BIO-EFFICACY OF BARK EXTRACTS OF TECOMELLA UNDULATA AGAINST PATHOGENIC MICROORGANISMS

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ABSTRACT

The present study was designed to evaluate the bio-efficacy of crude extracts of Tecomella undulata bark by examining their antimicrobial potential against twenty pathogens, including twelve bacterial (five Gram- positive and seven Gram- negative) and eight fungal strains. For determination of the antimicrobial activity, the crude aqueous and organic solvent (hexane, chloroform, ethyl acetate, acetone and methanol) extracts of bark of Tecomella undulata were prepared in increasing and decreasing order of solvent polarity, and were subjected to agar well diffusion assay. The extracts were found to exhibit variable inhibition zone (ranging between 7 to 27 mm) against most of the tested microbes; organic extracts showing a more potent activity as compared to the aqueous extract. Among the organic solvent extracts, chloroform and ethyl acetate extracts were found to be most active against the different microorganism species tested. The broth microdilution assay gave minimum inhibitory concentration (MIC) values ranging between 250 to 1000 µg/ml. The lowest MIC values were obtained against E. faecalis indicating the susceptibility of this organism to all extracts. The effectivity of a few extracts when compared with the antimicrobial response exhibited by the standard antibiotics (ampicillin, penicillin, streptomycin, tetracycline, fluconazole, ketoconazole and miconazole) was found to be comparable, or even higher than the antibiotics against a few microorganisms. The study thus promises an interesting future for designing a potentially active antimicrobial agent from Tecomella undulata.
KEYWORDS: *Tecomella undulata*, antibacterial activity, antifungal activity, pathogenic microorganisms.

INTRODUCTION
In developing countries like India, infectious diseases account for a high proportion of health problems. More lately, the treatment of infectious diseases has become an immense clinical problem, the problem being aggravated constantly by an ever-increasing resistance of microorganisms to many antibiotics. [1, 2] The spread of drug resistant pathogens has thus become one of the most serious threats to successful treatment of microbial diseases. [3] This situation has generated a need for the search of new and more effective therapeutic agents. [4] The past few years have seen much interest in the use of natural materials as a source for new antimicrobial agents. Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources. [5, 6]

Utilization of plants for medicinal purposes in India has been documented long back in ancient literature. [7, 8, 9] In 1998, the World Health Organization (WHO) also noted that majority of the world’s population (especially 80% of the people living in developing countries) depends on traditional medicine for primary healthcare, and most of the traditional medicine relies heavily on medicinal plants. [10] Plants have therefore become one of the most important sources of modern medicine too. Many recent reports show the effectiveness of traditional plants against microorganisms. [11, 12] Extracts prepared from different parts of several medicinal plants have been tested for their antimicrobial efficacy by several researchers in different parts of the world. [13, 14, 15, 16, 17] But, the full potential of higher plants as a source for new drugs still remains largely unexplored. Hence, in the present investigation, *Tecomella undulata*, commonly known as Rohira, Rohitaka and Phaphugh has been tested for its efficacy to inhibit several pathogenic microorganisms.

*Tecomella undulata*, belonging to the family Bignoniaceae, is an important agro-forestry tree found in the western parts of India. Different parts of this plant are used for the cure of syphilis and eczema, as antiwrinkle agent and skin nourisher; the bark possesses mild relaxant, cardiotonic and chloretic activities; whereas the whole plant methanol extract reportedly has analgesic potential. [18] The plant has also been used for the treatment of various diseases like urinary troubles, spleen enlargement, liver and abdominal diseases and leucorrhoea. Although the leaves have been reported to possess antimicrobial activity, [19] but no such studies have been conducted with the bark extracts. Therefore, the present study has
been designed to explore the antimicrobial potential of the bark extracts of this plant against a large number of pathogenic microorganisms.

**MATERIALS AND METHODS**

**Plant material and extract preparation:** Fresh bark of *Tecomella undulata* was collected from the plant growing in regions adjoining Sirsa, Haryana. Immediately after collection, the bark was washed thoroughly, initially with tap water and then with distilled water to remove any debris or dust particles and was then allowed to dry in an oven at 35°C. The dried plant material was ground to a fine powder and stored at room temperature in airtight containers. Aqueous and organic solvent extracts (prepared in both increasing and decreasing order of solvent polarity) were prepared using distilled water and five organic solvents (Flow chart 1).

