



Antimicrobial Potential of *Helicanthus elastica* (Desr.) Danser - A less explored Indian mistletoe Growing on Mango Trees

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ABSTRACT

Helicanthus elastica (Desr.) Danser (Loranthaceae) is a less-known medicinally important mistletoe species occurring in India. It is used to check abortion, and also in vesical calculi and kidney affections. There are no detailed studies reporting the antimicrobial potential of this plant. Based on the traditional use and the rich phenolic composition of the whole plant, the antimicrobial property of the alcohol extract was analyzed and the results are outlined in the present paper. For the analysis, zone of inhibition, and minimum inhibitory concentration were used, and the total activity was assayed by standard methodologies. The antimicrobial activity was studied against bacteria like *Aeromonas hydrophila*, *Bacillus subtilis*, *Escherichia coli*, *Klebsiella pneumoniae*, methicillin-resistant *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus pyogenes*, *Vibrio fischeri*, and a fungus *Candida albicans*. Of the eight tested bacteria, the alcoholic extract of *H. elastica* was found to be active against *K. pneumoniae*, *A. hydrophila*, *E. coli*, and *V. fischeri* at concentration ranging from 250 to 500 µg/ml. *C. albicans* showed inhibition only at a concentration of 2000 µg/ml.

Key words: Ethyl gallate, Gallic acid, *Helicanthus elastica*, Loranthaceae, Mango mistletoe

INTRODUCTION

Screening of medicinal plants for antimicrobial activities is important for finding new compounds for therapeutic use. In particular, the antimicrobial activity of plant oils and extracts has formed the basis of many applications, including raw and pro-

cessed food preservation, pharmaceuticals, alternative medicine, and natural therapy.^[1] The development and spread of resistance to the existing antibiotics by microorganisms call for increased efforts in the development of new antibiotics.^[2] Although a number of plants with antimicrobial activities have been identified, great number still remains unidentified. Many plants are considered as

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antimicrobial agents for bactericidal or fungicidal applications.^[3]

Natural products like quinones, terpenes, alkaloids, and tannins have been reported to curtail the growth of vegetative cells and spores of bacteria and fungi.^[4] The word mistletoe is applied for hemiparasitic plants, with the exception of the genera *Nuytsia*, *Atkinsonia*, and *Gaiadendron*, producing parasitic roots on the aerial shoots of other higher plants.^[5] *Helicanthus elastica* (Desr.) Danser (Loranthaceae) is a common mistletoe species growing on mango trees in India. Its leaves are used to check abortion, and also in vesical calculi and kidney affections.^[6] The hemiparasite is found to be a rich source of phenolic compounds.^[7] Hence, a thought has been given to assess the antimicrobial properties of the plant. The prime objective of this work is to evaluate the action of the alcoholic extract of *H. elastica* on different microbial strains to create direct comparable, quantitative, antimicrobial data on these less-known Indian mistletoes.

MATERIALS AND METHODS

Laboratory materials

Chemicals and solvents used in the study were of laboratory and analytical grade and were obtained from Merck chemicals, Mumbai, India. Growth media and their components were obtained from HiMedia, Mumbai, India. Glassware of Borosil were used throughout the study. They were cleaned properly prior to the experiment by soaking them in Lysol for 24 h followed by detergent and were washed thoroughly in running water, rinsed with distilled water, and air dried. Glassware, growth media, and other accessories used in this study were sterilized in a vertical autoclave at 121°C and 15 lbs/sq. inch pressure for 15 min.

Preparation of extract

Fresh plants of the mistletoe growing on *Mangifera indica* were collected during flowering in August 2009 from Kasaragod District of Kerala. Voucher specimen of the plant collected was deposited (voucher specimen number 00637) at the Pharmacognosy Department of Captain Srinivasa Murti Drug Research Institute for Ayurveda, Chennai. Fifty grams of the coarsely powdered air-dried plant material of *H. elastica* was soaked overnight in absolute ethanol (250 ml). Next day the flask was slightly warmed over a water bath and filtered. The marc was again treated with more of alcohol and filtered. The filtrates were combined and concentrated on a water bath till it became a semi-solid mass (9.5 g) at a temperature not more than 105°C. Further drying was done in a vacuum dryer. The dried extracts were stored in an air-tight amber glass container and kept in a refrigerator until the studies were completed.

