

Antimicrobial Activity of *Bridelia retusa* Against Human Pathogenic Microorganisms

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Abstract

Bridelia retusa is one of the essential medicinal plant have their extensive pharmacological properties. Extracts of *Bridelia retusa* possess some phyto-chemical components which can act against both bacteria and fungi. This situation has forced scientists to search new antimicrobial agents in selected plants. This is a monoecious, deciduous plant belonging to family Euphorbiaceae. By this experiment we observed that hydro-methanolic extract is the most effective as compared to the extract of water. Antimicrobial studies of each extracts of *Bridelia retusa* are performed which includes various disease causing pathogenic fungi. This experiment point out the presence of antimicrobial agent in selected medicinal plants.

Key words:- Medicinal Plants, *Bridelia retusa*, Antibacterial activity, antifungal activity.

1. Introduction

This is the commonest Indian species of *Bridelia*, obtained in dry deciduous to moist deciduous forests,

mixed forest, riverbanks, rocky places, up to 2000 m in South India, 600 m in central and Central-East India, 1600 m on Himalayas and 1000 m in North East India. Found throughout the country excluding Andaman and Nicobar Islands. *Bridelia retusa* is a small or moderate sized deciduous tree up to 7 m in height, armed with long conical thorns when young and having dark brown bark. Exfoliation is irregular flakes, lanceolate or ovate – lanceolate leaves, flowers present in long axillary or terminal spikes and greenish yellow fruits¹.

Plant medicine is still the mainstay of about 75-80% of World population, mainly in the developing countries for primary health care because of better cultural acceptability, better compatibility with human body and lesser side effects. The chemical phyto-constituents present in them are a part of the physiological function of living flora and hence they are believed to have better compatibility with human body and also showed various pharmacological effects²

Scientific classification

<u>Kingdom</u>	<u>Plantae</u>
<u>Order</u>	<u>Angiosperms</u>
<u>Family</u>	<u>Malpighiales</u>
<u>Genus</u>	<u>Bridelia</u>
<u>Specie</u>	<u>B. Retusa</u>



The Indian subcontinent is enriched by several flora-both aromatic and medicinal plants. This extensive flora has been greatly used as a source of many drugs in the Indian traditional system of therapy. In India, the earliest mention of the use of medicinal plants is to be found in Rigveda which was written between 4500-1600 BC³.

Medicinal values³

Bridelia retusa is used in Ayurveda and Siddha medicines.

- **young leaves** are chewed for the treatment of mouth ulcer.
- **Leaves** are warmed and applied on swellings.

- **Leaves paste** is applied in conjunctivitis.
- **Fruit** is very astringent and a decoction is given as an emmenagogue.
- **Root decoction** is taken orally in dysentery and abdominal pain.
- **Stem bark** is antiviral, hypoglycemic, hypersensitive, hypotensive, anthelmintic, astringent.
- It is also used as a postpartum remedy and for tuberculosis.
- **Powdered stem bark** is given with water in abdominal pain.
- **Bark juice** is given to treat peptic ulcer.
- **Pounded bark mixed with oil** is applied externally in rheumatism and wounds.

2. Material & methods

Collection of Material: Plant material (leaves) was collected from the place called Chitrakoot satna district, Madhya Pradesh. The material was air dried, chopped into pieces and pulverized in grinder and stored in closed plastic containers.

Preparation of extracts: Hydro-methanolic extracts were prepared from previously dried and powdered plant material by Soxhlet extraction method. About 300g of powdered leaf was extracted until drug exhaustion. The extract obtained were evaporated to yield semisolid material which was completely dried in desiccators in vacuum to yield 85.5g. The extracts were stored in closed plastic containers at 4°C temperature.

Preliminary Phytochemical Screening⁴: The wide spectrum antimicrobial activity of leaf extracts of *Bridelia retusa* as shown in the table no. 1 indicates the presence of biologically active compounds in the leaf extract. Therefore, preliminary chemical analysis of the leaf extract of *Bridelia retusa* was carried out. Different standard tests were performed to find out the active components.

Test for Alkaloids

Mayer's test: 1ml of sample was added to a few drops of Mayer's reagent. Formation of white or pale yellow precipitate indicates the presence of alkaloids in the sample.

Wagner's test: 1.5% of HCl was added in 1 ml of extract and a few drops of Wagner's reagent were added to it. Appearance of yellow/ brown precipitate indicates the presence of alkaloids.

Dragendroff test: 5ml of distilled water was added to the 2 ml of sample, then 2M HCl and 1 ml of

Dragondrof's reagent was added. Orange / orange red precipitate indicates the presence of alkaloids.

Test for Carbohydrates

Molisch test: 1 ml of sample is placed in a test tube and two drops of Molisch reagent was added. 2ml solution of concentrated H₂SO₄ was added in test tube. Formation of Red violet ring in the interface gave the positive Molisch test.

