Anti-enteric bacterial activity of the traditional medicinal plants of Kanyakumari coast, Tamilnadu, India

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Abstract

Objective: To evaluate the antimicrobial potentials of 6 traditionally used medicinal plants to treat gastrointestinal infection against pathogenic bacteria, as most of the pathogens develop drug resistance against commonly used antibiotics.

Methods: Crude extracts from different parts of different plants were tested against bacterial strains of clinical significance. Extraction of bioactive principles was done with water and ethanol. Evaluation of antibacterial activity was done by disc diffusion assay against selected bacterial stains.

Results: Of the 6 different plant materials tested, extracts prepared from Psidium guajava leaves showed significantly higher efficacy. Extracts prepared using alcohol exhibited higher antibacterial activity when compared to their corresponding aqueous extracts.

Conclusions: The findings of the present study suggested that phytochemical extracts of the presently studied plant materials possess significant anti-enteric bacterial activity, and thus lend pharmacological credibility to the suggested traditional use of the plant as a natural remedy for the treatment, management and/or control of gastrointestinal diseases in the coastal tracts of Kanyakumari district, Tamilnadu, India.

1. Introduction

Development and spread of drug-resistant pathogenic microorganisms create a challenge to public healthcare services. In particular, emergence of resistance to antibiotics has hampered the pace by which newer antibiotics are being introduced into the public domain[1]. This drives the discovery of novel antimicrobial therapeutic agents from the biological resources[2]. Global attention has been shifted towards hunting novel bio-molecules of biological origin for the development of new drugs. Despite ever increasing advancement in the field of medicine and molecular diagnosis, it is estimated that 80% of the world population is still dependent on plant derived pharmaceuticals. World Health Organization report depicted that plant based products or its derivatives account for nearly 28% of drugs available in the market[3]. A large proportion of plant-based compounds are used as lead molecules in drug discovery to produce synthetic molecular analogs that have similar skeletons yet intricate structures. This implicates that phytochemicals play a critical role in diversity-oriented synthesis of natural product-like pharma compounds[4]. Human beings have exploited the plants for curing ailments since antiquity. Tribal people acquire knowledge of medicine from their parents in non-coded form and practice it effectively. Ethnobotanical and ubiquitous plants serve as a rich resource of natural drugs for research and development[5-9]. Medicinal plant-based drugs owe the advantage of being simple, effective and exhibit broad spectrum activity[10-15]. Indigenous systems of medicine that use plant-based drugs could provide both concepts of therapy as well as therapeutic agents to complement modern medicine in management of diseases[16-21]. Considering the medicinal value of medicinal plants, present study evaluated the antibacterial potentials of aqueous and alcoholic extracts of Psidium guajava (P. guajava) leaves, Punica granatum (P. granatum) fruit rind, Aegle marmelos (A. marmelos) fruit pulp, Hemidesmus indicus (H. indicus) root, Mangifera indica (M. indica) seed kernel and Saraca asoca (S. asoca) stem bark against selected clinical bacterial strains. These plants were selected after repeated discussions and consultations with people and healers of Puthalam village, situated in the southwest coast of Kanyakumari district, Tamilnadu, India.

2. Materials and methods

2.1. Plant material

P. guajava leaves, P. granatum fruit rind, A. marmelos fruit pulp, H. indicus root, M. indica seed kernel and S. asoca stem bark
were collected from the fields in Puthalam village (N 08°06.086' and E 077°28.372'; altitude 17 m [mean sea level]), Kanyakumari district, Tamilnadu, India, and taken to the laboratory. Flora of the Presidency of Madras[22] and The flora of Tamilnadu Carnatic[23] were used for identification and authentication of the plants. Plant materials were washed separately under running tap water, followed by rinse using sterile distilled water. Excess of water was removed from the plant material using filter paper before they were used for powdering. Shade dried plant materials were powdered by making use of mechanical blender (Smith, India). Dried powder was stored in an airtight container and was used for extraction.

2.2. Preparation of extracts

The powdered plant material (150 g) was extracted with water and alcohol using cold maceration method. Both the extracts were filtered with a muslin cloth and the filtrate was concentrated in vacuum evaporator. Dried extracts were used for further studies.

