



## Antibacterial activity of the fruits of *Careya arborea* Roxb. (Lecythidaceae)

Manjima Prabhakaran, Bincy Reejo, D. Suresh Kumar\*

CARe Keralam Ltd, KINFRA Small Industries Park, KINFRA Park P.O., Koratty-680309, Thrissur District, Kerala, India

Article history: Received: 5December 2013, revised: 25 January 2014, accepted: 12 February 2014, Available online: 3 April 2014

### ABSTRACT

**Plan:** The present study was planned to investigate the antibacterial activity of ethyl acetate, ethanol and hexane extracts of the fruits of *Careyaarborea*Roxb.

**Methodology:** Agar diffusion assay was carried out using the extracts.

**Outcome:** All the tested bacterial strains viz., *Escherichia coli*, *Salmonella typhimurium*, *Listeria monocytogenes*, *Staphylococcus aureus* and *Staphylococcus epidermidis*) were found to be sensitive to all the 3 concentrations of ethyl acetate and ethanolic extracts of fresh and dry fruit, in ascending order.

**Keywords:** *Careya arborea*, antibacterial activity, Lecythidaceae

### 1. INTRODUCTION

*Careya arborea* is a medicinal plant used in Oriental medical traditions. The bark, leaves and fruits are used in Ayurveda in the treatment of ulcers, haemorrhoids, tumors, etc<sup>1</sup>. In Chinese medicine the tree is known as *Ka Li Yu Rui*<sup>2</sup>. The tree is also commonly found in the North East of Thailand, where people traditionally eat the shoots, young leaves and fresh flowers. Known as *Kradonbok* in Thai language, its leaf is used in the treatment of wounds and flowers are used for relieving cough<sup>3</sup>. In India, the tree grows in deciduous forests and grasslands.



Figure 1. Fruits of *Careya arborea* Roxb.

Known as *Katabhi* in Sanskrit, it is a medium sized deciduous tree growing up to a height of 15 metres, with thick, dark grey bark having shallow cracks. Fruits of *Careya arborea*are large, globose, green, glabrous berries, crowned with persistent calyx and style .Persistent calyx is a distinguishing feature of Lecythidaceae.



For Correspondence: dventu21@yahoo.com

Contact: 91-9449348897

Hygeia.J.D.Med. Vol.6 (1), April 2014© 2014 all rights reserved.

Hygeia journal for drugs and medicines, 2229 3590, 0975 6221

Rid: D-2044-2014

There are some reports on the antibacterial activity of leaf and stem bark of *Careya arborea*<sup>4-6</sup>. The antibacterial activity of the fruits of *Careya arborea* was studied, considering the paucity of information on this subject.

## 2. MATERIALS AND METHODS

Fresh fruits of *Careya arborea* (Figure 1) were plucked from a tree in the campus of CARE Keralam Ltd, authenticated by the botanist at Kerala Agricultural University, Mannuthy, and assigned herbarium accession number (Care K 101/herb/13). They were cut into small pieces. One portion was dried under shade and the other portion was refluxed at 35-40°C, for twenty minutes after boiling. Ethyl acetate, ethanol and hexane were used as solvents<sup>7</sup>. The extracts were filtered, concentrated by evaporating the solvents and used for antibacterial study.

### 2.1 Agar Diffusion assay

Brain heart infusion broth (HIMEDIA) 10 ml was inoculated with the test cultures, incubated at 37°C for 18 hours<sup>8,9</sup>. Muller Hinton agar (HIMEDIA) was prepared, autoclaved and about 15-20ml poured into Petri dishes. The agar was allowed to set and harden. Pure cultures of all experimental bacterial cultures including *Escherichia coli* (433), *Salmonella typhimurium* (3231), *Listeria monocytogenes* (657), *Staphylococcus aureus* (9886) and *Staphylococcus epidermidis* (3086) were obtained from the Microbial Type Culture Collection (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh<sup>10</sup>. The cultures were swabbed on the agar surface using sterile cotton swab. 8mm cork borer was used to make wells. The extracts were added into the wells at 100µl, 150µl, and 200 µl concentrations. Gentamicin 10mcg concentration, a standard antibacterial drug was used as positive control. The plates were left at room temperature for 2 hours to allow diffusion of extract and incubated overnight at 37°C with face upwards<sup>11</sup>. The diameter of the zones of inhibition was measured with measuring scale. Inhibition of the bacterial growth was measured in mm<sup>8</sup>.

