Antimicrobial activity of *Careya arborea* Roxb., leaf extracts was determined using cup-plate diffusion, two-fold serial dilution method. In cup-plate method, inhibition zone sizes were used to determine the susceptibility to extracts. The results showed that *C. arborea* leaf extracts showed potential antibacterial activity against *S. aureus* and *B. subtilis*. Ethyl acetate and ethanol extracts showed the highest zones of inhibition for Gram positive and Gram negative bacteria and for fungus *C. albicans*. *Careya arborea* leaf extracts were able to have a MIC range of 0.938-15 mg mL⁻¹, in two-fold dilution method. The ethylacetate extract exhibited significantly better inhibition compared to other extracts. Gram-positive *B. subtilis*, Gram-negative *E. coli* and fungus *C. albicans* were found to be most susceptible to ethylacetate extract. The extracts have shown to be bacteriostatic and fungistatic at low concentrations. Phytochemical screening of the extracts revealed the presence of phyto-compounds such as triterpenoids, steroids, flavonoids and tannins as major phytoconstituents with known antimicrobial agents. These phyto-constituents may be responsible for the antimicrobial activity of *C. arborea*.

**Key words:** Antimicrobial, *C. arborea*, MIC, zone of inhibition

**INTRODUCTION**

*Careya arborea* Roxb., commonly known as ‘wild guava’, is a medium-sized deciduous tree; exhibiting dark grey colour and exfoliating in thin strips. It is widely available in India, Sri Lanka and Malaysia Peninsula. The plant has a variety of traditional uses. The leaves are used orally for fever while applied locally to relieve swellings (Maheshwari *et al*., 1986). The juice of leaves is applied in ulcers and skin diseases in India (Sharma *et al*., 1985). Leaves are found to contain triterpenoids and steroids, such as taraxerol, n-hexacosanol, "-spinasterol, taraxerol, taraxeryl acetate, 2" hydroxy ursolic acid, triterpene ester-careaborin and $\$-sitosterol. It is also reported to contain tannins and flavonoids as ellagic acid and quercetin (Mahato *et al*., 1967; Das and Mahato, 1982; Gupta *et al*., 1975; Bani *et al*., 1981). The bio-activity guided fractionation of methanol extract of leaves reported to present triterpenoids and saponins with good antileishmanial activity. Previous study has shown that *C. arborea* bark possess antimicrobial activity (Kumar *et al*., 2006). Despite traditional claims, the leaves of *C. arborea* have not been evaluated for their antimicrobial potential. Thus, the present study was undertaken to evaluate these traditional claim of the leaves of *C. arborea*. 

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**ABSTRACT**

Antimicrobial activity of *Careya arborea* Roxb., leaf extracts was determined using cup-plate diffusion, two-fold serial dilution method. In cup-plate method, inhibition zone sizes were used to determine the susceptibility to extracts. The results showed that *C. arborea* leaf extracts showed potential antibacterial activity against *S. aureus* and *B. subtilis*. Ethyl acetate and ethanol extracts showed the highest zones of inhibition for Gram positive and Gram negative bacteria and for fungus *C. albicans*. *Careya arborea* leaf extracts were able to have a MIC range of 0.938-15 mg mL⁻¹, in two-fold dilution method. The ethylacetate extract exhibited significantly better inhibition compared to other extracts. Gram-positive *B. subtilis*, Gram-negative *E. coli* and fungus *C. albicans* were found to be most susceptible to ethylacetate extract. The extracts have shown to be bacteriostatic and fungistatic at low concentrations. Phytochemical screening of the extracts revealed the presence of phyto-compounds such as triterpenoids, steroids, flavonoids and tannins as major phytoconstituents with known antimicrobial agents. These phyto-constituents may be responsible for the antimicrobial activity of *C. arborea*.
MATERIALS AND METHODS

Plant material and extraction: The leaves of *C. arborea* (CA) were collected in June 2010, at Nanded District, Maharashtra, India. The plant was authenticated by Dr. Vishal R. Marathe, Science College, Nanded, where a herbarium voucher specimen was deposited. The plant material was air-dried on the laboratory bench for five days, then ground to a coarse powder and extracted with increasing polarity of solvents with the help of soxhlation (Kalaskar and Surana, 2011) with slight modification. The petroleum ether (60-80°) (PE-CA), chloroform (CH-CA), ethyl acetate (EA-CA) and ethanol (EO-CA) extracts were successively collected, evaporated and stored in desiccators for further use. The extracts were qualitatively evaluated for the presence of different secondary metabolites using standard qualitative chemical tests (Kalaskar and Surana, 2012).

Test organisms: The test organism included the following gram positive bacteria; *Staphylococcus aureus*, *Bacillus subtilis* and Gram negative bacteria-*Pseudomonas aeruginosa*, *Escherichia coli*. The fungal strains; *Aspergillus niger* and *Candida albicans* were used.

Anti-microbial activity: The antibacterial activity of successive extracts was performed using agar cup-plate method (Mallemlula *et al.*, 2013). Twenty milliliter of the sterile nutrient agar medium was poured into sterile Petri-dishes and allowed to solidify. The Petri dishes were incubated at 37°C for 24 h and 28°C for 48 h to check for sterility. The medium was seeded with 0.1 mL of Gram positive/negative and fungi test microorganisms by spread plant method. The 5 mm bores were made on the medium using sterile borers. Dried extracts of *C. arborea* leaves was dissolved in dimethyl sulfoxide (DMSO) to obtain a concentration of 500 µg mL⁻¹ and sterilized by filtration, through a Whatman filter paper No. 1 and 0.1 mL of different concentrations of extract were added to the respective bores. 0.1 mL of streptomycin and gentamycin at a concentration of 50 µg mL⁻¹ was taken as standard references. The Petri-dishes were kept in a refrigerator at 4°C for 1 h for diffusion. After diffusion, the Petri-dishes were incubated at 37°C for 24 h for antibacterial study and at 28°C for 48 h for antifungal evaluation. The zones of inhibition were measured. The DMSO was used as the control.