Fehling test: 2ml solution of Fehling A and Fehling B were taken in a test tube then dropwise sample were added. The mixture was shaken well and kept in a water bath for 10-15 minuts at 100 :C. A rusty brown or brick red colour precipitate confirms the presense of carbohydrates in the sample.

Anthron test: 2 ml of anthron reagent was added to 500 µl of extract. Formation of green blue colour gives a positive anthron test.

Test for Glycosides

To 1 ml of plant extract, 1 ml FeCl₃ (5%), and equal amount of acetic acid is added, then few drops of H₂SO₄ is added to the mixture. Greenish blue colour indicates the presence of glycosides.

Test for Steroids

Salvoski test: 1 ml of test sample was dissolved in 1 ml of chloroform and equal amount of concentrated H₂SO₄. Formation of Bluish red to cherry colour in chloroform layer shows the presence of steroids.

Test for phenols

1ml of plant extract, when treated with few drops of FeCl₃ solution; it gives blue green colour and confirms the presence of phenols.

Test for Proteins

Biuret test: 1% of NaoH was added to 1 ml of extract and few drops of 1% CuSO₄ were then added. Blue/ purple or violet/ pinkish colour indicates the presence of proteins.

Millon's test: 1 ml of test extract was mixed with H₂SO₄ then Millon's reagent was added dropwise. White/ yellow precipitate appears which turns into red colour precipitate, after heating the mixture. This indicates the presence of proteins.

Ninhydrin's test: 2 drops of freshly prepared Ninhydrin reagent (0.1% in n- butanol) is added to 1ml of extract and heat and observed for blue or red orange colour.

Test organisms: The test microorganisms used in this study included *S. aureus*, *Streptococcus* Sp. and *Bacilus subtilis*, *E.coli*, *Shigella dysenterae*, *Salmonella typhi*, *Pseudomonas aeruginosa* and *K. pneumonia*. *Aspergillus niger*, *Penicillum sp.* ,

Helmintho sporium sp., *Fusarium sp.* , *Collectotrichum sp.*, *Rhizoctonia sp.* were maintained on SDA at pH 5.5 to 6.

Antimicrobial Testing: Antimicrobial activity of leaf extract of *B. retusa* was determined by agar well diffusion method. The extract obtained by successive extraction of leaves by Soxhlet method were used for antimicrobial testing. Stock solutions of the crude extract was prepared by suspending 200mg of extract (DMSO+ Tris mixture). Further dilutions were made with sterile distilled water. Antimicrobial activity was determined by agar well diffusion method⁵ (Perez et al, 1990). Activity was measured in terms of zone of inhibition in mm around the well. The results obtained are shown in graphical mode.

3. Result & Discussion

The preliminary phytochemical tests of extracts were carried out method prescribed by Tripathi et al.⁴ (2017).The results of preliminary test are tabulated in Table no 1.

Table no 1:-

S. No	Phytochemicals	<i>B. retusa</i> (leaves)				
		Pe	Be	Chl	Et	Wt
1	Alkaloids	+	+	-	-	-
2	Carbohydrates	-	-	-	+	+
3	Glycosides	-	-	+	+	+
4	Phytosteroids	+	+	-	+	+
5	Phenolic compounds	-	-	-	+	+
6	Proteins	-	-	-	-	+

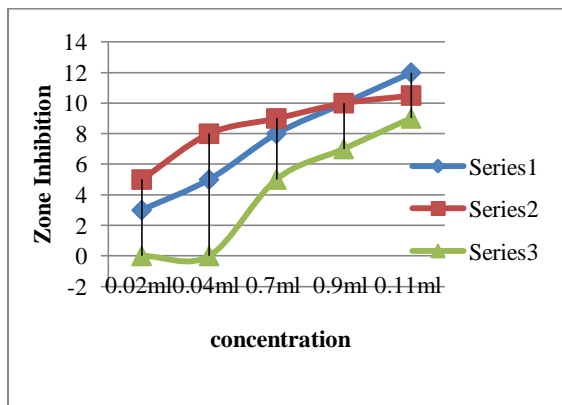
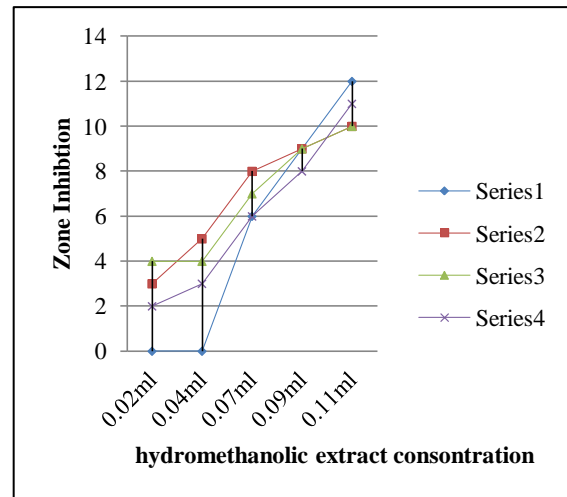
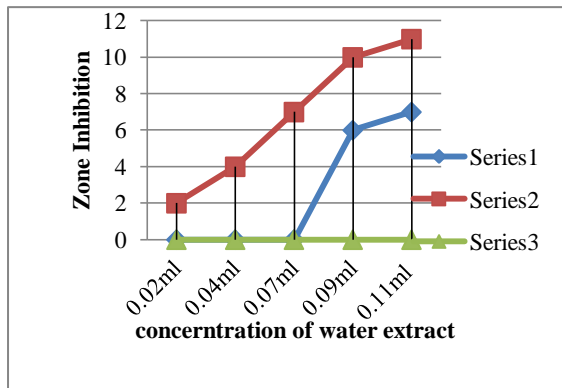
Pe; Petroleum ether extract, Be; Benzene extract, Chl; Chloroform extract, Et; Ethanol extract, Wt; water extract

