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# Phytochemical Screening and Antibacterial Activity of *Homonoia Retusa* (Graham ex Wight) Müll. Arg.

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## ABSTRACT

Our current study evaluated the leaf and barks of *Homonoia retusa* (Graham ex Wight) Müll. Arg. to screen the phytochemical constituents and the antimicrobial potentiality of this plant species. Powdered leaf and bark were subjected to the Soxhlet extraction process by using Ethanol and water as solvents, obtained crude drug was used for the screening of secondary metabolites and antimicrobial capacity. Tannins and Phenols, Saponins and Phytosterols were detected in all four extracts. The antibacterial efficiency of *Homonoia retusa* leaf and bark ethanolic extracts was studied on pathogens *Pseudomonas aeruginosa* and *Staphylococcus aureus* in different concentrations. The maximum zone of inhibition by *Pseudomonas aeruginosa* (6mm & 5mm) was observed at 120 µg/ml in leaf and bark extracts respectively. The bark extract showed a maximum zone of inhibition 6mm against *Staphylococcus aureus* at 120 µg/ml and the leaf showed a 5mm zone of inhibition in a similar concentration.

***KEYWORDS:*** *Homonoia retusa; Secondary metabolites; Phytomedicines; Soxhelet Extraction*

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## INTRODUCTION

Plants have formed the basis of sophisticated traditional medicine systems among which are Ayurvedic, Unani, and Chinese amongst others. These systems of medicine have given rise to some important drugs still in use today. The traditions of yesterday are the drugs of tomorrow (Gurib-Fakim, 2006). The traditional medicine system of AYUSH (Ayurveda, Yoga, Unani, Siddha, and Homeopathy) in the healthcare sector was popularized by India through global networks (Shakya, 2016). The various medicinal plant extracts and their secondary metabolites have served as antioxidants to protect against various diseases for a long ago (Nile & Khobragade, 2009).

The plants of the family Euphorbiaceae are traditionally used to cure several diseases. *Homonoia riparia* Lour. is well known for its medicinal properties. The powdered root of the plant is laxative, diuretic and emetic. A decoction of the root is given for piles, stones in bladder, chest pain, gonorrhoea and syphilis. Powdered leaves and fruits are applied as poultice for skin diseases (Bapat & Mhapsekar, 2014). The genus *Homonoia* Lour. which belongs to the family Euphorbiaceae. Globally, the genus Homonoia Lour. comprises three species, *H. intermedia*, *H. retusa* and *H. riparia*. (Paradesi, *et al*., 2020).

*Homonoia retusa* is typically found in the tropics, west coast semi-evergreen and Southern moist mixed deciduous forests, mostly seen along the banks of streams and rocky places. A shrub known as 'Pashanabheda' in Ayurveda. *Pashanabedha* is a derived word from 'pashana' meaning a stone and 'bhedha' means to break. This meaning is attributed to the drug since it is claimed to possess the property of disintegrating the calculi or stones in the bladder as well as in the Kidney (Kumar, *et al*., 2010). Root is laxative and diuretic. Decoction of the root is used in the treatment of piles, stones in the bladder, gonorrhoea and syphilis. The root is used against

ulcers and vesical calculi (Pattasseril, *et al*., 2015). It has been long used in traditional medicines to treat a broad range of illnesses, i.e., Malaria, bladder stone, urinary discharge, inflammation, ulcer, uterine disorders and blood disorders. Additionally, the plant's roots have emetic, anti-urolithiasis, diuretic, and laxative properties. The fruits, flowers and leaves are used to treat inflammation, cuts, antibacterial, antifungal, anticancer and dermatitis (Porika and Reddy, 2022).

## Materials and Methods

### Collection

*Homonoia retusa* (Graham ex Wight) Müll. Arg. is a riparian shrub located along the bank of rivers, it was collected from the bank of river Tungabhadra, Udagatti village (14˚42'08.0" N 75˚44'24.8" E), Ranebennur taluk, Haveri district, Karnataka. Fresh plant materials (twigs) were collected sustainably brought to the laboratory and washed with water. Further, the leaves were separated, and bark was peeled off from the fresh plant materials were air dried under shade to remove moisture. After drying then blended into fine powder. The powder was stored in airtight containers for further use. About 200 g of dried leaf and bark material was crudely powdered and subjected to extraction by a Soxhlet extractor. The extraction was done using ethanol and water as solvents. All the extracts were concentrated by rotary vacuum evaporator (Super Fit – 2.0 model) and the left-over solvent was evaporated to dryness using a water bath.

