SCREENING OF SOME MEDICINAL PLANTS USED IN NEPALESE TRADITIONAL MEDICINE AGAINST ENTERIC BACTERIA

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Abstract: Antibacterial properties of ethanolic extracts of 8 medicinal plants used in Nepal to treat gastrointestinal disorders were tested against ten different species of enteropathogenic bacteria: Escherichia coli, Klebsiella spp, Citrobacter spp, Enterobacter spp, Salmonella typhi, Salmonella paratyphi, Shigella spp, Proteus vulgaris, Proteus mirabilis and Pseudomonas spp. Among the selected medicinal plants: Punica granatum, Woodfordia fruticosa, Psidium guajava and Syzygium cumini were found effective against all enteric bacteria whereas Mimosa pudica, Acorus calamus, Aegle marmelos and Anethum somniferum were found ineffective against all. The minimum bactericidal concentration (MBC) of these plant extracts found against Salmonella typhi, Salmonella paratyphi, Proteus mirabilis and Proteus vulgaris were lower (0.39-25mg/ml) so are more susceptible whereas the plants showed lethal effect against Pseudomonas spp, Citrobacter spp, Enterobacter spp, E. coli, Shigella spp. and Klebsiella spp. at MBC value of around 25-50mg/ml.

Key words: Medicinal plants; Antibacterial activity; Plant extracts.

1. INTRODUCTION

Many efforts have been made to discover new antimicrobial compounds from various kinds of sources such as microorganisms, animals, and plants. One of such resources is folk medicines. Systematic screening of them may result in the discovery of novel effective compounds (Tomoko et al., 2002). Medicinal plants have been used as traditional treatments for numerous human diseases for thousands of years. Diarrhoeal diseases continue to be a major cause of morbidity and mortality throughout the world. Thus, their treatment by using medicinal plant is an important public health issue. Medicinal properties of plants are due to the active chemical constituents present in different parts of the plant (Mitscher et al., 1980).

Even though pharmacological industries have produced a number of new antibiotics in the last three decades, resistance to these drugs by microorganisms has increased (Nascimento et al., 2000). The increasing prevalence of multidrug resistant strains of bacteria and the recent appearance of bacteria with reduced susceptibility to antibiotics raises the specter of untreatable bacterial infections and adds urgency to the search for new infection-fighting strategies (Sieradzki et al., 1999). According to WHO, medicinal plants would be the best source to obtain a variety of drugs. Contrary to the synthetic drugs, antimicrobials of plant origin are not associated with many side effects and have an enormous therapeutic potential to heal many infectious diseases (Iwu et al., 1999).

In Nepalese traditional medicine, there is a rich local ethnomedical bibliography describing the species most frequently used by the population to cure gastrointestinal, respiratory, urinary and skin infections (HMG, 1993; Rajbhandari, 2001). However, there is a lack of experimental scientific studies confirming the possible antibiotic properties of a great number of these remedies. In vitro antifungal screening methods provide the required preliminary observations to select, among the crude plant products, those with potentially useful properties for further chemical and pharmacological studies.

The eight plant species here selected are reported to be used for the treatment of diarrhea, dysentery, cholera, fever and other gastrointestinal disorders classified under Medicinal plants of Nepal (HMG, 1993) and Ethnobotany of Nepal (Rajbhandari, 2001).

Based on local use for common diseases and ethnomedical knowledge, an attempt to assess the antibacterial property of medicinal plants was made in this study.

2. MATERIALS AND METHODS

2.1 Plant material

Different parts of selected medicinal plants were collected from different parts of Kathmandu Valley and were identified according to various literatures like Medicinal plants of Nepal by HMG/N (1993), Ethnobotany of Nepal (Rajbhandari, 2001) and including other pertinent taxonomic literature. The list of

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medicinal plants, their corresponding part used in this study, month of sample collection and location/place of the sample plants was given in the Table 1.