Preparation of bacterial inoculum

The quality and quantity of inoculums have only second-order effects upon the response of an assay system, which is the inhibition of the growth (antibiotics) or stimulation of the growth (vitamins). The suitability of the test organism for the purpose is more important than the quality and quantity of inoculums. The inoculums usually are grown in a medium similar to or identical to the assay medium.

A single colony was picked from the plate, suspended in 100 ml sterile nutrient broth, and incubated overnight at 37°C. The turbidity was measured in a calibrated photometer, and a suitable quantity was used for inoculating the assay broth to give 5 million cells/ml.

Microbial cultures

Bacterial strains

The strains used for screening antibacterial and antifungal activity were purchased from National Chemical Laboratory (NCL), Pune. The bacterial strains were methicillin-resistant (MR) *Staphylococcus aureus*, *Bacillus subtilis* (MTCC 441), *Pseudomonas aeruginosa* (ATCC 27853), *Aeromonas hydrophila* (ATCC 7966), *Streptococcus pyogenes* (ATCC 19615), *Vibrio fischeri* (ATCC 7744), *Klebsiella pneumoniae* (ATCC 15380), and *Escherichia coli* (ATCC 25922).

Fungal strains

A fungal strain *Candida albicans* (MTCC 227) was obtained from Madras Medical College, Chennai.

Preparation of media

Preparation of nutrient broth

Nutrient broth (NB) was used for the preparation of suspensions of bacterial cultures which were used as inoculums. NB was composed of peptic digest of animal tissue 5 g/l, sodium chloride 5 g/l, beef extract 1.5 g/l, and yeast extract 1.5 g/l. Exactly 1.3 g of accurately weighed NB was suspended in 100 ml of distilled water and sterilized in an autoclave at 121°C and 15 lbs pressure for 15 min.

Preparation of medium for antibacterial activity

Mueller Hinton Agar (MHA) from HiMedia Laboratories was used for antibacterial sensitivity test. MHA is composed of beef infusion 300 g/l, casein acid hydrolysate 17.50 mg/l, starch 1.50 g/l, and agar 17 g/l. Exactly 3.8 g of accurately weighed MHA medium was suspended in 100 ml of distilled water and sterilized in an autoclave at 121°C, 15 lbs pressure for 15 min.

Preparation of medium for antifungal activity

Sabouraud's Dextrose Agar (SDA) from Himedia Laboratories was used for the yeast sensitivity test. SDA is composed of mycological peptone 0.2 g/l, dextrose 4.0 g/l, and agar 1.5 g/l. Exactly 6.8 g of accurately weighed SDA medium was suspended in 100 ml of distilled water and sterilized in an autoclave at 121°C, 15 lbs pressure for 15 min.

Preparation of fungal inoculums

The fungal cultures were grown on SDA (Himedia Laboratories) by incubating at 25°C for 4 days.

Sterilization

The microbiology laboratory was fumigated with potassium permanganate and formaldehyde once in a month. Inoculation hood was cleaned with 95% ethyl alcohol and sterilized using UV lamp for 15 min each time before use.

Preparation of sample

Fifty milligrams of the extract was weighed in a sterile Eppendorf tube and 500 µl of dimethyl sulfoxide (DMSO) was added and mixed well.