### Preliminary Phytochemical analysis

The phytochemical analysis was carried out by using ethanolic and aqueous extracts of leaf and bark and qualitatively tested for different phytochemical constituents namely Carbohydrates, Alkaloids, Flavonoids, Coumarins, Anthocyanins, Emodin, Proteins and amino acids, Saponins, Cardiac-glycosides, Gums and Mucilage, Tannins and Phenols, Terpenoids, Diterpenes, Triterpenes and Phytosterols by following the standard procedures followed by standard procedures (Deepti, *et al*., 2012; Shaikh & Patil, 2020).

### Antibacterial activity

The *Homonoia retusa* leaf and bark ethanolic extracts were resuspended in distilled water and tested for antibacterial activity against pathogens Gram-negative (*Pseudomonas aeruginosa)* and Gram-positive (*Staphylococcus aureus*) on nutrient agar medium respectively, using the agar well diffusion method. The stock suspension was prepared and different volumes like 30, 60, 90 and 120 µl of stock suspension were poured in their respective wells. Appropriate volumes of positive controls such as, streptomycin (antibacterial) were used along with distilled water as a negative control. After the incubation time of 24 hours at 37°C, the inhibition zones were measured and recorded in mm using a standard antibiotic zone scale (Hudzicki, 2009; Aritonang, *et al*., 2019).

## Results and Discussion

### Preliminary phytochemical screening:

**Table. 1: Preliminary phytochemical screening of bark and leaf extracts of *Homonoia retusa* (Graham ex Wight) Müll. Arg.**

| Constituents | Tests | Leaf extracts | | Bark extracts | |
|---|---|---|---|---|---|
| | | Ethanolic | Aqueous | Ethanolic | Aqueous |
| Carbohydrates | Molisch's | + | + | + | + |
| | Benedict's | + | - | + | - |
| | Fehling's | + | - | + | - |
| Alkaloids | Dragendroff's | + | - | + | - |
| | Mayer's | - | - | - | - |
| | Hager's | - | - | - | - |
| | Wagner's | - | - | - | - |
| Flavonoids | Shinoda | + | - | + | - |
| Coumarins | Alkaline | + | + | + | - |
| Anthocyanins | HCl | - | - | - | - |
| Emodin | Emodin test | - | - | - | - |
| Proteins and amino acids | Xanthoproteic | + | - | + | - |
| Saponins | Froth formation | + | + | + | + |
| Cardiac-glycosides | Keller - Killani | - | - | - | - |
| | Baljet's | - | - | - | - |

| | | | | | |
|---|---|---|---|---|---|
| Gums and Mucilage | Alcohol | - | - | - | - |
| Tannins and Phenols | $FeCl_3$ | + | + | + | + |
| | Gelatin | + | - | + | - |
| Terpenoids | Test - 1 | - | - | - | - |
| Diterpenes | Test - 2 | - | - | - | - |
| Triterpenes | Test - 3 | - | - | - | - |
| Phytosterols | Salkowski's | + | + | + | + |
| | Hesse's response | + | + | + | + |