**Flow Chart 1:** Schematic representation of the extraction procedure of bark of *Tecomella undulata* prepared in increasing and decreasing order of solvent polarity.
To 500 g of *Tecomella undulata* bark powder, 1500 ml of each solvent viz. hexane, chloroform, ethyl acetate, acetone, methanol and distilled water was added serially in increasing solvent polarity, and in reverse order for obtaining extracts in decreasing solvent polarity. Extraction with each solvent was allowed to carry out for 24 h at room temperature. After 24 h, the supernatant was recovered by filtering through Whatman No. 1 filter paper. The filtrate was concentrated by evaporating the supernatant in a rotary vacuum evaporator to obtain the crude extract. The process was repeated thrice with each solvent before proceeding with the same procedure with the next solvent in sequence. These extracts (prepared both in increasing and decreasing order of polarity) were stored at 4° C until used further for the evaluation of antimicrobial activity.

**Bacterial test organisms:** For evaluation of the antimicrobial response, the following twelve bacterial (five Gram positive and seven Gram negative), and fungal (eight) strains were used.


All the bacterial strains were procured from Microbial Type Culture Collection (MTCC), Chandigarh; the fungal strains used were either the indigenous strains maintained in the Department of Biotechnology, CDLU, Sirsa, Haryana, or were procured from Indian Agricultural Research Institute (IARI), Delhi.

**Antimicrobial assay**

The evaluation of antimicrobial activity of the various plant extracts was carried out using the agar well diffusion method [20]. In this method, 100 µl of 24 h old culture of the test organism was inoculated on the agar plates and then spread onto the surface of agar with the help of a
sterilized glass spreader. After 30 minutes of inoculation of the test microorganism, wells (5mm diameter) were prepared with the help of a sterilized steel cork borer. Out of five wells made in each plate, four were loaded with 60 µl of different concentrations of the test plant extract. Extraction solvent used as the negative control was loaded in the fifth well. Sixty µl each of standard antibiotics viz. ampicillin, penicillin, streptomycin, tetracycline, fluconazol, ketoconazole and miconazole were loaded in different wells in a separate plate and were used as positive control. The plates were then aerobically incubated at 30°C for 24 h for bacterial and at 25°C for 48 h for fungal test microorganisms. Antimicrobial activity was determined by measuring the diameter (in mm) of zone of inhibition and comparing the results obtained with those from the standard antibiotics. The diameter was measured at cross angles and mean of three independent measurements was taken.

**Minimum Inhibitory Concentration (MIC)**

Defined as the lowest concentration of the test sample that results in complete inhibition of visible growth, minimum inhibitory concentration (MIC) was determined by using the dilution method as recommended by the National Committee for Clinical Laboratory Standard [21]. Different concentrations (ranging from 25 µg/ml to 1000 µg/ml) of all the extracts prepared both in increasing and decreasing order of solvent polarity were tested separately for each microorganism species. A stock solution of each active extract was serially diluted in 96-well microtiter plate with Mueller Hinton broth to obtain a concentration ranging from 25 µg/ml to 1000 µg/ml (with a gap interval of 25 µg/ml). A standardized inoculum for each bacterial strain was prepared so as to give an inoculum size of approximately 5 x 10^5 Cfu/ml in each well. Microtiter plates were then kept at 37°C for an overnight incubation. Following incubation, MIC was calculated as the lowest concentration of the extract inhibiting visible growth of the bacterial strain. All experiments were carried out in triplicate.

**RESULTS AND DISCUSSION**

**Antimicrobial assay**

The results of antimicrobial response exhibited by different extracts and the standard antibiotics are summarized in figures 1 to 6. All the extracts prepared exhibited variable degree of antimicrobial activity against the tested microorganisms. However, the data indicates that the bark extracts prepared in organic solvents were more effective than the aqueous extracts. The aqueous extracts prepared both in increasing and decreasing order of
solvent polarity did not exhibit any antimicrobial activity against the tested microorganisms. On the other hand, the organic solvent extracts showed a remarkable activity against most of the tested microorganisms. The antimicrobial response of the extracts prepared in decreasing order of solvent polarity was slightly better than that of the extracts prepared in increasing order of solvent polarity (Figs. 1, 2, 3 and 4).

