Enumeration of Antibacterial Activity of Few Medicinal Plants by Bioassay Method

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Abstract: The present study was aimed to investigate the antibacterial activity of some common locally available plants, in order to estimate the biological potential of these herbs. The alcoholic extract of *Tagetes erecta* L. (Asteraceae), *Argemone mexicana* L. (Papaveraceae), *Datura stramonium* L. (Solanaceae) and *Tylophora indica* (Burm.f.) Merr. (Asclepiadaceae) were evaluated for antibacterial activity using broth dilution bioassay method. It is clear from the results that, the extracts of these plants acts as a good source of antibiotics against various bacterial pathogens tested and exhibited broad spectrum of antibacterial activity. These plant extracts were shown to be moderate to maximum inhibitory effect against different bacterial forms such as *Salmonella typhi*, *Proteus vulgaris*, *Pseudomonas aeruginosa* and *Escherichia coli*, where as, mild to moderate activity against *Klebsiella pneumoniae* and *Staphylococcus aureus*. The results of these studies revealed most valuable information and also support the continued sustainable use of these plants in traditional systems of medicine.

Keywords: Antimicrobial-bioassay method, *Argemone mexicana*, *Tylophora indica*, *Tagetes erecta*, *Datura stramonium*.

Introduction

It is evident that the use of plants for various diseases since from vedic period. documentation of the ayurvedic system recorded by Sushruta and Charaka dates from about 1000 BC¹. Plants are a goldmine of novel chemicals; much impressive number of modern drugs has been developed from them. There are more than 2,70,000 higher plants existing in this planet. But so far less than 10% of recorded flora has been explored phytochemically as well as clinical evaluation for various biological activities². While vast majority of the plant resource is waiting for discovery. So far there is no appropriate treatment in the modern system of medicine for many of the diseases such as, Cancer, AIDS, Jaundice, Hepatitis, SARS, Chikun-guniya and Diabetes. Some of the nucleotide analogues, the current antiviral drug use reverse transcriptase as target and are highly toxic compounds. Recently certain

polyphenolic and bioflavonoids have been found to be the potential source of reverse transcription inhibitors. Anti-hepatotoxicity, immunomodulatory, anti-inflammatory, antimicrobial, antifeedent and antioxidant activities are amongst other important properties of flavonoids³⁻⁴.

The green revolution in the industrialized world has convinced many companies that plant might also prove a gold mine profit, an endless source which leads to new drugs and environmental friendly pesticides. By considering these above and other factors the present investigation was undertaken to evaluate the antibacterial activity by bioassay method using the crude ethanolic extracts of *Tagetes erecta*, *Argemone mexicana*, *Datura stramonium* and *Tylophora indica*.

Experimental

The leaves of *Argemone mexicana*, *Tylophora indica*, *Tagetes erecta* and *Datura stramonium* were collected from in and around Bellary city, Karnataka, India. All these plants were authenticated⁵⁻⁷ and the voucher specimens were deposited in the Herbarium of Department of Botany, Gulbarga University, Gulbarga, India. Later leaves of these plants were subjected to surface sterilization using 50% alcohol and then shade dried for further analysis.

Preparation of the extracts

The 100 g leaves of *A. mexicana*, *T. indica*, *T. erecta* and *D. stramonium* were extracted with 300 mL of 95% alcohol at 60 - 80 °C in a soxhlet apparatus. The obtained extracts were collected in a separate container and concentrated to dryness in a flash evaporator (Buchi type) under reduced pressure and controlled temperature (40 - 50 °C).

Microorganisms

Clinical laboratory bacterial isolates of *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Salmonella typhi*, *Proteus vulgaris*, *Pseudomonas aeruginosa* and *Escherichia coli* clinical isolates were collected from the standard stock cultures of Microbiology Laboratory, Vijayanagar Institute of Medical Sciences, Bellary, India. These cultures were maintained on nutrient agar medium and were stored at 4 °C for determining antimicrobial activity of these selected medicinal plants.

Culture media

The nutrient agar and nutrient broth were purchased from HiMedia, Laboratories Limited, Mumbai. Streptomycin (powdered form) another standard antibiotic from Nanjing Asian Chemicals Co., Ltd. The solvents and other chemicals used were analytical grade.