+: Present; -: Absent

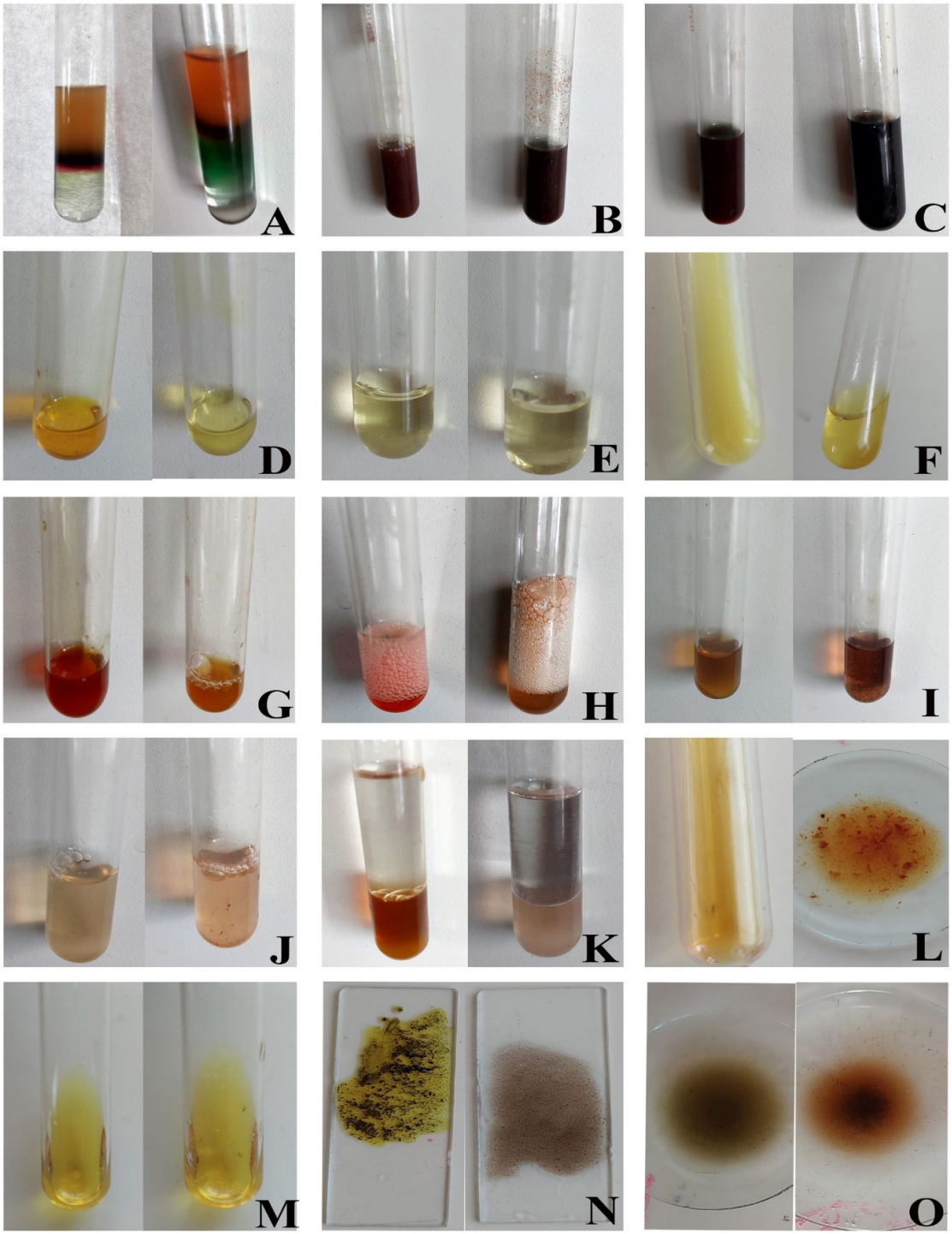


**Figure 1. Preliminary Phytochemical Screening of *H. retusa:* Ethanolic extracts:** Carbohydrates: **A.** Molisch`s test; **B.** Benedict`s test; **C.** Fehling`s test; Alkaloids: **D.** Dragendroff`s test; **E.** Mayer`s test; **F.** Hager`s test; **G.** Wagner`s test; Flavonoids: **H.** Shinoda test; Coumarins: **I.** Alkaline test; Anthocyanins: **J.**

HCl test; K. Emodin test; **L.** Xanthoproteic test; Cardiac Glycoside: **M**. Keller-Kilian test; **N.** Baljet`s test, **O.** Gums & Mucilage test.