Agar well diffusion method^[8]

The Petri plates and the medium were sterilized in an autoclave at 121°C and 15 lbs pressure for 15 min. About 25-30 ml of the sterile medium was transferred aseptically to the sterile Petri plates and labeled. The plates were allowed to solidify inside the laminar air flow chamber. Ten microliters of the bacterial inoculums (5 million cells/ml) was transferred using micropipette onto sterile media and a lawn culture was made using sterile “z” rod. Similarly, the SDA plates were inoculated with 72-h-old fungal culture maintained in SDA broth. The plates were labeled. Using separate sterile plungers, wells of 6 mm diameter were made on the solidified loaded media. Then, 20 µl, 10 µl, 5 µl, and 2.5 µl of the extract were aseptically transferred into separate wells. The different wells had the extract concentration of 2000 µg/ml, 1000 µg/ml, 500 µg/ml, and 250 µg/ml, respectively. Standard solution (Ciprofloxacin 5 µg) was used as the positive control. Care was taken to avoid tilting of the plates. The MHA plates were incubated at 37°C for 24 h and the SDA plates at 25°C for 48 h.

Minimum inhibitory concentration

Minimum inhibitory concentration (MIC) was determined for the plant extract showing antimicrobial activity against the tested pathogens. Broth microdilution method^[9] was followed for determination of MIC values. Plant extracts were re-suspended in DMSO to a concentration of 2000 µg/ml, and then subjected to twofold serial dilutions and added to broth media present in 96 wells of microtiter plates. Thereafter, 100 µl inoculum [1×10^8 colony forming units (CFU)/ml for bacteria and 1×10^7 cells/ml for fungus] was added to each well. The microtiter plates were incubated at 37°C for 24 h for bacteria and at 28°C for 48 h for fungi. Each extract was assayed in duplicate. A loop full of the broth from a different dilution was inoculated onto the surface of sterile nutrient agar and the plates were incubated at 37°C for 24 h for bacteria and the same was carried out on SDA plates for *C. albicans*. The MIC values were taken as the lowest concentration of the extracts in the well of the microtiter plate that showed no growth after incubation.

Total activity determination

Total activity (TA) is the volume at which the test extract can be diluted with the ability to kill microorganisms. It is calculated by dividing the amount of extract from 1 g plant material by the MIC of the same extract or compound isolated, and is expressed in milliliters per gram.^[10]

RESULTS

The antibacterial activity of the alcohol extract of *H. elastica* whole plant was studied against *A. hydrophila*, *B. subtilis*, *E. coli*, *K. pneumoniae*, MR *St. aureus*, *P. aeruginosa*, *Str. pyogenes*, and *V. fischeri*. Antifungal activity of the above-mentioned extract was tested against *C. albicans*.

Antimicrobial activity

The zones of inhibition of the test extract against the selected strains are presented in Figure 1 and Table 1.

The ethanol extract of *H. elastica* at a concentration of 250-2000 µg was screened against the selected pathogenic species. *K. pneumoniae* is the only organism that was susceptible to the extract at the lowest concentration tested (250 µg).

The observed zone of inhibition against the organisms tested was concentration dependent. *H. elastica* alcohol extract was found to have antifungal activity against *C. albicans* only at a higher concentration of 2000 µg/ml.

Minimum inhibitory concentration

The MIC values of the alcoholic extract of *H. elastica* are shown in Table 2.

DISCUSSION

The goal of this study was to ascertain the antimicrobial activity of the alcohol extract of the whole plant. In a previous study,^[11] the plant extracts were found to possess cytotoxic activity. The test extract was evaluated against several selected bacterial and fungal



1 - 250 µg; 2 - 500 µg; 3 - 1000 µg; 4 - 2000 µg; 5 - Ciprofloxacin 5 µg

Figure 1. Antimicrobial activity of the alcohol extract of *Helicanthus elastica* Graphic Summary

Table 1. Zone of inhibition of alcoholic extract of *H. elastica* on tested pathogens

