

## ANTIBACTERIAL EFFICACY OF LEAF EXTRACTS OF *PHYLLANTHUS WIGHTIANUS* MÜLL. ARG.

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The dried pulverized leaf extracts (ethanol, acetone and chloroform) from *Phyllanthus wightianus*, were screened for its antibacterial activity against *Escherichia coli*, *Bacillus subtilis*, *Proteus vulgaris*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Aeromonas hydrophila* and *Staphylococcus epidermidis*. Ethanol extracts exhibited significant activity against the selected bacterial strains compared to other extracts.

**Keywords:** Antibacterial activity; Human bacterial pathogens; *Phyllanthus wightianus*; Solvent extracts.

### Introduction

Angiospermic plants are storehouse of effective chemotherapeutants<sup>1</sup> and results of biological screening, revealed wide range of activities which proved that these can be used for treating diseases like asthma, cholera, diarrhoea, dysentery, dermatological infections, intestinal disorders, ulcers, rheumatism and uraemia etc<sup>2-4</sup>.

The traditional medicinal methods especially the use of plants, play a vital role in the basic health needs in the developing countries and the use of herbal remedies has enhanced in the developed countries<sup>5</sup>. In India, large numbers of medicinal plants are found in their natural habitats. Antimicrobial substances are also found in numerous plants. In some plants, the compounds present in tissues may provide protection against certain plant and human pathogenic microorganisms<sup>6</sup>. The active principles of many drugs are found in plants or produced as secondary metabolites. Phytochemical and biological analysis of medicinal plants is providing remarkable contribution to the drug industry all over the world<sup>7</sup>.

According to WHO estimate, more than 80% of the world's population relies on traditional medicinal practices for primary health care needs<sup>8</sup>. The importance of medicinal plants in India, providing healthcare against various ailments including infectious diseases are well documented. Considering the rich diversity of these plants, it is expected that screening and scientific evaluation of plant extract for their active principles may provide new antimicrobial substances. The genus *Phyllanthus* is a valuable medicinal plant having many properties. Recently, several investigators reported on their medical values such as antimicrobial<sup>9,10</sup>, antiviral<sup>11-13</sup>, antitumour and

anticarcinogenic<sup>14</sup>, antimalarial<sup>15</sup>, antiprotozoal<sup>16</sup>, antiplasmodial<sup>17,18</sup> and antihepatotoxic<sup>19-21</sup> properties. Hence, the present research was motivated to evaluate the efficacy of *Phyllanthus wightianus* Müll. Arg. (Euphorbiaceae) extracts against the selected bacterial strains.

### Materials and Methods

**Plant materials :** *Phyllanthus wightianus*, is a sub shrub, commonly found in floor and border of the shola vegetation in the Eastern Ghats and Western Ghats of Tamil Nadu, India. The leaves of this plant species were collected from Kolli hills, (above 1000m in height), one of the broken chains of Eastern Ghats of Tamil Nadu. The plants were shade dried for 10 days.

**Preparation of extracts :** The method of extract preparation was followed as per Trease and Evans<sup>22</sup> and carried out with slight modifications. The dried plant materials were powdered using a mixer grinder. About twenty-five grams of each powdered material was separately extracted with 100 ml of 50% ethanol, acetone and chloroform solvents respectively. All the solvents were kept at room temperature, for 7 days to allow the extraction of compounds from plants. Each mixture was stirred every 24 hours using sterile glass rod. The greenish extracts were obtained and passed through the Whatmann filter paper No.1 and the respective solvents were evaporated (at 40°C) with the help of heating mantle. The sticky black substances were obtained and stored in refrigerator and dissolved in DMSO (Dimethyl Sulfoxide) prior to use.