## 3. RESULT AND DISCUSSION

Three different concentrations of ethyl acetate (EA) extract, ethanolic extract and hexane extract of fresh and dry fruit of *Careya arborea* were tested and compared for their antibacterial effect against three Gram negative and two Gram positive organisms. The results showed that all the tested bacterial strains were found to be sensitive to all the 3 concentrations of EA and ethanolic extracts of fresh and dry fruit, in ascending order (Tables 1 and 2). EA extract of fresh fruit was shown to have potent antibacterial action than EA extract of dry fruit. But, ethanolic extract of both fresh and dry fruit were found to have somewhat similar antibacterial potential.

In case of hexane extract of fresh fruit, 150 and 200µl showed inhibitory activity against *S.typhimurium* in ascending order. *S. aureus* was found to be sensitive to 200 µl of hexane extract of fresh fruit and the remaining 2 concentrations were inactive. But in the case of hexane extract of dry fruit, *E.coli* was found to be sensitive to 200 µl concentrations only.

*S.typhimurium* had only intermediate action against all the 3 concentrations of hexane dry fruit extract and 200µl of the same was inhibitory in action on *L. monocytogenes* and *S. epidermidis*. Hexane dry fruit extract had no action on *S. aureus*. Both EA and ethanolic extracts of fresh and dry fruit of *C. arborea* were observed to have high antibacterial activity than their hexane extract. From Tables 1 and 2 it is evident that the standard antibacterial drug Gentamicin has either the same or less activity in inhibiting the growth of the test organisms. The EA and ethanolic extracts have considerably higher activity when compared to Gentamicin.

Three reports are available on the antibacterial activity of various parts of *Careyaarborea*. Daduanget al<sup>5</sup> reported the antibacterial activity of leaves of *Careya sphaerica* (Synonym: *Careya arborea*) and a similar study was reported by Behera et al<sup>6</sup>. Another study on stem bark was reported by Kumar et al., (2006). According to the study of Kumar et al<sup>4</sup> most of the bacterial and fungal species were inhibited by the extract. Methanolic extract of stem bark showed considerable broad spectrum antibacterial activity on all bacterial strains tested at 25-200 µg/disc.

Behera et al<sup>6</sup> explain that the leaf extract in the mixture of solvents contained polyphenols which exhibited significantly lower zones against *S. aureus* and *E.coli*. The activity against *E. coli* and *S. aureus* may be due to interaction of more lipophilic flavonoids with extracellular and soluble proteins to complex with bacterial cell wall for disruption.

These antibacterial effects are obviously brought about by well-defined chemical entities present in the leaf, stem bark or fruit of *Careya arborea*. Several characteristic compounds like careyagenolide, careaborin, hydroquinone, resorcinol, syringic acid, vanillic acid, 4-hydroxy-3-(p-hydroxyphenyl)-5,7-dimethoxy-coumarin, 3-(o-hydroxyphenyl) coumarin, 3', 4, 4', 7-tetra methoxy-, trans-2, 3, cis-2, 4-(+)-3-flavanol and 2-methoxydibenzofuran have been isolated from leaves and root of this tree<sup>13-15</sup>.

Search of literature reveals that very few studies have been carried out on the chemical entities in *Careya arborea* fruits. The present study shows that the fruit of *Careyaarborea* contains compounds with strong antibacterial activity. Therefore, there is an urgent need to isolate the antibacterial compounds from this fruit.

