



## Antimicrobial activity of methanolic & aqueous extracts of leaves & young stem bark of *Holoptelea Integrifolia* L.

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### Abstract

*Holoptelea integrifolia* L. belongs to family Urticaceae. Plants have a great potential to develop new drugs that would greatly benefit mankind and plant medicinal properties based on the existence of the specific physiologically active component contributing to the discovery for new biologically active therapeutic efficacy principles. The present study was carried out to evaluate the antimicrobial efficiency of methanolic and aqueous extract of leaves and young stem bark of *Holoptelea integrifolia* L. against the microorganisms *Staphylococcus aureus* bacteria and *Penicillium notatum* fungus. Aqueous and methanolic extracts concentrations (5%, 10%, 15% and 20%) were prepared from the leaves and young stem bark of *Holoptelea integrifolia*. The antimicrobial efficiency of the aqueous and methanolic extract concentration of each plant was tested using agar well diffusion method and zone of inhibition zone was measured in millimeters. Methanolic and aqueous extract of leaves and young stem bark showed positive results against *Staphylococcus aureus* from 5% to 20% concentration. 11mm zone of inhibition at 15% concentration for aqueous leaves extract, 11mm zone of inhibition at 15% and 20% concentration for methanolic leaves extract, 8.5mm zone of inhibition at 5% concentration, 9.5mm at 10% concentration, 9.5mm zone of inhibition at 15% concentration and 11mm zone of inhibition at 20% concentration of young stem bark of aqueous extract. Methanolic Young stem bark extract showed negative results. Methanolic and aqueous extract of leaves and young stem bark showed positive results against *Penicillium notatum* fungus from 5% to 20% concentration. The results obtained showed that the diameter of zone of inhibition increased with increase in concentration of extract and the antibacterial efficiency of aqueous and methanolic extracts of the plant was observed in the increasing order.

**Keywords:** Antimicrobial, Methanolic, *Holoptelea Integrifolia* L.

### 1. Introduction

Evidence of the use of plants for medicinal purpose dates some 60,000 years back in both western and eastern cultures. Medicine derived from plants have been a part of our traditional health care system. Indian civilization, as everyone is aware, is very ancient and rich in natural resources. The use of herbs and plant-derived products for treating various disease has been a common practice since ages (Pratap Gowd M. J. S., 2019) [1]. *Holoptelea integrifolia* is an ornamental road side tree. It belongs to family Ulmaceae which comprises 15 genera and 200 species. It is commonly known as chilbil, kanju in Hindi, chirivilva, poothigam in Sanskrit, njettaval in Malayalam and India elm tree in English. The plant species originated from Pacific island. It is distributed over tropical and temperate region of northern hemisphere. *Holoptelea integrifolia* is considered to be native to Asian-tropical region including India, Nepal, Sri Lanka, Cambodia, Laos, Myanmar, Vietnam and China (Nithya Babu., 2017) [2]. A large spreading glabrous deciduous tree, which grows from 15-18 m height with grey, pustular bark that is smooth when young, exfoliating in corky scales on older trees. Leaves elliptical-ovate, acuminate, base rounded or subcordate. Flowers greenish yellow, in short racemes or fascicles on the leafless branches. Fruit sub-orbicular samara with membranous wing. Seeds flat (Padmaa M Paarakh., 2011) [4]. In traditional system of medicine, bark and leaves are used as bitter, astringent, acrid, thermogenic, anti-inflammatory, digestive, carminative, laxative, anthelmintic,

depurative, repulsive, Urinary astringent and in rheumatism. The phytoconstituents isolated so far from stem bark are Holoptelin A and B, 2-aminonaphthaquinone, friedelin, Epifriedelin,  $\beta$ -sitosterol and its  $\beta$ -D-glucose (Nadella Drga and Paarakh padmaa M., 2011) [4]. It has many pharmacological activities like Antiobese Activity, Antidiarrhoeal Activity, Antidiabetic Activity, Antimicrobial Activity, Antitumour Activity, Antioxidant Activity, Wound Healing Activity, Antiemetic Activity, Anti-inflammatory Activity, Anthelmintic Activity, Adaptogenic Activity, Antigenic and Allergenic Activity etc. The present microbiological study was aimed to evaluate the antimicrobial efficiency of medicinal plant *Holoptelea integrifolia* against pathogenic microorganism (*Staphylococcus aureus*) and tested antifungal activity against *Penicillium notatum* fungus.