![Fig. 1. Antibacterial activity of various extracts of bark of *Tecomella undulata* prepared in the order of increasing solvent polarity.

- No activity was found in aqueous extract.]

![Fig. 2. Antibacterial activity of various extracts of bark of *Tecomella undulata* prepared in the order of decreasing solvent polarity.

- No activity was found in aqueous extract.]
Fig. 3. Antifungal activity of various extracts of bark of *Tecomella undulata* prepared in the order of increasing solvent polarity.

➢ No activity was found in aqueous extract.

Fig. 4. Antifungal activity of various extracts of bark of *Tecomella undulata* prepared in the order of decreasing solvent polarity.

➢ No activity was found in aqueous extract.

Among the five organic solvent extracts, chloroform and ethyl acetate fractions showed a higher zone of inhibition against the bacterial strains, followed by almost a comparable activity by the acetone and methanol fractions. Least activity was exhibited by the hexane fractions. Whereas, the antifungal activity was found to be strongest by the ethyl acetate extracts, followed by a similar response by the acetone and chloroform fractions, a slightly
lesser response by the methanol fractions, and a very low activity by the hexane fractions. Chloroform extracts exhibited a strong antimicrobial activity against all bacterial (Gram positive as well as Gram negative) and fungal (except \textit{A. vitis}) strains tested. The zone of inhibition for the bacterial strains was in the range of 8-23 mm, and for the fungal strains, it was in the range between 7-20 mm. Ethyl acetate extracts also showed a significant antimicrobial activity, exhibiting a response higher than that of chloroform extracts against the bacterial (forming a zone of inhibition in the range of 9-23 mm) and fungal (11-27 mm zone of inhibition) strains; but the response was observed against a fewer number of microorganisms as compared to the chloroform extracts. Two bacterial strains, \textit{viz.}, \textit{B. subtilis} and \textit{E. aerogenes} and one fungal strain, \textit{viz.}, \textit{A. alternata} were found to be resistant to the ethyl acetate extracts. Acetone and methanol extracts exhibited almost a similar pattern of antimicrobial activity, although the antibacterial activity was found to be weaker than the antifungal activity. The zone of inhibitions for the acetone and methanol extracts were observed to be in the range of 7-19 mm and 6-14 mm, respectively, against the bacterial strains; and between 9-27 mm and 10-23 mm, respectively, against the fungal strains. A few microorganisms, \textit{viz.}, \textit{S. aureus}, \textit{E. coli}, \textit{E. aerogenes}, \textit{S. epidermidis}, \textit{A. faecalis}, \textit{B. subtilis}, \textit{P. aeruginosa}, \textit{A. alternata} and \textit{A. brasicola} were found to be resistant to the two fractions. Compared to the other extracts, the hexane fractions were observed to be least effective (exhibiting a zone of inhibition in the range of 6-14 mm). Only 50\% of the microorganisms tested were found to be sensitive to the hexane extracts.

