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

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 aerugiosa*, *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

Correspondence to:
Dr. K. N. Sunil Kumar, Senior Research Officer – Pharmacognosy, SDM Centre for Research in Ayurveda and Allied Sciences, Laxminarayana Nagar, Kuthpady, Udupi - 574 118, India. Tel: +91-8050230864; Fax: +91-8202533970 E-mail: sunilkumararayanan@gmail.com

DOI: 10.4103/2225-4110.126183
antimicrobial agents for bactericidal or fungicidal applications.\textsuperscript{[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.\textsuperscript{[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.\textsuperscript{[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 calcui and kidney affections.\textsuperscript{[6]} The hemiparasite is found to be a rich source of phenolic compounds.\textsuperscript{[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 \textit{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 \textit{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 \textit{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) \textit{Staphylococcus aureus}, \textit{Bacillus subtilis} (MTCC 441), \textit{Pseudomonas aeruginosa} (ATCC 27853), \textit{Aeromonas hydrophila} (ATCC 7966), \textit{Streptococcus pyogenes} (ATCC 19615), \textit{Vibrio fischeri} (ATCC 7744), \textit{Klebsiella pneumoniae} (ATCC 15380), and \textit{Escherichia coli} (ATCC 25922).

**Fungal strains**

A fungal strain \textit{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 peptie 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 1.5 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 25ºC 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, 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.

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 Sta. 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 cytoxic activity. The test extract was evaluated against several selected bacterial and fungal...
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  |
| *A. hydrophila* | 16   | 14   | 12   | -    | 26  |
| *B. subtilis*  | -    | -    | -    | -    | -   | 28 |
| *C. albicans*  | 19   | -    | -    | -    | 28  |
| *E. coli*      | 18   | 14   | 12   | -    | 20  |
| *K. pneumoniae* | 18   | 15   | 14   | 30   |     |
| *P. aeruginosa* | 50.65| 500  | 500  | 3000 |     |
| *MR Sta. aureus* | -    | -    | -    | -    | 30  |
| *Stx. pyogenes* | 16   | 12   | 10   | -    |     |
| *V. fischeri*  | 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) |
|----------------------|----------|-----------|
| *A. hydrophila*      | 500      | 400       |
| *C. albicans*        | 2000     | 100       |
| *E. coli*            | 500      | 400       |
| *K. pneumoniae*      | 62.5     | 3200      |
| *P. aeruginosa*      | 2000     | 100       |
| *S. pyogenes*        | 1000     | 200       |
| *V. fischeri*        | 500      | 400       |

MIC: Minimum inhibitory concentration; TA: Total activity

portunistic infections. It is often present in the appendix abscesses, peritonitis, cholecystitis, and septic wounds, and causes bacteremia and endotoxemic 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.

### ACKNOWLEDGMENTS

The authors are highly grateful to the Director General, CCRAS, New Delhi for providing facilities. Help by Dr. Hairul, Project Scientist, Biozone Research Technologies, Chennai is gratefully acknowledged.

### REFERENCES

1. Reynolds JE, Martindale – the Extra Pharmacopoeia, 31st ed. London: Royal Pharmaceutical Society of Great Britain; 1996.
2. Walsh TR, Weeks J, Livermore DM, Toleman MA. Dissemination of NDM-1 positive bacteria in the New Delhi environment and its implications for human health: An environmental point prevalence study. Lancet Infect Dis 2011;11:355-62.
3. Friedman M. Antibiotic activities of plant compounds against non-resistant and antibiotic resistant food borne human pathogens. In: Juneja VK, Cherry JP, Tunick MS, editors. Advance in microbial food safety. Washington DC, USA: American Chemical Society; 2006. p. 167-83.
4. Cowan MM. Plant products as antimicrobial agents. Clin Microbiol Rev 1999;12:564-82.
5. Kuijt J. The Biology of Parasitic Flowering Plants. Berkeley and Los Angeles: University of California Press; 1969. p. 246.
6. Kirikar KR, Basu BD. Indian Medicinal Plants. Vol. 3. Dehradun: M/S. Bishen Singh Mahendra Singh Pall; 1935. p. 2178-85.
7. Sunil Kumar KN. Pharmacognostical, Phytochemical and Medicinal Activity Profile of *Helicanthus elastica* (Desr.) Danser (Mango Mistletoe) –Loranthaceae. PhD Thesis, Chennai, India: The University of Madras 2011. p. 45-63.
8. Collee JG, Fraser AG, Marmion BP, Simmons A, editors. Mackie and Mc

Kumar, et al./ Journal of Traditional and Complementary Medicine 4 (2014) 258-262
Cartney Practical Medical Microbiology, 14th ed. New York: Churchill Livingstone; 1996. p. 364-5, 369.
9. Basri DF, Fan SH. The potential of aqueous and acetone extracts of galls of *Quercus infectoria* as antibacterial agents. Indian J Pharmacol 2005;37:26-9.
10. Eloff JN. Quantifying the bioactivity of plant extracts during screening and bioassay guided fractionation. Phytomedicine 2004;11:370-1.
11. Mary KT, Girija K, Ramadasan K. Partial purification of tumour reducing principle from *Helicanthus elasticus* (Fam. Loranthaceae). Cancer Lett 1994;81:53-7.
12. Cunningham MW. Pathogenesis of group A streptococcal infections. Clin Microbiol Rev 2000;13:470-511.
13. Hoi H, Thong HD, Tony TH, Derek L. Vibrio infections. Emedicine Specialties 2007 January. Available from: http://www.emedicine.com/med/topic2375.htm. [Last accessed on 2011 Dec 8].
14. Cueva C, Mingo S, Muñoz-González I, Bustos I, Requena T, del Campo R, *et al.* Antibacterial activity of wine phenolic compounds and oenological extracts against potential respiratory pathogens. Lett Appl Microbiol 2012;54:557-63.