake	-500ml

8.3: Disc Diffussion Method for Antifungal Activity:

The bark extract of *T. Bellerica* was tested by well diffusion method for the detection of its antifungal activity. The sterilization of media was done by autoclaving at 121^0C under 15lbspressure for 15 min and the media was poured in petri plate under laminar flow with suitable sterile condition. 1ml of culture is placed in petri plate and sabouraud's agar was poured , mix thoroughly and plates were allowed to solidify. Prepare well in plates by using cork borer. All extract were separately dissolved in 100%Dimethyl sulfoxide (DMSO) solution with respect to10 mg/ml. Plant extract of different solvent i.e. aqueous, methanol and chloroform added in labeled well and incubated at 30^0 C for 24 hours. The test material having antifungal activity inhibit the growth of microorganism and clear distinct zone of inhibition was seen around the well. The diameter of inhibited zone around each disc was measured.

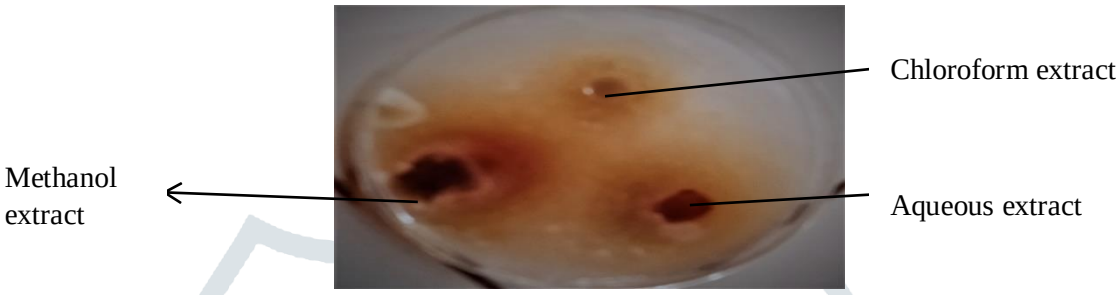


Fig 25: Antifungal activity against A.niger

Table No 5: Antifungal activity of the bark extract of *T.Bellerica*

	Zoneof inhibition		
Extract ofbarkof *T.Bellerica*	Methanol	Chloroform	Aqueous
Aspergillusniger	17	11	15

9.0 RESULT AND DISCUSSION:

Plants and humans are inseparable. From the thousands of years' plants are used as a source of herbal medicine. The important advantages of medicinal plants in various treatment as they safe, less expensive, efficacious and availability throughout the world.

Infectious disease has become the major cause and serious concern in public health issues. The occurrence of drugs drug resistance strain with less susceptibility to antibiotics due to is challenging amongst the researchers to invent new drug. The current study was made due to resistance development in bacteria and fungi to available drugs. Extract were active against bacterial and fungal isolates tested may indicate a broad spectrum of activity.The extracted plant antibiotics are safe, effective, and have no or little side effects. There are many antibacterial, antioxidant, antifungal, anticancer carry out on fruit of *T. bellerica*, which also important constituent of 'Triphala' formulation.

The present study is an attempt to evaluation of pharmacological activities such as antibacterial and antifungal of the extract of bark of T. bellerica. The photochemical analysis of *T.bellerica* showed that the bark contains alkaloids, flavonoids, saponins, tannins and phenols. These secondary metabolites are liable for their antibiotic and antifungal activities.

The photochemical alkaloid present in bark extract of *T.bellerica* might have inhibit the growth of microorganisms by impairing of enzymes used in energy production, interfering the cell membrane and structural components synthesis. The growth of fungus might have been inhibited due to the presence of phenol which might have induced the swelling., the plasma seeping and leakage, distortion and wrinkling of hyphae.

The antibacterial study was done by using Disc Diffusion Method. The extract of bark of behera of different solvents shows zone of inhibitions formed around the well indicates the extent of antibacterial

and antifungal activity. The methanol extract shows zone of inhibition against S.aureus of 20mm, aqueous extract shows zone of inhibition of 19mm and chloroform shows zone of inhibition of 16mm. The methanol extract is more potent against bacterial strain S. aureus then chloroform and aqueous.

The Antifungal study was done by using well diffusion method. It shows that zone of inhibition of methanol against A. Niger of 17 mm, aqueous extract shows zone of inhibition of 15mm and chloroform shows 11mm.

From this study it reveals that the bark extract of *T. bellerica* shows significant antibacterial and antifungal activity. Methanolic extract shows more inhibitory action than aqueous and chloroform extract. We can use the bark extract of bahera as an alterative to antibiotics.

10.0 CONCLUSION:

The plant extract used in present study exhibit the presence of various active components which shows antibacterial and antifungal activity which may be useful against different infections or diseases.

The antibacterial and antifungal activity of *T.bellirica* studies were done by using bark extract with different solvents. According to the results we can conclude that the bark extracts have antibacterial activity against S.aureus and antifungal effect against A.niger. Thus the *T.bellerica* is useful medicinal plants and its further assessment is important, which can help in discovery of new antibiotics and antifungal drug development in market.

The present work can also extended towards the development of compounds with antimicrobial properties that can be used innovel drugs for the treatment of antibacterial and antifungal diseases.

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