Antimicrobial-bioassay method

Nutrient broth media was prepared and distributed into fourteen different test tubes, then autoclaved and cooled. To these tubes, 0.1 mL of 24 h old cultures of test microorganisms, different concentrations (0.8 mg/mL, 1.2 mg/mL/ and 1.6 mg/mL) of the of four plants extracts and 0.2 mg/mL of streptomycin (standard positive control) were added separately into thirteen different tubes, only the fourteenth tube was not treated with any of the drug and used as a negative control. These tubes were incubated at 37 °C for 24 h in a BOD incubator. Subsequently after 24 h 0.1 mL of formaldehyde solution was added to each inoculated tube as a growth stopping agent. Then the optical density of each tube was measured at 530 nm. Same procedure was repeated to all six different bacterial species separately. Nutrient medium devoid of plant extract and microorganisms was served as Blank⁸. This experiment was repeated thrice and found out the average mean and standard error using SPSS software package.

Results and Discussion

Antibacterial assay

In the present investigation, all these plants furnished evidence in favor of the antimicrobial activity. The alcoholic extract of the leaves of *Datura stramonium* was found to be most efficient in controlling the growth of certain bacterial forms such as *Salmonella typhii*, *Pseudomonas aeruginosa*, *Proteus vulgaris* and moderately effective against *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Escherichia coli* (Table 1). Similar observations were noticed with the extract of *Argemone mexicana*, but *Pseudomonas aeruginosa* was very much susceptible and highly sensitive to the extract of *A. Mexicana* (Table 2). Where as *S. typhii*, *P. vulgaris*, *P. aeruginosa* and *E. coli* were found to be highly sensitive, but *K. pneumoniae* and *S. aureus* were moderately inhibited to *Tagetes erecta* (Table 3). Where as, similar observations were noticed with *Tylophora indica*, except *S. aureus* was found to be less sensitive (Table 4).

Table 1. Evaluation of the antimicrobial bioassay of ethanolic extract of *Datura stramonium* L

Microorganisms	Optical density at λ_{530} nm				
	Control	Standard, 0.2 mg/mL	<i>D. stramonium</i> 0.8 mg/mL	<i>D. stramonium</i> 1.2 mg/mL	<i>D. stramonium</i> 1.6 mg/ml
<i>Klebsiella pneumoniae</i>	1.525 $\pm$ 0.089	0.410 $\pm$ 0.021	1.300 $\pm$ 0.028	1.196 $\pm$ 0.040	1.130 $\pm$ 0.028
<i>Staphylococcus aureus</i>	0.985 $\pm$ 0.044	0.108 $\pm$ 0.024	0.740 $\pm$ 0.005	0.636 $\pm$ 0.027	0.670 $\pm$ 0.005
<i>Salmonella typhii</i>	1.142 $\pm$ 0.1158	0.119 $\pm$ 0.033	0.568 $\pm$ 0.008	0.568 $\pm$ 0.008	0.5668 $\pm$ 0.008
<i>Proteus vulgaris</i>	1.189 $\pm$ 0.0800	0.223 $\pm$ 0.007	0.711 $\pm$ 0.005	0.701 $\pm$ 0.025	0.676 $\pm$ 0.005
<i>Pseudomonas aeruginosa</i>	1.788 $\pm$ 0.3085	0.449 $\pm$ 0.058	0.676 $\pm$ 0.002	0.660 $\pm$ 0.008	0.657 $\pm$ 0.002
<i>Escherichia coli</i>	1.125 $\pm$ 0.0649	0.212 $\pm$ 0.050	1.092 $\pm$ 0.001	1.091 $\pm$ 0.000	0.992 $\pm$ 0.001

Table 2. Evaluation of the antimicrobial bioassay of ethanolic extract of *Argemone mexicana* L

Microorganisms	Optical density at λ_{530} nm				
	Control	Standard 0.2 mg/mL	<i>A. mexicana</i> 0.8 mg/mL	<i>A. mexicana</i> 1.2 mg/mL	<i>A. mexicana</i> 1.6 mg/mL
<i>Klebsiella pneumoniae</i>	1.525 $\pm$ 0.089	0.410 $\pm$ 0.021	1.452 $\pm$ 0.226	1.084 $\pm$ 0.044	1.013 $\pm$ 0.076
<i>Staphylococcus aureus</i>	0.985 $\pm$ 0.044	0.108 $\pm$ 0.024	0.767 $\pm$ 0.003	0.739 $\pm$ 0.014	0.616 $\pm$ 0.007
<i>Salmonella typhii</i>	1.142 $\pm$ 0.1158	0.119 $\pm$ 0.033	0.737 $\pm$ 0.018	0.741 $\pm$ 0.008	0.720 $\pm$ 0.008
<i>Proteus vulgaris</i>	1.189 $\pm$ 0.0800	0.223 $\pm$ 0.007	0.812 $\pm$ 0.037	0.733 $\pm$ 0.013	0.606 $\pm$ 0.010
<i>Pseudomonas aeruginosa</i>	1.788 $\pm$ 0.3085	0.449 $\pm$ 0.058	0.448 $\pm$ 0.012	0.382 $\pm$ 0.004	0.347 $\pm$ 0.003
<i>Escherichia coli</i>	1.125 $\pm$ 0.0649	0.212 $\pm$ 0.050	0.986 $\pm$ 0.014	0.917 $\pm$ 0.024	0.888 $\pm$ 0.029