2.3. Bacterial strains

A total of fourteen clinically isolated bacterial strains including both Gram-negative and Gram-positive bacteria like Escherichia coli (E. coli), Shigella sp., Salmonella typhi (S. typhi), Lactobacillus sp., Streptococcus pyogenes (S. pyogenes), Pseudomonas fluorescenc (P. fluorescenc), Enterobacter sp., Citrobacter sp., Xanthomonas campestris (X. campestris), Xanthomonas citri (X. citri), Pseudomonas aeruginosa (P. aeruginosa), Klebsiella sp., Streptococcus faecalis (S. faecalis) and Staphylococcus aureus (S. aureus) were selected to assess susceptibility patterns. The bacterial cultures were maintained in nutrient agar slants at 37 °C. Each of the microorganisms were freshly cultured prior to susceptibility testing by transferring them into a separate test tube containing nutrient broth and incubated overnight at 37 °C.

2.4. Antibacterial sensitivity assay of extracts

2.4.1. Preparation of disc

Known quantity of the extract was dissolved in dimethylsulfoxide:polybutylene succinate in the ratio of 1:1. It was then filtered by making use of syringe filter of pore size (0.42 µm). Sterile discs of 6 mm diameter were loaded with various concentrations of extracts and were dried. Dried discs were stored in sterile containers till use. Solvent loaded discs were also prepared and were used as negative control and tetracycline loaded discs were used as positive control.

2.4.2. Antibacterial assay

Petri plates containing 20 mL of nutrient agar were seeded with a 24 h old culture of the bacterial strain. Different concentrations (100 µg, 200 µg, 400 µg and 800 µg) of plant extracts were impregnated into the sterile 6 mm diameter discs. Discs were dried and dispensed on the solidified nutrient agar medium previously inoculated with test microorganisms. Tetracycline and vehicle loaded (dimethylsulfoxide:polybutylene succinate) discs were used as positive and negative control respectively. Then it was incubated at 37 °C for 24 h. The assessment of antibacterial activity was based on the measurement of zone of inhibition formed around the discs. Antibiotic zone scale was used to measure zone of inhibition (HIM edia, Mumbai).

2.4.3. Determination of minimum inhibitory concentration (MIC)

A agar dilution method was used to find out the MIC. MIC was recorded based on the growth of the test organism at the particular concentration.

2.5. Statistical analysis

All data were expressed as mean ± SD. Statistical analysis was performed by using origin version 6.0 software.

3. Results

Tribal and country people acquire incredible knowledge of herbal medicine from their parents in non-coded form. With the repeated discussions and consultation with people of East Puthalam village, Kanyakumari District, Tamilnadu, India 6 plant parts were selected to screen antibacterial activity. All these 6 plants have been traditionally used to treat gastrointestinal infections.

Antibacterial activity of aqueous and alcoholic extracts of 6 different plants were assessed by disc diffusion and drug dilution method. Aqueous extract of *P. guajava* exhibited best antibacterial activity against *Klebsiella* sp. and the zone of inhibition ranged from 20–23 mm. Similarly aqueous extracts of other plants also showed antibacterial activity and the zone of inhibition ranged from 12–15 mm for *H. indicus*, 15–21 mm for *M. indica*, 11–26 mm for *P. granatum*, 15–20 mm for *S. asoca* and 12–15 mm for *A. marmelos* (Table 1).

Table 2 reveals antibacterial potentials of alcoholic extracts of different plant materials. Among the microorganisms used, *S. faecalis* was susceptible to alcoholic extracts of all plants and the average zone of inhibition was 19.16 mm followed by *S. aureus* and *E. coli*.