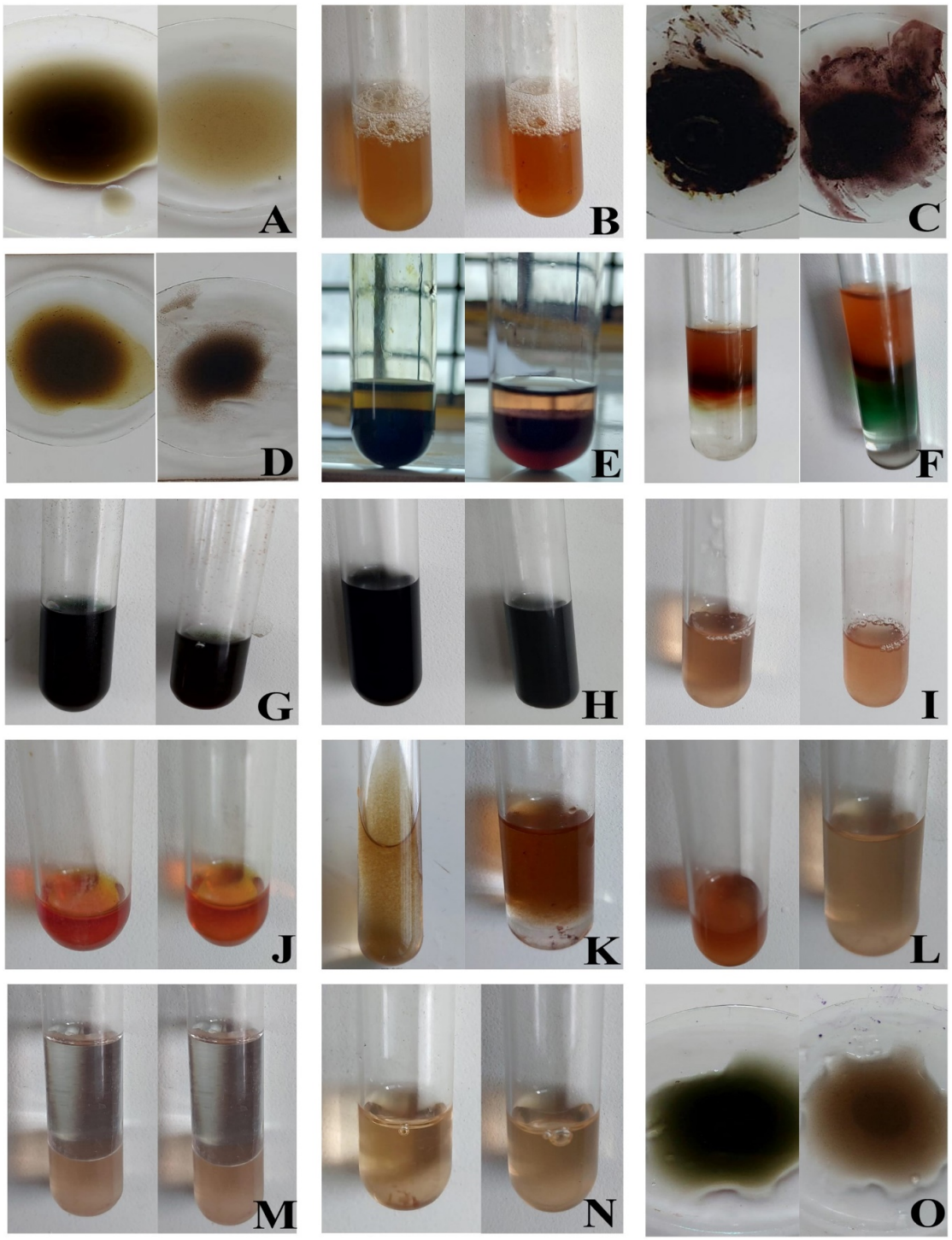


**Figure 2. Preliminary Phytochemical Screening of *H. retusa:* Ethanolic extracts:** Tannins & Phenols **A.** $FeCl_3$ test; **B**. Gelatin test; **C**. Terpenoids test; **D.** Diterpenes test; Phytosterols: **E.** Hesse`s response; **Aqueous Extract**: Carbohydrates: **F.** Molisch`s test; **G.** Benedict`s test; **H**. Fehling`s test; Alkaloids: **I.** Mayer`s test; J. Wagner`s, test; **K.** Coumarins; **L**. Anthocyanins, **M.** Emodin test; **N**. Xanthoproteic test; Tannins & Phenols: **O.** $FeCl_3$ test.