Organism	Zone of inhibition in mm (µg)				
	2000	1000	500	250	PC 5
<i>A. hydrophila</i>	16	14	12	-	26
<i>B. subtilis</i>	-	-	-	-	28
<i>C. albicans</i>	19	-	-	-	28
<i>E. coli</i>	18	14	12	-	20
<i>K. pneumoniae</i>	18	15	14	14	30
<i>P. aeruginosa</i>	-	-	-	-	31
MR <i>Sta. aureus</i>	-	-	-	-	30
<i>Str. pyogenes</i>	16	12	10	-	-
<i>V. fischeri</i>	15	13	10	-	32

PC: Positive control ciprofloxacin

Table 2. Minimum inhibitory concentration of the alcoholic extract of *H. elastica*

Name of the organism	MIC (µg)	TA (ml/g)
<i>A. hydrophila</i>	500	400
<i>C. albicans</i>	2000	100
<i>E. coli</i>	500	400
<i>K. pneumoniae</i>	62.5	3200
<i>P. aeruginosa</i>	2000	100
<i>S. pyogenes</i>	1000	200
<i>V. fischeri</i>	500	400

MIC: Minimum inhibitory concentration; TA: Total activity

pathogens which are considered to be the cause of many common infections. The results obtained indicate that the test extract was quite active against *K. pneumoniae*, *A. hydrophila*, *E. coli*, and *V. fischeri* in decreasing order (MIC 62.5, 500, 500, and 500 µg/ml). It produced only a weak effect against other strains tested.

A. hydrophila is a heterotrophic, gram-negative, rod-shaped bacterium, mainly found in areas with a warm climate. It can survive in aerobic and anaerobic environments. It is also highly resistant to multiple medications, chlorine, and cold temperature. Gastroenteritis is the common disease caused by these bacteria, especially in children. It is also associated with cellulitis and can cause myonecrosis and eczema in people with compromised immune system. The pathogenic mechanisms of *Aeromonas* spp. still remain unknown. The recently proposed type III secretion system (TTSS) mediated pathogenic mechanism has been proven to play a pivotal role in the pathogenesis of *Aeromonas*. It is a specialized protein secretion machinery that exports virulence factors delivered directly to host cells. These factors subvert normal host cell functions in some ways that are beneficial to the invading bacteria.^[12]

V. fischeri is a gram-negative rod frequently found in symbiotic relationships with marine animals like the bobtail squid. Infections associated with non-cholera *Vibrio* species are gastroenteritis, wound infection, and septicemia.^[13]

K. pneumoniae is a non-motile Gram-negative rod. This organism is commonly isolated from water and human and animal feces. It causes infection of the surgical wounds and the urinary tract, and is also commonly found in the respiratory tract.^[8]

E. coli is a motile Gram-negative rod. It is one of the most important members of Enterobacteriaceae. It causes frequent op-

portunistic infections. It is often present in the appendix abscesses, peritonitis, cholecystitis, and septic wounds, and causes bacteremia and endotoxic shock, and occasionally meningitis in neonates. It is the commonest cause of uncomplicated infections of the lower urinary tract (cystitis). An increasing number of strains are recognized as primary gastrointestinal pathogens.^[8]

CONCLUSION

From the above account, it can be suggested that the tested extract is likely to contain important phytoconstituents possessing antibacterial activity against four important Gram-negative bacteria. This can be considered as a lead for further purification and activity-guided fractionation. Phytochemical study of the extract has shown the presence of numerous constituents. A detailed chemical investigation may result in isolation of therapeutically important antimicrobial agents. Ethyl gallate and gallic acid, isolated from this plant,^[7] are reported to possess antimicrobial activity.^[14] It is possible that they may be responsible at least in part to the observed antimicrobial activity of the tested extract. It remains to be elucidated if the other constituents found to be present contribute to the observed activity. Of the eight tested bacteria, the alcoholic extract of *H. elastica* was found to be active against *K. pneumoniae*, *A. hydrophila*, *E. coli*, and *V. fischeri* at concentrations ranging from 250 to 500 µg. When tested on *C. albicans*, the alcoholic extract showed inhibition only at a concentration of 2000 µg.

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