Determination of Minimum Inhibitory Concentration (MIC): The Minimum Inhibitory Concentration (MIC) value was considered as the lowest extract concentration with no visible growth, for each plant extract and the test pathogen. To measure the MIC values, various concentrations of the stock of extracts (15, 7.5, 3.75, 1.875, 0.938, 0.469, 0.234, 0.117, 0.059 and 0.029 mg mL⁻¹) were assayed against the test pathogens. Plant extracts were re-suspended in DMSO to make a 15 mg mL⁻¹ final concentration and were then serially diluted, two-fold; 1 mL of each extract was added to test tubes containing 1 mL of sterile Nutrient Agar Medium (for bacteria) Sabouraud Dextrose Agar Medium (for fungi). The tubes were then inoculated with the standard size of microbial suspension (1x10⁶ CFU mL⁻¹ for bacteria and 1x10⁷ cell mL⁻¹ for fungi) and the tubes were incubated at 37°C for 24 h for bacteria and at 28°C for 48 h for fungi in a BOD incubator and were observed for change in turbidity after 24 h and compared with the growth in controls (Demarsh *et al.*, 2001). A tube containing nutrient broth and inoculums but no extract was taken as the control. Bacterial and fungal suspensions were used as negative controls while broth containing standard drug was used as positive controls. After the incubation period, the tubes were observed for the MICs by checking the concentration of the first tube in the series (ascending extract and antibiotic concentrations) that showed no visible trace of growth. The first tube in the series with no visible growth after the incubation period was taken as the MIC.
RESULTS AND DISCUSSION

Preliminary phyto-profiling: The preliminary phyto-profiling for different extracts of *C. arborea* were carried out, wherein the consistency was found to be sticky in the petroleum ether and chloroform solvent extracts, compared to ethyl acetate and ethanol extracts. The petroleum ether and chloroform extracts showed the presence of triterpenoids, steroids while ethyl acetate and ethanol extracts have shown the presence of flavonoids and tannins as major phytoconstituents.

Antimicrobial activity: Antimicrobial activity (denoted in terms of inhibition zones) of the plant extracts, tested against selected microorganisms were recorded (Table 1). In the present study total of 4 successive plant extracts were selected and tested for their bioactivity.

Chloroform and ethyl acetate extracts showed significant antimicrobial potential against test microbes. However, petroleum ether extract showed least activity against the selected microorganisms at the tested concentration. The most susceptible organism in the investigation was *B. subtilis* against which, extracts showed good inhibition zones. Maximum antimicrobial activities were recorded for ethyl acetate extracts against *B. subtilis*.

In general, *C. arborea* leaf extracts expressed significant antimicrobial activities by suppressing the growth of microbes under investigation. Excellent antibacterial activities were observed for ethyl acetate extract of *C. arborea* while with chloroform and ethanol extracts, significant activity indicated by low MIC values against microorganisms tested (Table 2).

This result suggests the bacteriostatic/fungistatic effects of the extracts. Ethyl acetate extract of *C. arborea* leaves was recorded as bactericidal against Gram-positive bacteria *B. subtilis*. Earlier studies reported that Gram-positive bacteria were more susceptible to plant extracts than Gram-negative (Lin et al., 1999; Palombo and Semple, 2001). It is well documented that the

| Extracts/zone of inhibition (mm) |
|----------------------------------|
| Microorganisms                  |
| S. aureus                       |
| B. subtilis                     |
| E. coli                         |
| P. aeruginosa                   |
| C. albicans                     |
| A. niger                        |
| PE-CA                           |
| CH-CA                           |
| EA-CA                           |
| EO-CA                           |
| SMC                             |
| GMC                             |

Values are expressed as Mean±SD (n = 3), PE-CA: Petroleum ether (60-80 C) *C. arborea* leaf extract, CH-CA: Chloroform *Careya arborea* leaf extract, EA-CA: Ethyl acetate *Careya arborea* leaf extract, EO-CA: Ethanol *Careya arborea* leaf extract, SMC: Streptomycin GMC: Gentamycin

| Minimum Inhibitory Concentration (MIC) of *Careya arborea* successive extracts |
|--------------------------------------------------------------------------------|
| Microorganisms                  |
| S. aureus                       |
| B. subtilis                     |
| E. coli                         |
| P. aeruginosa                   |
| C. albicans                     |
| A. niger                        |
| PE-CA                           |
| CH-CA                           |
| EA-CA                           |
| EO-CA                           |

PE-CA: Petroleum ether (60-80 C), CH-CA: Chloroform, EA-CA: Ethyl acetate extract, EO-CA: Ethanol extract of *Careya arborea* leaves
Gram-negative bacteria has difference in cell wall composition. In the present study there is susceptibility differences between Gram-positive and Gram-negative bacteria indicating the extract may have antibacterial activity due to cell wall inhibition as one of the mechanisms. Additionally, the study showed that the extracts have considerable potential antifungal activity. The plant extracts showed good activity against A. niger. The ethyl acetate extract exhibited potential activity while chloroform and ethyl acetate extract showed significant antifungal activity.

CONCLUSION

The present study proved the traditional claims of C. arborea leaves on modern scientific line. In conclusion, the C. arborea has the potential and broad spectrum antimicrobial activity and may help to discover new chemical classes of antibiotic substances that could serve as selective chemotherapeutic agents for infectious diseases and their control.

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