2.2 Preparation of extracts

Plant materials were dried in the dark at room temperature, powdered and extracted by soxhlet extraction method using ethanol as solvent. Afterwards, the solvent was distilled under reduced pressure in a rotary vacuum evaporator until the extracts became dry. The percentage yield for each extract was determined and the crude extract was then transferred in a bottle by sterile spatula. One-gram crude ethanol extract from each medicinal plant was mixed with 9ml of sterile distilled water in test tube & vortexed to make homogenous mixture/solution/suspension of 1g/10ml i.e. 100mg/ml working suspension or solution and stored in a refrigerator (2-8°C).

2.3 Microorganisms

The strains of microorganisms employed were *Escherichia coli*, *Klebsiella* spp, *Citrobacter* spp, *Enterobacter* spp, *Salmonella* typhi, *Salmonella* paratyphi, *Shigella* spp, *Proteus vulgaris*, *Proteus mirabilis* and *Pseudomonas* spp. All the microorganisms were maintained at 4 °C on nutrient agar slants.

2.4 Antimicrobial activity

The crude extract of medicinal plant was screened for its antibacterial activity i.e. determination of zone of inhibition against tested organism by agar well diffusion method as given by Dingle et al (1953). The crude extracts which showed antibacterial activity were subjected to two-fold serial dilution method to determine minimum bactericidal concentration (MBC). The fresh bacterial culture comparable with turbidity standard was prepared and swabbed on the surface of Muller-Hinton agar plates. Wells of diameter 6mm were made in the inoculated media plate. To evaluate the efficiency of the methodology, 50µl of each working suspension of extract was transferred into the well and the plates were incubated at 37°C for 24 hours. After this period, it was possible to observe zone of inhibition. Overall, cultured bacteria with halos greater than 8mm were considered susceptible to the tested extract. The extracts which showed antibacterial activity were subjected to two-fold serial dilution method to determine MBC.

3. RESULT AND DISCUSSION

The percentage yields of the ethanol crude extract of medicinal plants as obtained by soxhlet extraction process are shown in the figure 1. The amount of crude extracts varied among the medicinal plants. *Punica granatum* gave the highest yield (25.30%) and lowest yield was obtained from *Anethum sowa* (7.59%).

The data pertaining to the antibacterial potential of the plant extracts are presented in Table 2. In addition inhibition zone formed by extracts and MBC value against enteric bacteria are listed in Table 3. It was revealed from the result that each medicinal plant shows different degree of inhibition against different microorganisms. The diameter of zone of inhibition produced depends on several factors broadly classified as extrinsic and intrinsic parameters. The extrinsic parameters like pH of the medium, period and temperature of incubation, volume of the well, concentration of plant extracts and size of inoculums can be fixed and standardized during experiment, hence no error results due to extrinsic factors. However, intrinsic factors such as nature of medicinal plants including its components, solubility and diffusing property are predetermined. Due to variable diffusibility, the antibacterial with very high potency may not demonstrate a ZOI commensurate to its efficacy (Prasai et al, 2004).

The maximum zone of inhibition was observed in case of *Proteus mirabilis* (31mm) due to the action of *Syzygium cumini* and minimum was against *Pseudomonas* spp. (10mm) shown by *Psidium guajava*. The results revealed variability in the bactericidal concentrations of each extract for given bacteria. The minimum bactericidal concentration (MBC) of these plant extracts found against *Salmonella typhi*, *Salmonella paratyphi*, *Proteus mirabilis* and *Proteus vulgaris* were lower (0.39-25mg/ml) so are more susceptible whereas the plants showed lethal effect against *Pseudomonas* **

![Figure 1: Percentage yields of ethanol crude extracts of medicinal plants](image-url)
spp, \textit{Citrobacter} spp, \textit{Enterobacter} spp, \textit{E. coli}, \textit{Shigella} spp. and \textit{Klebsiella} spp. at MBC value of around 25-50mg/ml. The zone of inhibition (ZOI) and minimum bactericidal concentration (MBC) are two different attributes and there is absence of linear relationship between ZOI and MBC values.