### Materials and Methodology

**Plant Material:** The plant material stem bark and leaves of *Holoptelea integrifolia* L. were collected from Gujarat university campus, Navarangpura, Ahmedabad, Gujarat.

### Extract Preparation Method

The collected plant material young stem bark and leaves was air dried for 15 days. And grounded into fine powder with a mechanical grinder. The powdered samples were obtained after pulverisation then they were subjected to successive extraction with organic solvents such as methanol and aqueous by dry crude extraction. 10 g

weighed powdered material of each sample were treated with different solvents including methanol and aqueous and incubated for 24 hrs on shaker. The extracts were filtered through the Whatman filter paper no 1. The filtered extracts were kept at room temperature for evaporation of solvents. After 3 days we got the crude extract of each sample.

**Preparation of pure culture**

The bacterial culture of *Staphylococcus aureus* was collected from department of Microbiology and Biotechnology, school of science, Gujarat University. In the sterile condition the broth was prepared and bacteria were grown with the help of LAF and shaker. After 24 hrs the bacterial culture was ready for the inoculation in experimental plates.

**Methodology for antibacterial activity**

The antibacterial activity was determined by well diffusion method. 6.5 gm nutrient agar and 7.5 gm agar- agar were mixed with 500ml distil water in conical flask. After that, the media was sterilized with the autoclave. After 30 minutes the flask was carefully taken out from the autoclave. 30ml of sterile nutrient agar medium was poured into sterile petri-dishes. This whole process was done in laminar air flow cabinet and between two spirit lamps. The petri dishes were incubated at 37 ° C for 24 hours to check for sterility. The *Staphylococcus aureus* bacteria were inoculated on nutrient agar plate with help of inoculating loop. Nutrient agar plates were prepared for aqueous and methanolic extracts of young stem bark and leaves of *Holoptelea integrifolia* (L.). wells were punched into it with a sterile corn borer. All the wells were labelled through markers and the wells were filled with 5mg/2ml, 10mg/2ml, 15mg/2ml and 20mg/2ml concentration extracts.

**Methodology for antifungal activity:**

Agar well diffusion methodology was selected for the experiment. Potato dextrose agar was used and this PDA, (Potato Dextrose Agar) was dissolved in distilled water.

After that, the media was sterilized with the help of autoclave. After 30 minutes the flask was carefully taken out from the autoclave. 25 to 30 ml of PDA media was poured into sterilized petri plates. This whole process was done in laminar air flow cabinet and between two spirit lamps. Then after it was allowed to solidify at room temperature for 24 hours. The *Penicilium notatum* fungus was inoculated on nutrient agar plate with help of inoculating loop. Nutrient agar plates were prepared for methanolic and aqueous extracts of young stem bark and leaves of *Holoptelea integrifolia* (L). Using the cork borer several wells of 1.5mm in diameter were punched. The equal volume (100ml) of aqueous and methanolic extracts with particular concentration were poured into the wells. Then the plates were incubated at 37°C for 24 hours. Inhibition zone was measured with zone scale.

**Results and Discussion**

**Antibacterial activity of aqueous extracts of young stem bark and leaves**

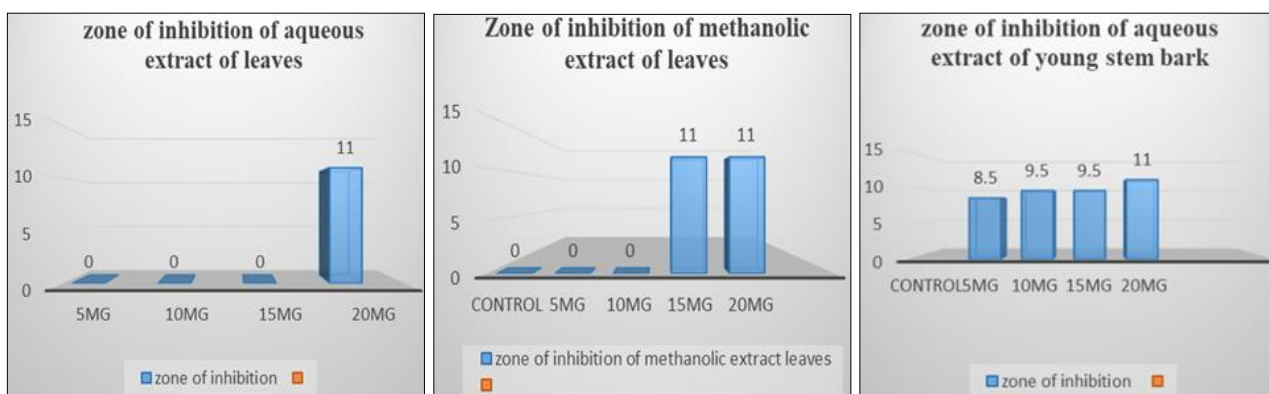
The aqueous extracts of young stem bark of *Holoptelea integrifolia* obtained by infusion has provided inhibitory activity against *Staphylococcus aureus*. The zone of inhibition of 8.5mm seen at 5mg concentration and the zone of inhibition of 9.5mm seen at 10mg and 15 mg Concentration and the maximum zone of inhibition of 11mm seen at 20mg concentration. And aqueous leaves extract of *Holoptelea integrifolia* showed only maximum zone of inhibition of 11mm seen at 20mg concentration. aqueous leaves extract of *Holoptelea integrifolia* showed only maximum zone of inhibition of 11mm seen at 20mg concentration.