Among the bacterial strains tested, \textit{E. faecalis} was found to be the most sensitive strain (exhibiting a larger zone of inhibition against most of the extracts), whereas, \textit{S. aureus} was found to be the most resistant strain (forming a smaller zone of inhibition and against the least number of extracts. Among the fungal strains, most of the fungi showed almost a similar inhibition pattern, however, \textit{A. niger} and \textit{A. vitis} were more sensitive as compared to the other strains. \textit{A. alternata} and \textit{A. solani} were the most resistant fungi, showing least activity with the extracts. All the microorganisms were more sensitive to the ethyl acetate and chloroform extracts as compared to the other extracts. In previous studies also, ethanol extracts have been reported to be effective against several microorganisms \cite{22, 23}. It has been reported that the most active components are generally water insoluble, hence it is expected that low polarity organic solvents would yield more active extracts \cite{24}. On a similar pattern, in the present study also, the aqueous extract exhibited no antibacterial activity.
The effectiveness of the extracts against the various microorganisms was compared with the antimicrobial response shown by the antibiotics, *viz.*, ampicillin, penicillin, streptomycin, tetracycline, fluconazole, ketoconazole and miconazole used as standard drugs (Figs. 5 and 6). All the five organic solvent extracts showed a higher activity against several microorganisms when compared with ampicillin and penicillin, and with streptomycin to some extent. However, when compared to the response exhibited by tetracycline, only ethyl acetate and chloramphenicol extracts were found to be slightly better in case of some microorganisms. Among the three antifungal drugs tested, ketoconazole was found to be least effective, followed by a slightly higher response by miconazole, and the maximum activity was shown by fluconazole. Compared with the zone of inhibition formed by the standard drugs, all the solvent extracts of *T. undulata* showed an appreciably greater zone than that formed by ketoconazole. The antifungal response exhibited by the ethyl acetate, chloroform, methanol and acetone extracts against some of the fungi was also comparable to that of the other two standard drugs (miconazole and fluconazole).

**Fig. 5. Antibacterial activity of standard antibiotics**
The results in the present study thus show that *Tecomella undulata* extracts exhibited significant activity against most of the tested microorganisms which was comparable to that of the standard drugs.

**Minimum Inhibitory Concentration (MIC)**

All the active extracts (bark extracts prepared in organic solvents) were further subjected to determination of minimum inhibitory concentration, the results being shown in Table 1. The lowest MIC was exhibited by chloroform extracts against the tested bacterial strains; and by ethyl acetate extracts against the tested fungal strains (250-500 µg/ml), followed by the acetone and methanol (between 250-1000 µg/ml for most of the microorganisms) extracts. Hexane extracts exhibited comparatively higher MIC, indicating less effectiveness of these extracts. Among the various microorganisms tested, lowest MIC values were obtained for *E. faecalis*, followed by *E. coli* and *K. pneumoniae*, indicating that these bacteria were most sensitive to the *T. undulata* bark extracts. Among the various fungi, the most sensitive strain was *A. niger* having MIC values in the range of 250-500 µg/ml. The results of MIC assay confirmed the findings of antimicrobial assays, wherein it was reported that ethyl acetate and chloroform extracts were more potent inhibitors of the microorganisms tested; and that *E. faecalis* and *A. niger* were among the most sensitive strains.
Table 3. Minimum inhibitory concentration (MIC) values of different bark extracts of *T. undulata* against the tested bacterial and fungal strains.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Bacterial/ Fungal Strains</th>
<th>Minimum inhibitory concentration (µg/ml)</th>
<th>Hexane (I)</th>
<th>Hexane (D)</th>
<th>Chloroform (I)</th>
<th>Chloroform (D)</th>
<th>Ethyl acetate (I)</th>
<th>Ethyl acetate (D)</th>
<th>Acetone (I)</th>
<th>Acetone (D)</th>
<th>Methanol (I)</th>
<th>Methanol (D)</th>
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<td><strong>Bacterial Strains</strong></td>
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<td>6.</td>
<td><em>E. coli</em></td>
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<td><em>K. pneumoniae</em></td>
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<td>8.</td>
<td><em>M. luteus</em></td>
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<td>9.</td>
<td><em>P. aeruginosa</em></td>
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<td>10.</td>
<td><em>S. typhimurium</em></td>
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<td>11.</td>
<td><em>S. aureus</em></td>
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<td>12.</td>
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<td><em>A oryzae</em></td>
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<td><em>A alternata</em></td>
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<td>6.</td>
<td><em>A. brasicola</em></td>
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All the values are an average of three determinations

- No activity. (I) - increasing solvent polarity, (D) decreasing solvent polarity.
CONCLUSION
The present study scientifically validates the antimicrobial potential of the traditionally important plant, *Tecomella undulata*. The results provide an important basis for the use of ethyl acetate, chloroform, acetone and methanol extracts of the tested plant species for the treatment of infections associated with the pathogens used in this study, and for the development of new antimicrobial drugs; thus confirming the traditional therapeutic claims of this plant. However, further studies related to the isolation and identification of the particular compounds responsible for the antimicrobial activity are underway.

REFERENCES