The table clearly shows that presence of various phytochemicals in differect extracts of *B. retusa* leaves.. The extract of Soxhlet method shows the greater extraction because on addition of reagents it produces the intense color.

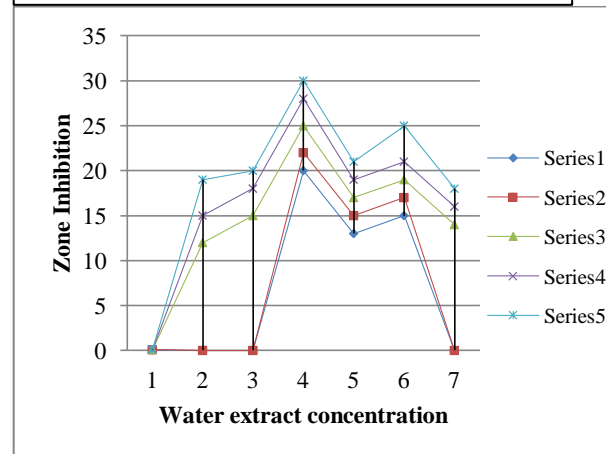
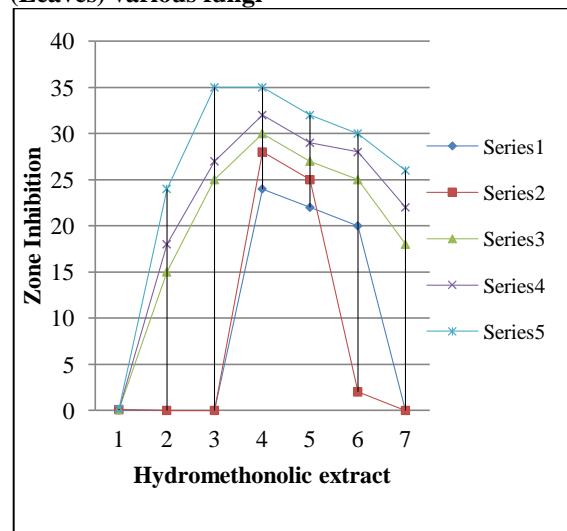
Antimicrobial activity

Zone inhibition

- a) **Antimicrobial testing of each extracts of *B. retusa* (Leaves) against Gram Positive Bacteria.**



c) Antifungal testing of each extract of *B. retusa* (Leaves) various fungi



Results of present antimicrobial investigation were presented in graph 1,2,3,4 & 5. It was found that hydro-methanolic extract of *B. retusa* showed significant antimicrobial activity against gram positive bacteria *S. aureus* with inhibition zone 12.1 mm at the concentration of 0.12 ml, whilst the extract showed inhibition against *Streptococcus Sp*, *Bacillus subtilis* and also for selected gram negative bacteria. When the concentration of the extracts was decreased, slight decrease in inhibition zone was observed. *B. retusa* also represent moderate inhibitory activity for fungal colonies.

b) **Antimicrobial testing of each extracts of *B.retusa* (Leaves) against Gram Negative Bacteria**

4. Conclusion

Our experimental results provide further information regarding the organic solvent utilized for the extraction of plants. It was highlighted in our study that the Hydro-methanolic and water extracts of *B.*

retusa showed remarkable antibacterial effect against gram positive and gram negative bacteria and fungus. Hence, it may be recommended that this plant could be used in the treatment of human infectious diseases caused by the previously mentioned organisms. As *B. retusa* is an important medicinal plant and very little work has been done on this aspect, these findings will be new in research field in area of medical microbiology (mycology).

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