As can be seen from Table 2, only four of plant extracts viz. \textit{Punica granatum}, \textit{Woodfordia fruticosa}, \textit{Psidium guajava} and \textit{Syzygium cumini} showed an antimicrobial effect against all ten enteric bacteria. Antibacterial activity due to \textit{Punica granatum} was previously reported. (Perez and Anesini, 1994; Bhatta, 1998; Rani and Khullar, 2003; Alanis et al, 2005) Similarly, activity due to \textit{Psidium guajava} (Ahmad & Beg, 2001; Holetz et al 2002), \textit{Syzygium cumini} (Nascimento et al, 2000; Baidya, 2001) and \textit{Woodfordia fruticosa} (Timsina, 2003) was also reported.

Considering that in this study only crude ethanolic extracts

\begin{table}
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\begin{tabular}{|c|c|c|c|c|c|c|}
\hline
S.N & Local Name & Botanical Name & Part used & Month of collection & Location /District \\
\hline
1 & Saunp & \textit{Anethum sowa} & seeds & April & Market /Kathmandu \\
2 & Bel & \textit{Aegle marmelos} & Fruit & May & Rudrapipal/Baglung \\
3 & Anar & \textit{Punica granatum} & rind of fruit & June & Market /Kathmandu \\
4 & Dhanvero & \textit{Woodfordia fruticosa} & leaves & May & Godawari/Lalitpur \\
5 & Laijawati & \textit{Mimosa pudica} & leaves & June & Khumaltar/Lalitpur \\
6 & Guava & \textit{Psidium guajava} & leaves & June & Tinmi/Bhaktapur \\
7 & Jamun & \textit{Syzygium cumini} & leaves & June & Chyasal/Lalitpur \\
8 & Bojho & \textit{Acorus calamus} & rhizome & May & Godawari/Lalitpur \\
\hline
\end{tabular}
\caption{List of medicinal plants used in antimicrobial assay.}
\end{table}

\begin{table}
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\begin{tabular}{|c|c|c|c|c|c|c|c|c|c|}
\hline
S.N & Medicinal Plants & \textit{E. coli} & \textit{Klebsiella} spp. & \textit{Citrobacter} spp. & \textit{Enterobacter} spp. & \textit{Salmonella typhi} & \textit{Salmonella paratyphi} & \textit{Shigella} spp. & \textit{Proteus vulgaris} & \textit{Proteus mirabilis} & \textit{Pseudomonas} spp. \\
\hline
1 & \textit{Anethum sowa} & - & - & - & - & - & - & - & - & - \\
2 & \textit{Aegle marmelos} & - & - & - & - & - & - & - & - \\
3 & \textit{Punica granatum} & + & + & + & + & + & + & + & + \\
4 & \textit{Woodfordia fruticosa} & + & + & + & + & + & + & + & + \\
5 & \textit{Mimosa pudica} & - & - & - & - & - & - & - & - \\
6 & \textit{Psidium guajava} & + & + & + & + & + & + & + & + \\
7 & \textit{Syzygium cumini} & + & + & + & + & + & + & + & + \\
8 & \textit{Acorus calamus} & - & - & - & - & - & - & - & - \\
\hline
\end{tabular}
\caption{Antibacterial activity caused by plant extracts through agar diffusion method.}
\end{table}