**Microorganisms used :** Seven bacterial cultures namely *Escherichia coli*, *Bacillus subtilis*, *Proteus vulgaris*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Aeromonas hydrophila* and *Staphylococcus epidermidis* were used in



**Table 1.** Antibacterial efficacy of leaf extracts from *Phyllanthus wightianus*.

| S. No | Organisms Tested                  | Diameter of inhibition zone in mm* (Mean ± SD) |            |            |
|-------|-----------------------------------|--|------------|------------|
|       |                                   | Solvents Used                                  |            |            |
|       |                                   | Ethanol  | Acetone    | Chloroform |
| 1.    | <i>Aeromonas hydrophila</i>       | 9.33±0.82                                      | 8.0±0.00   | 11.33±0.82 |
| 2.    | <i>Bacillus subtilis</i>          | 8.66 ± 0.81                                    | 12.0±3.74  | 10.66±0.81 |
| 3.    | <i>Salmonella typhi</i>           | 11.33±1.63                                     | —          | 13.33±0.82 |
| 4.    | <i>Staphylococcus epidermidis</i> | 17.0± 2.45                                     | 14.66±0.81 | 10.0±0.00  |
| 5.    | <i>Proteus vulgaris</i>           | 12.66±1.63                                     | 13.66±2.16 | 9.66±0.81  |
| 6.    | <i>Escherichia coli</i>           | 9.33±0.82                                      | —          | —          |
| 7.    | <i>Pseudomonas aeruginosa</i>     | 12.66±3.56                                     | 13.33±2.16 | 10.33±0.82 |

-- No activity

\* - Average value of triplicate

this investigation. All the cultures were procured from Microbial Type Culture Collection (MTCC), IMTECH, Chandigarh, India.

**Media and Inoculum preparation :** The media used for antibacterial test were Nutrient Agar/Broth and Mueller Hinton agar. All the media were obtained from Himedia Pvt. Ltd., Mumbai, India. The test bacterial strains were inoculated onto nutrient broth and incubated at 37°C for 24 hours. After the incubation period, the culture tubes were compared with the turbidity (opacity) standard.

**Antibacterial activity test :** Antibacterial activity test was carried out by the disc diffusion method<sup>23</sup> with little modifications. A sterile swab was dipped into the culture, the swab saturated with suspension of bacteria was used to inoculate the entire surface of the Mueller Hinton agar medium containing petridishes. The sterile disc (0.5 mm in diameter) were loaded with each extract (50 µl/ disc) and kept in an incubator to dry (for an hour) before being placed on top layer of the plates. The plates with discs were incubated for 24 hours at 37°C. The inhibition of bacterial growth was determined by measuring the diameter of the clear zone around each disc. Each extract was tested in triplicate for the calculation of mean value of the zone of inhibition<sup>24</sup>.

### Results and Discussion

Antibacterial efficacy of the leaf extracts of *Phyllanthus wightianus*, were presented in table 1. The results suggested that ethanolic extracts of *P. wightianus* showed an excellent antibacterial activity against *Staphylococcus epidermidis* followed by *Proteus vulgaris* and

*Pseudomonas aeruginosa*, which is clearly documented. The other tested organisms like *Salmonella typhi*, *Aeromonas hydrophila*, *Escherichia coli* and *Bacillus subtilis* exhibited moderate activity. However, the acetone extracts reflected good activity against *S. epidermidis*, *P. vulgaris* and *P. aeruginosa*. The same extract exhibited fairly significant activity towards *B. subtilis* and no inhibition was seen in *S. typhi*. But in case of *A. hydrophila* expressed least activity against the extract tested. Chloroform extracts showed good to moderate inhibitory activity against the tested bacteria namely *S. typhi*, *A. hydrophila*, *B. subtilis*, *P. aeruginosa*, *S. epidermidis* and *P. vulgaris*. Both the acetone and chloroform extracts found to be inactive against the growth of *E. coli*. This study is also comparable to the antibacterial activity of organic solvent extracts from *Phyllanthus emblica*<sup>25,26</sup>, *P. niruri*<sup>27</sup>, *P. amarus* and *P. muellerianus*<sup>28</sup> showed remarkable activity against several bacterial pathogens.

The results clearly indicate that ethanolic leaf extract was found to have better activity against all the bacterial strains tested followed by acetone and chloroform extracts. This may be due to the presence of chemical constituents, which is soluble in organic solvents and responsible for bioactivity. It can be interpreted that the antibacterial activity against microorganisms is due to one or more alkaloids found in plants<sup>29</sup>. The present findings support the use of *Phyllanthus wightianus* in traditional system of medicine to treat many diseases like fever, inflammations, ulcers, wound infections etc. Further research is necessary to isolate and purify the active compounds in this plant, which allow the scientific community to recommend their utilization as an accessible alternative to synthetic antibiotics.

### Acknowledgements

Authors are grateful to the Management, Kandaswami Kandar's College, Velur for their constant support during this research work.

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