Preliminary screening of *H. retusa* leaf and bark extracts (ethanol and aqueous) showed the presence of a diversity of phytochemical constituents. Tannins and Phenols, Saponins, and Phytosterols were detected in all four extracts (**Fig. 1 & 2**). Coumarins were present in all other extracts except the aqueous bark extract. For the detection of carbohydrates, Molisch's, Benedict's, and Fehling's tests were conducted in ethanolic extracts both leaf and barks showed their presence. In alkaloid detection, only Dragendroff's test of ethanolic extracts bark and leaf showed a positive response, while in others no response. The Shinoda test was conducted for the

detection of flavonoids only in ethanolic extracts. Alkaline test conducted for detection of Coumarins showed their presence in both extracts of leaf and absence in aqueous extract of bark. Xanthoproteic tests conducted for the detection of proteins and amino acids were present in only ethanolic extracts. The froth formation test to detect saponins resulted in the presence of all extracts. In the $FeCl_3$ test for the detection of Tannins and Phenols, all the extracts showed presence. In the Gelatin test, only ethanolic extracts showed their presence. Salkowski's and Hesse's responses conducted for the detection of Phytosterols showed their presence in all extracts. Anthocyanins (HCl) and Emodin, Cardiac-glycosides (Keller–Kiliani & Baljet's test), Gums and Mucilage (Alcohol) Terpenoids, Diterpenes, Triterpenes were absent in all extracts **(Table 1).**

**Antibacterial activity**

The antibacterial efficiency of *Homonoia retusa* leaf and bark ethanolic extracts was studied on pathogens *Pseudomonas aeruginosa* and *Staphylococcus aureus* **(Fig. 3).** The inhibition zones formed by *H. retusa* leaf and bark extracts in different concentrations (30, 60, 90 and 120 µg/ml) against *Pseudomonas aeruginosa* were recorded in **Table 2**. The maximum zone of inhibition 6mm & 5mm was observed at 120 µg/ml in leaf and bark extracts respectively. The bark extract showed a maximum zone of inhibition 6mm against *Staphylococcus aureus* at 120 µg/ml and the leaf showed a 5mm zone of inhibition in a similar concentration **Table 3.**