\begin{table}
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\begin{tabular}{|c|c|c|c|c|c|c|c|}
\hline
S.N & Test organisms & \textit{Punica granatum} & \textit{Psidium guajava} & \textit{Syzygium cumini} & \textit{Woodfordia fruticosa} \\
\hline
1 & \textit{E. coli} & ZOI (mm) & MBC (mg/ml) & ZOI (mm) & MBC (mg/ml) & ZOI (mm) & MBC (mg/ml) \\
2 & \textit{Klebsiella} spp. & 21 & 50 & 16 & 50 & 21 & 50 & 16 & 50 \\
3 & \textit{Citrobacter} spp. & 27 & 6.25 & 25.5 & 50 & 25 & 50 & 20 & 50 \\
4 & \textit{Enterobacter} spp. & 25 & 50 & 19.5 & 50 & 22.5 & 50 & 18.5 & 50 \\
5 & \textit{Salmonella typhi} & 19 & 3.12 & 13 & 6.25 & 10.5 & 3.12 & 26.5 & 1.56 \\
6 & \textit{Salmonella paratyphi} & 25 & 1.56 & 20 & 3.12 & 20 & 0.39 & 15 & 25 \\
7 & \textit{Shigella} spp. & 23 & 50 & 14 & 50 & 19.5 & 50 & 15 & 25 \\
8 & \textit{Proteus vulgaris} & 16 & 6.25 & 15 & 25 & 18 & 1.56 & 22 & 1.56 \\
9 & \textit{Proteus mirabilis} & 30 & 12.5 & 24 & 12.5 & 31 & 12.5 & 23 & 25 \\
10 & \textit{Pseudomonas} spp. & 11 & 50 & 10 & 50 & 11.5 & 25 & 15 & 50 \\
\hline
\end{tabular}
\caption{Antimicrobial activity of crude ethanol extracts.}
\end{table}
were employed and that Gram-negative organisms are in general terms more resistant than Gram positive ones to antimicrobial agents, we considered a strong response to exist when the extracts produced an effect at concentrations of 25mg/ml or below for enteric bacteria. Thus Salmonella typhi, Salmonella paratyphi, Proteus mirabilis and Proteus vulgaris are more susceptible having MBC value (0.39-25mg/ml) whereas the plants showed lethal effect against Pseudomonas spp, Citrobacter spp, Enterobacter spp, E. coli, Shigella spp and Klebsiella spp. at MBC value of around 25-50mg/ml.

The results obtained indicate the existence of antimicrobial compounds in the crude ethanolic extracts of these plants and show a good correlation between the reported uses of these plants in Medicinal plants of Nepal against different diseases and the experimental data of such extracts toward the most common pathogens. The cidal activities of medicinal plants like Punica granatum, Woodfordia fruticosa, Psidium guajava and Syzygium cumini are due to the active constituents present in them. The compounds like ellagittannins and alkaloids in the rind of fruit Punica granatum, flavonoids and tannins in leaf of Syzygium cumini; comarins, essential oils, flavonoids, triterpenes and ellagittannins in leaf of Psidium guajava and tannins, flavonoids, anthraquinone glycosides and polyphenols in Woodfordia fruticosa have been reported (Nascimento et al 2000). Other four plants namely Mimoso pudica, Acorus calamus, Aegle marmelos and Anethum sowa, though were selected on the basis of their use in common diseases like diarrhea, dysentery, fever etc, they didn’t show antimicrobial activity against enteric bacteria isolates. This can be due to the inability of water to dissolve the active components of alcoholic extracts of the plants during preparation of working solution or microorganisms used are not susceptible to these extracts.

The results of the present study provide a scientific validation for the popular use of the medicinal plants studied and serve as a guide which may help in selection of plants with antimicrobial activities for further phytochemical work on the isolation and the identification of the active compounds.

4. CONCLUSIONS

Result express that plant extracts have great potential as antimicrobial compounds against microorganisms. Thus, they can be used in the treatment of infectious diseases. Punica granatum, Woodfordia fruticosa, Psidium guajava and Syzygium cumini extracts possess a broad spectrum of activity against a panel of bacteria responsible for the most common bacterial diseases. These promissory extracts open the possibility of finding new clinically effective antibacterial compounds.

5. ACKNOWLEDGEMENTS

Acknowledgements are due to Nepal Academy of Science and Technology, Khumaltar for the laboratory facilities and instruments provided by them.

6. REFERENCE