Table 1: The antibacterial activity of fresh fruit of *Careya arborea* (Zone size in mm)

| Organism                | Ethyl acetate (EA) |     |           |    |           |    | Ethanol (E) |           |    |           |    |           | Hexane (H) |   |           |    |           |    | #Gentamicin (GEN) |    |   |           |    |
|-------------------------|--------------------|-----|-----------|----|-----------|----|-------------|-----------|----|-----------|----|-----------|------------|---|-----------|----|-----------|----|-------------------|----|---|-----------|----|
|                         | 100<br>µl          | *In | 150<br>µl | In | 200<br>µl | In | EA          | 100<br>µl | In | 150<br>µl | In | 200<br>µl | In         | E | 100<br>µl | In | 150<br>µl | In | 200<br>µl         | In | H | 10<br>mcg | In |
| <i>E.coli</i>           | 24                 | *S  | 28        | S  | 29        | S  | -           | 25        | S  | 28        | S  | 29        | S          | - | -         | *R | -         | R  | -                 | R  | - | 22        | S  |
| <i>S.typhimurium</i>    | 24                 | S   | 26        | S  | 28        | S  | -           | 19        | S  | 20        | S  | 22        | S          | - | -         | R  | 15        | R  | 18                | R  | - | 24        | S  |
| <i>L.mono cytogenes</i> | 22                 | S   | 23        | S  | 24        | S  | -           | 27        | S  | 28        | S  | 28        | S          | - | -         | R  | -         | R  | -                 | R  | - | 24        | S  |
| <i>S.aureus</i>         | 30                 | S   | 33        | S  | 36        | S  | -           | 16        | S  | 17        | S  | 22        | S          | - | -         | R  | -         | R  | 18                | R  | - | 27        | S  |
| <i>S.epidermidis</i>    | 23                 | S   | 25        | S  | 26        | S  | -           | 22        | S  | 23        | S  | 25        | S          | - | -         | *I | 17        | S  | 19                | S  | - | 25        | S  |

\*In - Inference, \*S – Sensitive, \*R – Resistant, \*I – Intermediate # Gentamicin zone interpretation 12- Resistant (R), 13-14 -Intermediate (I), 15 - Sensitive (S)<sup>12</sup>

Table 2: Antibacterial activity of dry fruit of *Careya arborea* (Zone size in mm)

| Organism                | Ethyl acetate (EA) |     |           |    |           |    | Ethanol (E) |           |    |           |    |           | Hexane (H) |   |           |    |           |    | #Gentamicin (GEN) |    |   |           |    |
|-------------------------|--------------------|-----|-----------|----|-----------|----|-------------|-----------|----|-----------|----|-----------|------------|---|-----------|----|-----------|----|-------------------|----|---|-----------|----|
|                         | 100<br>µl          | *In | 150<br>µl | In | 200<br>µl | In | E           | 100<br>µl | In | 150<br>µl | In | 200<br>µl | In         | E | 100<br>µl | In | 150<br>µl | In | 200<br>µl         | In | H | 10<br>mcg | In |
| <i>E.coli</i>           | 15                 | *S  | 18        | S  | 31        | S  | -           | 24        | S  | 26        | S  | 27        | S          | - | -         | *R | -         | R  | 17                | S  | - | 23        | S  |
| <i>S.typhimurium</i>    | 15                 | S   | 17        | S  | 19        | S  | -           | 19        | S  | 20        | S  | 28        | S          | - | 12        | R  | 13        | R  | 14                | *I | - | 25        | S  |
| <i>L.mono cytogenes</i> | 12                 | R   | 14        | I  | 15        | S  | -           | 20        | S  | 22        | S  | 23        | S          | - | -         | R  | 13        | I  | 15                | S  | - | 23        | S  |
| <i>S.aureus</i>         | 15                 | R   | 20        | S  | 24        | S  | -           | 25        | S  | 30        | S  | 33        | S          | - | -         | R  | -         | R  | -                 | R  | - | 31        | S  |
| <i>S.epidermidis</i>    | -                  | R   | 14        | I  | 16        | S  | -           | 22        | S  | 24        | S  | 26        | S          | - | -         | R  | -         | R  | 17                | R  | - | 30        | S  |