Table 3. Evaluation of the antimicrobial bioassay of ethanolic extract of *Tagetes erecta* L

Microorganisms	Optical density at λ_{530} nm				
	Control	Standard 0.2 mg/mL	<i>T. erecta</i> 0.8 mg/mL	<i>T. erecta</i> 1.2 mg/mL	<i>T. erecta</i> 1.6 mg/mL
<i>Klebsiella pneumoniae</i>	1.525 ± 0.089	0.410 ± 0.021	1.203 ± 0.001	1.120 ± 0.008	1.049 ± 0.004
<i>Staphylococcus aureus</i>	0.985 ± 0.044	0.108 ± 0.024	0.775 ± 0.009	0.643 ± 0.005	0.608 ± 0.008
<i>Salmonella typhi</i>	1.142 ± 0.1158	0.119 ± 0.033	0.811 ± 0.005	0.796 ± 0.007	0.773 ± 0.032
<i>Proteus vulgaris</i>	1.189 ± 0.0800	0.223 ± 0.007	0.654 ± 0.011	0.633 ± 0.006	0.604 ± 0.002
<i>Pseudomonas aeruginosa</i>	1.788 ± 0.3085	0.449 ± 0.058	0.650 ± 0.005	0.638 ± 0.012	0.537 ± 0.008
<i>Escherichia coli</i>	1.125 ± 0.0649	0.212 ± 0.050	0.911 ± 0.005	0.712 ± 0.005	0.707 ± 0.005

Table 4. Evaluation of the antimicrobial bioassay of ethanolic extract of *Tylophora indica* (Burm.f.) Merr

Microorganisms	Optical density at λ_{530} nm				
	Control	Standard 0.2 mg/mL	<i>T. indica</i> 0.8 mg/mL	<i>T. indica</i> 1.2 mg/mL	<i>T. indica</i> 1.6 mg/mL
<i>Klebsiella pneumoniae</i>	1.525 ± 0.089	0.410 ± 0.021	1.124 ± 0.026	1.170 ± 0.030	1.227 ± 0.204
<i>Staphylococcus aureus</i>	0.985 ± 0.044	0.108 ± 0.024	0.895 ± 0.045	0.856 ± 0.016	0.701 ± 0.004
<i>Salmonella typhi</i>	1.142 ± 0.1158	0.119 ± 0.033	0.537 ± 0.016	0.476 ± 0.007	0.424 ± 0.012
<i>Proteus vulgaris</i>	1.189 ± 0.0800	0.223 ± 0.007	1.107 ± 0.003	1.103 ± 0.002	1.080 ± 0.026
<i>Pseudomonas aeruginosa</i>	1.788 ± 0.3085	0.449 ± 0.058	0.517 ± 0.124	0.618 ± 0.007	0.560 ± 0.026
<i>Escherichia coli</i>	1.125 ± 0.0649	0.212 ± 0.050	1.159 ± 0.017	1.139 ± 0.055	1.089 ± 0.004

The present investigations revealed that, the different concentrations of the alcoholic extracts of *D. stramonium*, *T. indica*, *T. erecta* and *A. mexicana* were proved to be effective and concentration dependent antimicrobial activity against both Gram positive and Gram negative bacteria tested. This is also evidenced by several research groups, and supporting the presence of antibacterial activity of *D. stramonium* and *T. indica* against Gram positive bacteria by dose dependent manner⁹⁻¹². Where as, the inhibition of Gram positive and negative bacterial growth by *Argemone mexicana* and *Tagetes erecta* based on agar diffusion method¹³. So far there are no reports on the clinical antibacterial and antifungal activity on these plants.

Conclusion

In conclusion, the alcoholic extracts of these plants showed promising antimicrobial activity against all the selected pathogens. However, further experiments including detail evaluation of antimicrobial potentials are required to elucidate their mechanism of action at cellular and molecular levels.

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