**Antibacterial activity of methanolic extracts of young stem bark and leaves**

The methanolic extracts of young stem bark showed negative results against different concentration while methanolic extract of leaves showed maximum zone of inhibition of 11 mm seen at 15-20 mg concentration.

**Table 1:** showing antibacterial activity of methanolic and aqueous extracts of *Holoptelea integrifolia* L

Part of plant	Extract	Extract concentration (mg/2ml)					mm= millimetre
		control	5mg/2ml	10mg/2ml	15mg/2ml	20mg/2ml	
Leaves	methanol	0.0mm	0.0mm	0.0mm	11mm	11mm	
	aqueous	0.0mm	0.0mm	0.0mm	0.0mm	11mm	
Young stem bark	methanol	0.0mm	0.0mm	0.0mm	0.0mm	0.0mm	
	aqueous	0.0mm	8.5mm	9.5mm	9.5mm	11mm	



**Fig 1:** Results of antibacterial activity of *Holoptelea integrifolia* L

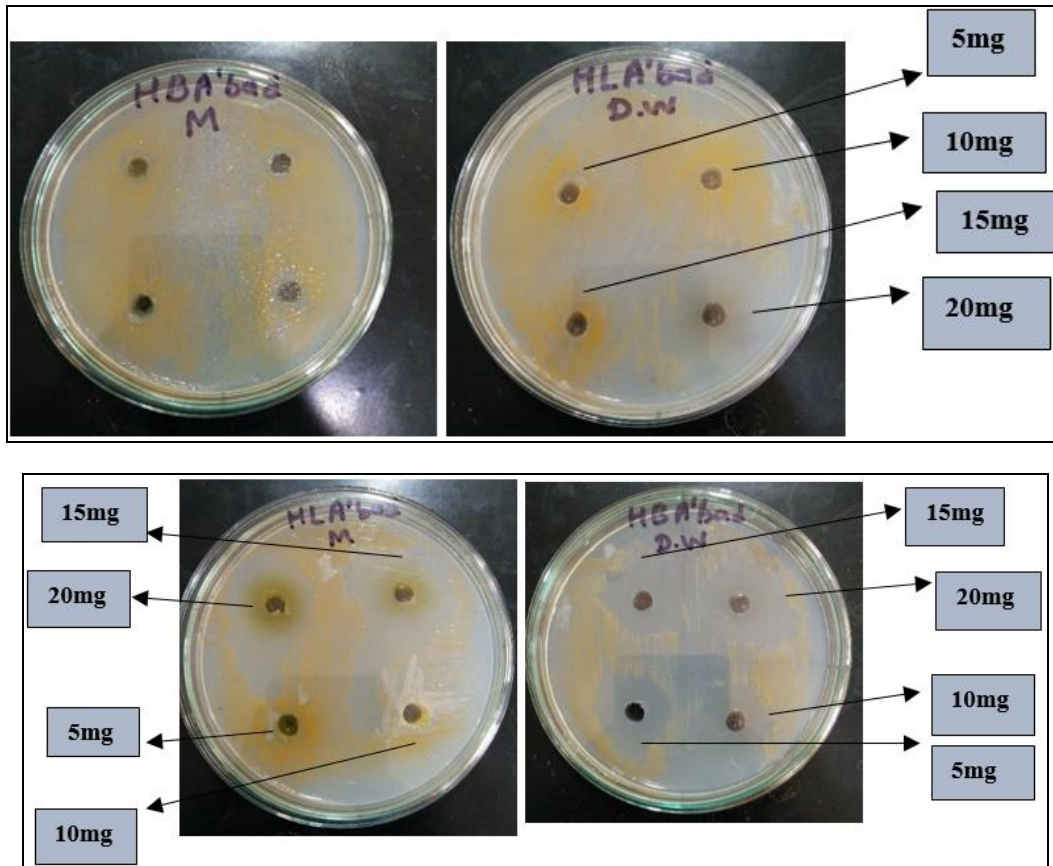


Plate 1: Antimicrobial activity of *Holoptelea integrifolia* L against *Staphylococcus aureus*

**Antifungal activity of methanolic extracts of leaves and young stem bark**

The result obtained that the methanolic extract of leaves showed zone of inhibition of 6.5mm at 15 mg concentration and 7.5mm at 20mg concentration.

The methanolic extract of young stem bark showed negative result.

**Antifungal activity of aqueous extract of leaves and young stem bark**

The result obtained that the aqueous extract of leaves showed zone of inhibition of 5.5mm at 20mg concentration and the aqueous extract of young stem bark showed zone of inhibition of 2.5mm at 5mg concentration, zone of inhibition of 4.5mm at 10 mg concentration, zone of inhibition of 4.5mm at 15mg concentration and zone of inhibition of 4.5mm at 20mg concentration.

Table 2: Antifungal activity of methanolic and aqueous extract of *Holoptelea integrifolia* L. against *Penicillium notatum*.

Plant Parts	Extract concentration (mg/2ml) mm(millimeter)					
	Extract	Control	5mg/2ml	10mg/2ml	15mg/2ml	20mg/2ml
Leaves	Methanol	0.0mm	0.0mm	0.0mm	5.5mm	7.5mm
	Aqueous	0.0mm	0.0mm	0.0mm	0.0mm	5.5mm
Young stem bark	Methanol	0.0mm	0.0mm	0.0mm	0.0mm	0.0mm
	Aqueous	0.0mm	2.5mm	4.5mm	4.5mm	4.5mm