\*In – Inference, \*S – Sensitive, \*R -Resistant, \*I – Intermediate , # Gentamicin zone interpretation , 12- Resistant (R), 13-14 -Intermediate (I), 15 -Sensitive (S)<sup>12</sup>

## ACKNOWLEDGEMENTS

The authors are thankful to Dr. Joy T. Verghese, Executive Director of Care Keralam Ltd for keen interest in this study.

## REFERENCES

1. Warriar P.K., Nambiar VPK, Ramankutty C. Indian Medicinal Plants, Orient Longman Private Ltd, Hyderabad. **2005**; pp 380-382.
2. Zhou J., Xie G., Yan X. Encyclopedia of Traditional Chinese Medicines, Indexes, Springer Verlag, Berlin. **2011**; pp 508.
3. Maisuthisakul P. Phenolic constituents and antioxidant properties of some Thai plants. In Phytochemicals- A Global Perspective of their role in Nutrition and Health, Venketeshwer Rao, Editor. *In Tech Croatia* **2012**; pp 187-212.
4. Kumar R.S., Sivakumar S., Shanmuga Sundaram R., Sivakumar P., Nethaji R., Gupta M., Mazumdar U.K. Antimicrobial and antioxidant activities of *Careya arborea* Roxb. Stem bark. *Iranian J. Pharmacol. Therapeut* **2006**; 5, 35-41.
5. Daduang J., Vichitphan S., Hongsprabhas P., Boonsiri P. High phenolics and antioxidants of some tropical vegetables related to antibacterial and anti-cancer activities. *Afr. J. Pharm. Pharmacol.* **2011**; 5, 608- 615.
6. Behera P.C., Bisoi P.C., Parija S.C. HPTLC detection of polyphenols and flavonoids of *Careya arborea* leaves and study of antimicrobial effect. *Int. J. Phytopharmacol* **2012**; 3, 36-41.
7. Lavekar G.S. Laboratory Guide for the Analysis of Ayurveda and Siddha Formulations, C.C.R.A.S., New Delhi. **2010**; pp 88.
8. Valgas C., De Souza S.M., Smania E.F.A., Smania Jr A. Screening methods to determine antibacterial activity of natural products. *Braz. J. Microbiol* **2007**; 38, 369-380.
9. Forbes B.A., Sahn D.F., Weissfeld A.S. Bailey and Scott's Diagnostic Microbiology, Elsevier's Health Sciences, Philadelphia, U.S.A. **2007**; pp 187.
10. Sen A., Batra A. Evaluation of antimicrobial activity of different solvent extracts of the medicinal plant, *Melia azadirach* L. *Int. J. Curr. Pharma* **2012**; Res. 4, 67-73.
11. Irshad S., Mahmood M., Perveen F. In vitro antibacterial activities of three medicinal plants using agar well diffusion method. *Research J. Biol.* 2012; 2,1-8.
12. Anonymous. Index of Bioscience. HIMEDIA Laboratories Pvt Ltd., Mumbai. **2012**; pp. 366.
13. Talapatra B., Basak A., Talapatra S.K. Terpenoids and related compounds. Part XX. Careborin, a new triterpene ester from the leaves of *Careya arborea*. *J. Ind. Chem. Soc.* **1981**; 58, 814-815.
14. Das M.C., Mahato S.B. Triterpenoid saponins from the leaves of *Careya arborea* structure of careyagenolide. *Phytochem* **1982**; 21, 2069-2073.
15. Kalita J.C., Haque A., Kalita E., Sharma R., Rahman M.M. Antifertility effects of the methanolic root extract of *Careya arborea* Roxb in albino mice. *The Bioscan* **2011**; 6, 701-706.