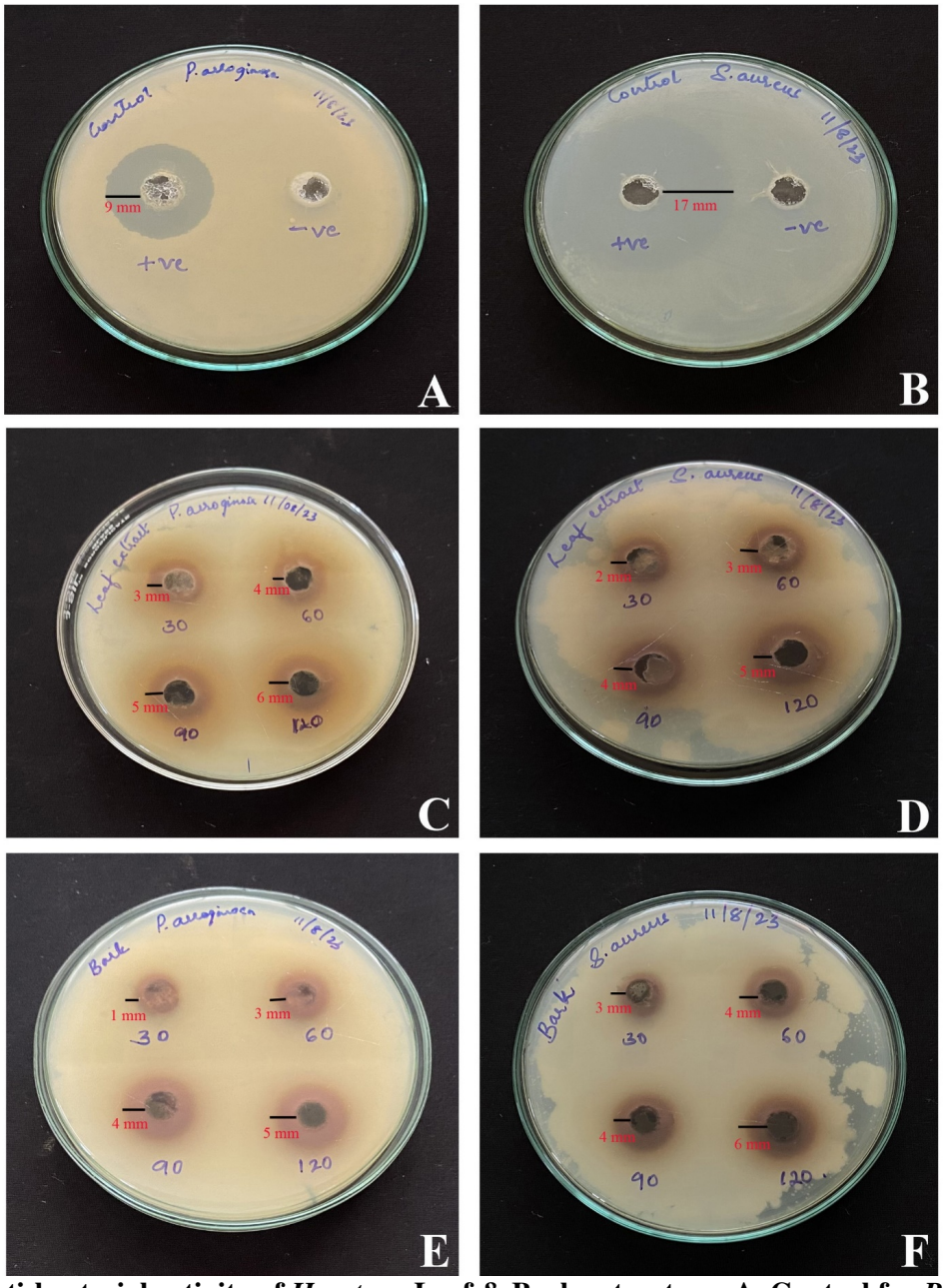


**Figure 3. Anti-bacterial activity of *H. retusa* Leaf & Bark extracts on A. Control for *Pseudomonas aeruginosa*, C. & E. Leaf & Bark extracts activity against *P. aeroginosa*: B. Control for *Staphylococcus aureus,* D. & F. Leaf & Bark extracts activity against *S. aureus***

**Table 02. Determination of the antibacterial activity of *H. retusa* leaf and bark ethanolic extracts against *Pseudomonas aeruginosa*.**

| Concentration in µg/ml | 30 | 60 | 90 | 120 |
|---|---|---|---|---|
| Leaf extract | 3mm | 4mm | 5mm | 6mm |
| Bark extracts | 1mm | 3mm | 4mm | 5mm |

**Table 03. Determination of the antibacterial activity of *H. retusa* leaf and bark ethanolic extracts against *Staphylococcus aureus*.**

| Concentration in µg/ml | 30 | 60 | 90 | 120 |
|---|---|---|---|---|
| Leaf extract | 2mm | 3mm | 4mm | 5mm |
| Bark extracts | 3mm | 4mm | 4mm | 6mm |

Antibacterial activity of *Morinda tinctoria* Roxb. leaf ethyl acetate extracts showed a maximum zone of inhibition of 20 mm against *Staphylococcus aureus* and Chloroform extract of leaf showed their maximum zone of inhibition of 15 mm against *Pseudomonas auregenosa* (Deepti, *et al*., 2012). Antibacterial activity of fresh and dry *H. riparia* entire plant, root, leaves, male flowers, and fruit extracts using different solvents, the highest zone of inhibition of 11 mm and 12 mm in dry and fresh male flowers respectively in DMSO extracts was observed against *Staphylococcus aureus* (Bapat and Mhapsekar, 2014). The aqueous and isopropanol extracts of different parts concluded that *E. hirta* was highly effective to growth inhibition against *E. coli*, *S. aureus* and *K. pneumonia*. It is suggested that different parts of this plant have great potential efficacy against different human pathogenic diseases (Ali, *et al.,* 2023).

## CONCLUSION

It was observed that the plant *Homonoia retusa* contains a wide variety of secondary metabolites like Saponins, Tannins and Phenols, Phytosterols, and Carbohydrates. Antibacterial capacity study showed that leaf extract was effective against *Pseudomonas aeruginosa* and bark extract was effective against *Staphylococcus aureus,* which gives scientific evidence to conduct further studies and to investigate the lead compounds present in the plant and evaluate its potentiality *in vivo* animal models and put forward an attempt to carry out trials on human beings.

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