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# *In vitro* Antibacterial Activity in Seed extracts of *Phoenix sylvestris* Roxb (Palmae), and *Tricosanthes dioica* L (Cucurbitaceae)

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**Abstract**

Seeds of *Phoenix sylvestris* Roxb (Palmae), and *Tricosanthes dioica* L (Cucurbitaceae) were investigated for their *in vitro* antibacterial activity against few gram-negative and gram-positive bacteria. Extracts of the plant seeds were prepared in methanol, ethanol, and chloroform by microwave assisted extraction method. Their antibacterial activity was investigated by disc diffusion and broth dilution methods. Ethanol extract of *P. sylvestris* was found active (bacteriostatic) against both gram-positive and gram-negative organisms with MIC (minimum inhibitory concentration) values of 481 and 410 µg/mL against *Salmonella paratyphi A* and *Staphylococcus epidermidis*, respectively. Phytochemical screening revealed presence of phenols, alkaloids, and flavones in it. This study indicated various extracts of *P. sylvestris,* and *T. dioica* seeds to possess antibacterial activity.

**Key words**: Antibacterial, *Phoenix sylvestris, Tricosanthes dioica,* MAE (microwave assisted extraction)

## Introduction

Plants are rich source of drugs, either as direct remedies or template for production of synthetic drugs. Numerous studies made throughout the world have demonstrated wide occurrence of antimicrobial compounds in higher plants (1). Due to emergence of drug resistant strains of pathogenic bacteria, it has become important to investigate plants as sources of novel antimicrobials, as they may inhibit bacteria by a mechanism different than that of currently used antibiotics (2).

Present study was aimed at screening various extracts of seeds from two plants namely- *Phoenix sylvestris* Roxb (Palmae; common name- Khajur), and *Tricosanthes dioica* L (Cucurbitaceae; common name- Parvar) for their antibacterial activity. Seeds of the latter has been reported to possess haemagglutinating and antifungal activity, and are rich in fatty acids (3).

## Material and Methods

Seeds of both the plants *P. sylvestris,* and *T. dioica* were procured from local market of Ahmedabad city. They were authenticated for their unambiguous identity by Prof. Y. T. Jasrai, Head of Botany Dept., Gujarat University, Ahmedabad.

***Extraction:*** Seeds were extracted in three different solvents (Merck, Mumbai, India) – methanol, ethanol (50%), and chloroform using the microwave assisted extraction (MAE) method (4). Dry seed powder was soaked into the solvent in a ratio of 1:50, and subjected to microwave

heating (Electrolux EM30EC90SS) at 720 W. Total heating time was kept 90, 70, and 180 second for methanol, ethanol, and chloroform respectively, with intermittent cooling. This was followed by centrifugation (at 10,000 rpm for 15 min.), and filtration with Whatman paper # 1 (Whatman International Ltd., Maidstone, England). Solvent was evaporated from the filtered extract and then the dried extracts were reconstituted in: (i) their respective solvents for disc diffusion assay, and (ii) dimethyl sulfoxide (DMSO) for broth dilution assay. Reconstituted extracts were stored under refrigeration for further use. Extraction efficiency was calculated as percentage weight of the starting dried plant material. Extraction efficiency ranged from 2.8-13.6 %, with highest (13.6%) being in case of methanol extract of *T. dioica* seeds.

***Bacterial strains:*** *Staphylococcus aureus* MTCC 737, *Streptococcus pyogenes* MTCC 442, *Staphylococcus epidermidis* MTCC 435, *Escherichia coli* MTCC 723, *Aeromonas hydrophila* MTCC 1739, *Salmonella paratyphi A*, *Shigella flexneri* MTCC 1457, *Vibrio cholerae* MTCC 3906, and *Pseudomonas oleovorans* MTCC 617. *S. paratyphi A* was procured from Department of Microbiology, Gujarat University, Ahmedabad, while the rest were obtained from Microbial Type Culture Collection (MTCC), Chandigarh.

***Disc diffusion assay (DDA) :*** This was performed by Kirby-Bauer method as per NCCLS guidelines (5). 500 µL of inoculum (adjusted to 0.5 McFarland standard) was spread on surface of Muller-Hinton agar medium (HiMedia, Mumbai, India). Sterile discs (6 mm diameter) made of Whatman paper # 1 were dipped into the test extract and were put onto the agar surface after complete drying. Discs dipped into pure solvents (separate disc for each solvent) after drying were applied as negative control. Commercially available discs of either streptomycin or Ofloxacin (HiMedia) served as positive control. Plates were then incubated at 35°C for 24 h. After incubation plates were observed for zones of inhibition, and their diameter were measured. Studies were performed in triplicates.