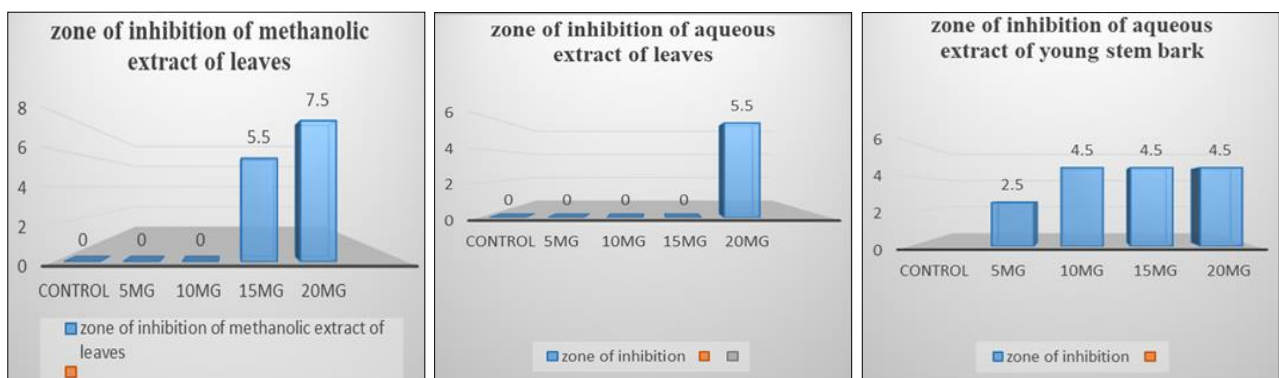
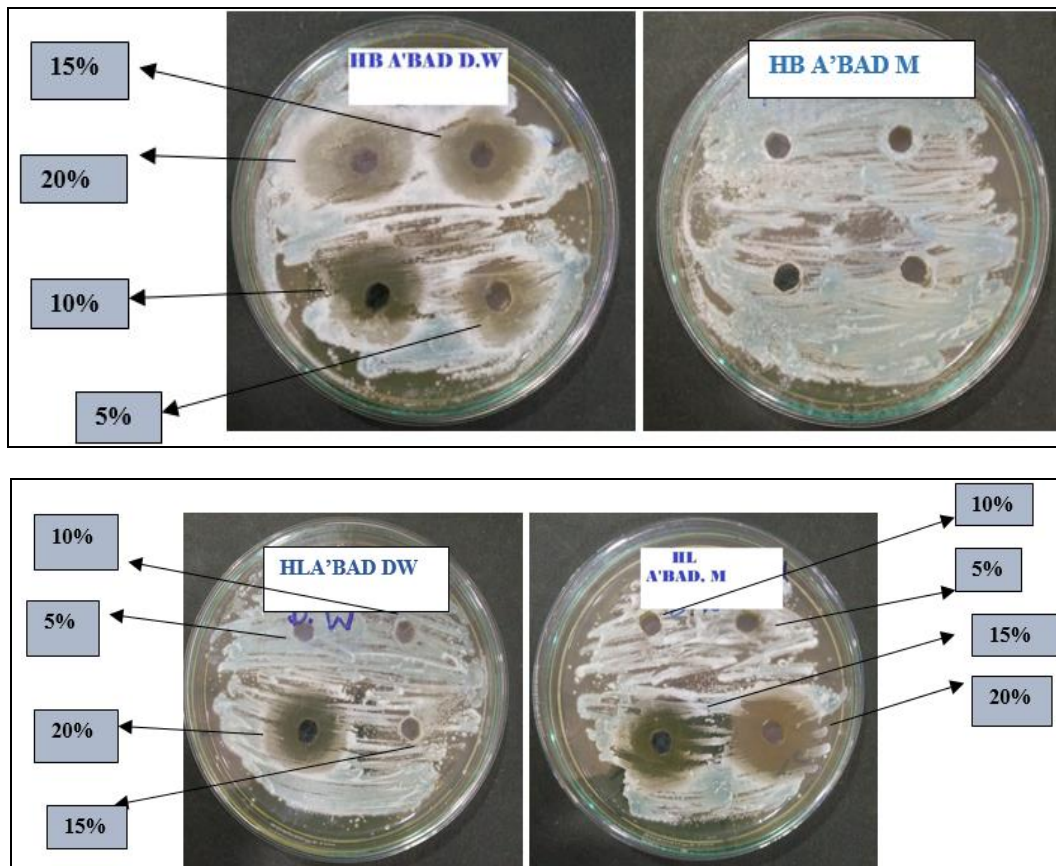


Fig 2: Results of antifungal activity of *Holoptelea integrifolia* L.



**Plate 2:** Antifungal activity of *Holoptelia integrifolia* L. against *Penicillium notatum*.

Phytochemicals are chemical compound produced by plants that help the plants protect themselves from harmful agents such as bacteria. They include alkaloids, flavonoids, phenols, tannins and saponins (UB Akuru and BA Amadi., 2018) [5]. The most important mechanism of the beta-lactam antibiotic resistance is the destruction of the antibiotics by the enzyme beta-lactamase. Use of beta-lactamase inhibitors in combination with antibiotics is one of the successful antibacterial strategies. The aqueous extract of leaves of *Holoptelea integrifolia* was evaluated for antimicrobial activity against various bacteria viz. *Staphylococcus pyogenes*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhi*. The antibacterial activity of aqueous extract of *Holoptelea integrifolia* in different concentration was evaluated where zone of inhibition was observed against all most resistant bacterial strains (Nadella Durga and Padmaa M Paarakh., 2011) [4]. A number of studies have reported the antibacterial activities of naphthalenedione derivatives from plants. Nafcillin, a synthetic  $\beta$ -lactam ring. This indicates that 1,4-naphthalenedione may also act as an inhibitor to  $\beta$ -lactamase, due to the structural similarity and explains the activity of the compound against  $\beta$ -lactamase-resistant *Staphylococcus aureus* (N V vinod *et al.*, 2010) [6].

### Conclusion

*Holoptelia integrifolia* L. is an important medicinal plant which is rich in phytochemical constituents and has so many pharmacological activities. On the basis of obtained results of the research it can be concluded that *Holoptelia integrifolia* L. has antimicrobial activity. In future another microbial strains can be used to assess anti-microbial activity and plant pathogenic organism's activity can be

analysed, which will definitely be useful to make herbal pesticide or insecticide.

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