| Test organisms | A. marmelos | H. indicus | M. indica | P. granatum | P. guajava | S. asoca |
|----------------|-------------|------------|-----------|-------------|------------|---------|
| E. coli        | 13.33 ± 1.15 | 14.66 ± 1.15 | 20.00 ± 2.00 | 21.66 ± 1.15 | 24.60 ± 1.52 | 18.00 ± 1.00 |
| Shigella sp.   | 12.66 ± 1.52 | 12.00 ± 2.00 | 21.00 ± 1.00 | 20.33 ± 2.08 | 21.33 ± 2.08 | 16.00 ± 1.73 |
| S. typhi       | 11.60 ± 0.57 | 13.60 ± 0.57 | 20.60 ± 0.57 | 20.60 ± 0.57 | 21.30 ± 0.57 | 15.30 ± 0.57 |
| Lactobacillus  | 11.60 ± 1.52 | 11.60 ± 1.15 | 16.00 ± 2.00 | 16.00 ± 3.60 | 13.00 ± 3.00 | 13.60 ± 3.05 |
| S. pyogenes    | 11.50 ± 2.12 | 13.30 ± 2.30 | 13.30 ± 2.80 | 20.00 ± 1.00 | 14.30 ± 1.15 | 16.00 ± 3.00 |
| P. fluorescens | 14.00 ± 1.00 | 13.00 ± 3.00 | 15.00 ± 2.64 | 18.60 ± 1.52 | 20.30 ± 0.57 | 15.30 ± 2.08 |
| Enterobacter sp. | 11.50 ± 2.12 | 13.00 ± 0.00 | 16.30 ± 1.52 | 16.30 ± 3.05 | 10.50 ± 0.70 | 14.60 ± 3.21 |
| Citrobacter sp. | 11.60 ± 2.08 | 12.30 ± 0.57 | 14.30 ± 1.52 | 18.30 ± 0.57 | 12.30 ± 2.08 | 19.00 ± 1.70 |
| X. campestris  | 12.50 ± 0.70 | 13.30 ± 1.52 | 19.00 ± 1.73 | 17.30 ± 1.15 | 16.00 ± 2.64 | 17.60 ± 0.57 |
| X. citri       | 11.30 ± 1.52 | 12.00 ± 0.00 | 15.00 ± 2.00 | 15.30 ± 0.57 | 15.60 ± 3.21 | 19.30 ± 2.51 |
| P. aeruginosa  | 12.60 ± 2.30 | 11.30 ± 1.52 | 13.00 ± 1.00 | 13.60 ± 4.61 | 17.00 ± 1.73 | 15.30 ± 2.08 |
| Klebsiella sp. | 12.60 ± 2.08 | 11.30 ± 1.15 | 12.30 ± 2.30 | 20.00 ± 1.00 | 23.60 ± 2.08 | 20.60 ± 0.57 |
| S. faecalis    | 13.00 ± 1.00 | 13.60 ± 1.15 | 16.60 ± 1.15 | 20.30 ± 0.57 | 19.60 ± 1.15 | 16.00 ± 1.00 |
| S. aureus      | 12.60 ± 0.57 | 15.00 ± 0.00 | 14.30 ± 1.15 | 21.00 ± 2.64 | 18.60 ± 2.30 | 17.00 ± 2.00 |
(18.66 mm each). The antibiotic tetracycline showed minimum zone of inhibition than that of plant extracts (average zone of inhibition 0–18 mm) and the difference was statistically significant. P. granatum extract produced (24.60 ± 3.21) mm inhibition zone against E. coli, whereas alcoholic extract of P. granatum exhibited best activity against Shigella sp. [(23.30 ± 1.52) mm].

MIC was used to confirm the antimicrobial nature of plant extracts. It also represented minimum quantity of antimicrobial compound required to kill or arrest multiplication of all microorganisms present in the medium or body fluid. Aqueous extract of H. indicus showed least MIC value [(841.66 ± 38.18) µg/mL] against Citrobacter, whereas P. granatum showed a MIC of (808.33 ± 38.18) µg/mL against Citrobacter sp. (Table 3). P. granatum extract also showed best MIC against Klebsiella sp. and S. aureus [(50.00 ± 25.00) µg/mL].

### Table 2

| Test organisms | Zone of inhibition |
|----------------|--------------------|
| A. marmelos  | 12.30 ± 2.08 |
| H. indicus   | 15.30 ± 2.08 |
| M. indica    | 15.60 ± 0.57 |
| P. granatum  | 1.16 ± 1.52 |
| P. guajava   | 13.60 ± 1.52 |
| S. asoca     | 14.00 ± 2.00 |
| Enterobacter sp. | 13.00 ± 1.41 |
| Citrobacter sp. | 12.50 ± 0.70 |
| X. campestris | 12.60 ± 1.52 |
| X. citri     | 16.00 ± 0.00 |
| P. aeruginosa | 16.00 ± 0.00 |
| Klebsiella sp. | 12.00 ± 2.82 |
| S. faecalis  | 16.30 ± 3.05 |
| S. aureus    | 11.50 ± 0.70 |

Data were expressed as mean ± SD.