***MIC determination :*** MIC (minimum inhibitory concentration) determination was carried out using microbroth dilution method as per NCCLS guidelines (5). Assay was performed in a 96-well microtitre plate. Total volume of the assay system in each well was kept 200 µL. Cation-adjusted Muller-Hinton broth (HiMedia) was used as growth medium. Inoculum density of the test organisms was adjusted to that of 0.5 McFarland standard. Broth was dispensed into wells of microtitre plate followed by addition of test extract and inoculum. Extracts (reconstituted in DMSO) were serially diluted into each of the wells. A DMSO control was included in all assays (6). Gentamicin (HiMedia) served as positive control. Plates were incubated at 35°C for 16-20 h, before being read at 655 nm in a plate reader (BIORAD 680). MIC was recorded as the lowest concentration at which no growth was observed. All MICs were determined on three independent occasions. Concentration at which growth was inhibited by 50% was recorded as $IC_{50}$ value.

After reading the plates for MIC, subculturing was made on nutrient agar plate from the wells showing no growth, so as to determine whether the extract is bactericidal or bacteriostatic. Growth on the plate indicated bacteriostatic action, absence of growth indicates bactericidal action.

***Total activity:*** Total activity (mL/g) was calculated as (7): Amount extracted from 1 g (mg) / MIC (mg/mL).

***Activity index:*** Activity index was calculated as (8)- zone of inhibition by extract / zone of inhibition by antimicrobial agent used as positive control

***Phytochemical screening:*** Active extracts were tested for presence of alkaloids, flavones, phenolics, and flavonoids as described below (8,9).

***Alkaloids:*** Dragendorff reagent was used to test the presence of alkaloids. The presence of alkaloids was indicated by the appearance of yellow precipitate when few drops of reagent were added to the solution.

***Flavones:*** Test solution (500 µl) was mixed with 100 µl of absolute alcohol, 0.02 g of p-dimethyl amine benzaldehyde and two drops of conc. Hcl. Development of red or pink colour indicated the presence of flavones.

***Flavonoids:*** An aqueous solution of the extract was treated with 10% ammonium hydroxide solution. Yellow florescence indicates the presence of flavonoids.

***Phenols:*** Ferric chloride was used to test the presence of phenols. The presence of phenols was indicated by appearance of green colouration when the reagent was added to extract.

## Results and Discussion

Results of disc diffusion assay (table 1) indicated *S. paratyphi A* to be susceptible to all the extracts tested against it. *S. pyogenes*, and *S. auerus* exhibited no notable susceptibility to any of the test extracts. Ethanol extract of *P. sylvestris* (50 mg/mL) produced same size of inhibition zone as that produced by ethanol extract of *T. dioica* (100 mg/mL) against *S. paratyphi A*. As the former did it at exactly half the concentration of the latter, it can be said to be twice as potent as the latter. Methanolic extract of *P. sylvestris* produced smallest zone of inhibition against *S. paratyphi A*, despite its concentration being higher than all other test extracts. Activity index (AI) was calculated for the extracts which exhibited activity in disc diffusion assay, as written in parentheses in table 1. Methanolic extract of *T. dioica* registered highest values of AI against *S. paratyphi A*, and *P. oleovorans*. As ethanolic extract of *P. sylvestris* was found to be effective against both gram-positive as well as gram-negative bacteria, it can be said to have a broad spectrum of activity (10). It was further investigated for its MIC values against susceptible bacteria. It was capable of inhibiting the growth of *S. paratyphi A*, and *S. epidermidis* at concentrations of 481 and 410 µg/mL, respectively (Table 2). It was found to be bacteriostatic in action, as organisms were able to revive growth when transferred on nutrient agar free from extract. Total activity of this extract against both the organisms was above 100 mL/g. Total activity is a measure of the amount of material extracted from a plant in relation to the MIC of the extract, fraction or isolated compound. It indicates the degree to which the active fractions or compounds present in 1 g can be diluted and still inhibit growth of the test organism (7). Phytochemical tests indicated the presence of phenols, alkaloids, and flavones in ethanolic extract of *P. sylvestris* seeds.

## Conclusion

This study indicated various extracts of *P. sylvestris,* and *T. dioica* seeds to possess antibacterial activity. These extracts should be further investigated for identification of active constituent(s). Active constituent(s) once separated can be subjected to compatible techniques such as mass spectrometry, IR and NMR spectroscopy for structural studies.