### Table 3

| Test organisms | MIC value of aqueous extracts of the plant material |
|----------------|--------------------------------------------------|
| A. marmelos  | 766.66 ± 76.37 |
| H. indicus   | 758.33 ± 76.37 |
| M. indica    | 350.00 ± 50.00 |
| P. granatum  | 108.33 ± 38.18 |
| P. guajava   | 11.66 ± 44.33 |
| S. asoca     | 825.00 ± 50.00 |
| Enterobacter sp. | 758.33 ± 11.45 |
| Citrobacter sp. | 625.00 ± 66.14 |
| X. campestris | 725.00 ± 25.00 |
| X. citri     | 725.00 ± 25.00 |
| P. aeruginosa | 725.00 ± 25.00 |
| Klebsiella sp. | 725.00 ± 25.00 |
| S. faecalis  | 725.00 ± 25.00 |
| S. aureus    | 508.33 ± 25.00 |

### Table 4

| Test organisms | MIC value of alcoholic extracts of different plants (µg/mL) |
|----------------|----------------------------------------------------------|
| A. marmelos  | 608.33 ± 38.18 |
| H. indicus   | 575.00 ± 25.00 |
| M. indica    | 666.66 ± 28.86 |
| P. granatum  | 591.66 ± 38.18 |
| P. guajava   | 641.66 ± 14.40 |
| S. asoca     | 675.00 ± 25.00 |
| Enterobacter sp. | 475.00 ± 25.00 |
| Citrobacter sp. | 633.33 ± 28.86 |
| X. campestris | 608.33 ± 38.18 |
| X. citri     | 575.00 ± 25.00 |
| P. aeruginosa | 525.00 ± 25.00 |
| Klebsiella sp. | 508.33 ± 14.43 |
| S. faecalis  | 725.00 ± 25.00 |
| S. aureus    | 508.33 ± 14.43 |
The growth of \textit{S. flexneri}, most of the gastrointestinal infections.

Activity against \textit{Salmonella enteritis}, bacteria especially against pathogenic serogroups such as \textit{E. coli}, \textit{Salmonella}, \textit{Shigella}, \textit{Streptococcus}, and \textit{Pseudomonas} were the best for intestinal and invasive bacterium (Table 3).

Alcoholic extract of 6 different plants also showed good MIC value against all the microorganisms tested, than the aqueous extracts. \textit{P. granatum} arrested the growth of \textit{S. pyogenes} completely at a concentration of (25.00 ± 0.00) µg/mL. Least MIC value was exhibited by \textit{H. indicus} against \textit{E. coli} (285.00 ± 25.00) µg/mL MIC value. Among all the plants tested, \textit{H. indicus} and \textit{P. guajava} leaf alcoholic extract showed best activity (Table 4).

4. Discussion

All 6 plants used in the study were tested for various infections especially gastrointestinal infections. The results of present antibacterial study support the use of medicinal plants as anti-diarrhoeal, astrigent and anti-inflammatory. M. indica seed kernel, \textit{P. guajava}, \textit{S. asoca} stem bark, \textit{H. indicus} root and \textit{A. marmelos} fruit pulp extracts. Gram-negative bacteria were found more susceptible as compared to Gram-positive species. The efficacies of plant extracts were higher than the standard. Herbal medicines are a valuable and readily available resource for primary healthcare and complementary healthcare systems. Unfortunately, many species of plants containing substances of medicinal value have yet to be discovered, though large numbers of plants are constantly being screened for their antimicrobial effects. It has been suggested that phytochemical extracts from plants hold promise to be used in allopathic medicine as they are potential sources of antiviral, antitumoral and antimicrobial agents.

Conflict of interest statement

We declare that we have no conflict of interest.

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