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**Table 1.** Disc diffusion assay of *P. sylvestris* and *T. dioica* seed extracts

| Organism | *P. sylvestris* | | *T. dioica* | | Positive control | | |
|---|---|---|---|---|---|---|---|
| | Diameter of zone of inhibition (mm) | | | | | | |
| | M[1] | Et[2] | M[3] | Et[4] | Ch | O | S |
| *A. hydrophila* | 0 | 0 | 0 | 0 | 11±2.8 (0.61) | - | 18±2.0 |
| *E. coli* | 0 | 0 | 0 | 0 | - | 28±1.8 | - |
| *S. aureus* | FI | 0 | 0 | 0 | - | 17±2.0 | 28±2.5 |
| *S. epidermidis* | 0 | 9±1.2 (0.47) | FI | 0 | - | - | 19±1.8 |
| *S. paratyphi A* | 11±1.8 (0.5) | 17±2.0 (0.77) | 18±1.6 (0.81) | 17±2.0 (0.77) | - - | - - | 22±1.0 |
| *S. flexineri* | 0 | 9±1.8 (0.29) | FI | 20±2.0 (0.64) | 0 | 31±2.4 | - |
| *V. cholerae* | 9±1.0 (0.45) | FI | FI | FI | - | 27±1.4 | 20±2.2 |
| *P. oleoverans* | 0 | FI | 21±2.8 (0.80) | FI | FI | 26±1.4 | - |
| *S. pyogenes* | 0 | 0 | 0 | 0 | 0 | 15 | 20±2.2 |

All values are means±SD of three experiments.
FI: Faint inhibition without clear zone; O: Ofloxacin (5 µg /disc), S: Streptomycin (10 µg /disc)
M: Methanol; Et: Ethanol; Ch: Chloroform
Conc. of extract into which disc was dipped (mg/mL): [1]260, [2]50, [3]120, [4]100
Figures in parentheses indicate activity index. Negative controls did not cause any inhibition of growth.

**Table 2.** Results of broth dilution assay

| Extract | Organism | $IC_{50}$ (µg/mL) | MIC (µg/mL) | Total activity (mL/g) |
|---|---|---|---|---|
| Ethanol extract of | *S. paratyphi* A | <400 | 481 | 103.95 |
| *P. sylvestris* seeds | *S. epidermidis* | <380 | 410 | 121.95 |

## References

1. Joseph, I., and Singh, R. (2008). Antimicrobial activity of selected medicinal plants, *Craetva magna* (Linn.), *Pongamia glabra* (Linn.), and *Areca catechu* (Linn.). Ethnobotany Leaflets, 12: 995-1002.

2. Tomoko, N., Takashi, A., Hiromu, T., Yuka, I., Hiroko, M. Munekazu, I., Totshiyuki, T., Tetsuro, I., Fujio, A., Iriya, I., Tsutomu, N., and Kazuhito, W. (2002). Antibacterial activity of extracts prepared from tropical and subtropical plants on methicillin resistant *Staphylococcus aureus*. Journal of Health Sciences, 48: 273-276.

3. Khare, C.P. (2004). Encyclopedia of Indian medicinal plants. Springer, Germany, pp. 208-357.

4. Kothari, V.O., Punjabi, A.B., and Gupta, S.R. (2009). Optimization of microwave assisted extraction of *Anona squamosa* seeds. The Icfai University Journal of Life Sciences, 3(1): 55-60.

5. Jorgensen, J.H., and Turnidge, J.D. (2003). Susceptibility test methods: Dilution and disk diffusion methods. In: *Manual of clinical microbiology* (8th edition),. Ed, PR Murray, ASM International, New York, USA, pp. 1108-1127.

6. Wadhwani, T., Desai, K., Patel, D., Lawani, D., Bahaley, P., Joshi, P., and Kothari, V. (2009). Effect of various solvents on bacterial growth in context of determining MIC of various antimicrobials. The Internet Journal of Microbiology, 7(1).

7. Eloff, J.N. (2004). Quantifying the bioactivity of plant extracts during screening and bioassay-guided fractionation. Phytomedicine, 11: 370-371.

8. Borgio, J.F., Thorat, P.K., and Lonkar, A.D. (2008). Antimycotic and antibacterial activities of *Gynandropis pentaphylla* DC extracts and its phytochemical studies. The Internet Journal of Microbiology, 5(2).

9. Wagner, H., Bladt, S., and Zgainski, E. (1983). Plant Drug Analysis (2nd edition), Springer, Berlin, Germany.

10. Talaro, K.P. (2008). Foundations in Microbiology: Basic Principles (6th edition), McGraw Hill, New York, USA, pp. 350.