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Evaluation of Antibacterial Activities of Medicinal Plants

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Abstract

Plant and plant products are used as medicine from the beginning of human civilization. This study compares the antibacterial activity of crude hexane, ethylacetate and methanol extracts of nine different medicinal plants used in traditional Nepalese medicine, tested against 10 species of bacteria: *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 20063), *Klebsiella oxytoca, Proteus mirabilis (ATCC 49132), Proteus vulgaris, Pseudomonas aeruginosa* (ATCC 27853), *Salmonella typhi, Salmonella paratyphi* and *Shigella dysenteriae* by agar well diffusion method. The selected parts of these medicinal plants namely *Acorus calamus* (Rhizome), *Aegle marmelos* (Fruit), *Asparagus racemosus* (Tuberous root), *Mimosa pudica* (Root), *Terminalia bellirica* (Fruit), *Terminalia chebula* (Fruit), *Tinospora cordifolia* (Stem), *Woodfordia fruticosa* (Flower) and *Holarrhena antidysenterica* (Seed) were taken for study.The result showed that out of nine tested plants, four plant extracts (44%) showed activity against at least five or more tested bacteria and five plant extracts (56%) were active against three or less than three bacteria. None of the tested plant extracts was active against all the tested bacteria. *A. racemosus* was the least effective against bacterial species. *S. aureus* was the most susceptible bacteria being sensitive to 18 extracts from 9 medicinal plants. *P. vulgaris* was the most resistant bacteria being resistant to all selective plants. The MBC value ranges from 3.12 mg/ml to >50 mg/ml. Lowest MBC was shown by ethylacetate extract of *T. bellirica* against *E. coli* and ethylacetate extract of *W. fruticosa* against *S. dysenteriae*. Largest ZOI (31 mm) was produced by ethylacetate extract of *T. bellirica*.

Key words: antibacterial activity, medicinal plants, plant extracts

Introduction

Plants and plant products have been a source of food and medicines from the dawn of human civilization. The earliest mention of medicinal use of plants on Indian subcontinent has been found in the Rigveda which was written between 4500-1600 BC (HMG 1993). Nepal Himalaya, globally significant and biologically diverse ecosystem is rich in varieties of medicinal plants. Among the 7000 species of medicinal plants recognized all over the world, more than 900 types of precious medicinal plants are said to be found in Nepal (Manandhar 2000). In Nepal, about 75-80% of the rural populations use these traditional remedies (HMG 2007). The knowledge about the use of medicinal plants is deeply rooted in the traditional and culture of Nepalese people living in rural areas (Rijal 1994).

Infectious diseases caused by baceria, fungi, viruses and parasites are still a major threat to public health, despite the tremenodous progress in human medicine. The clinical efficacy of many existing antibiotics is being threatened by the emergence of multi-drug resistant pathogens (Kumar *et al*. 2002). Bacterial and fungal pathogens have evolved numerous defense mechanisms against anti-microbial and resistance to old and newly produced drugs is on rise. This increase has been attributed to indiscriminate use of broad-spectrum antibiotics and immunosuppressive agent (Kokoska *et al*.2002). In addition, in developing countries, synthetic drugs are not only expensive and inadequate for the treatment of diseases but also often with adulterations and side effects. Therefore, there is

need to search new infection- fighting strategies to control microbial infections (Sieradzki *et al*. 1999). Innumerable biologically active compounds are found in plants that posses antimicrobial properties which represent a vast untapped source of medicines (Branter & Grein 1994). These active compounds are found in various parts such as root, shoot, bark, fruit and sometime throughout whole plant.

The aim of this study was to evaluate the antimicrobial activity of some medicinal plants selected on the basis of their common use in Ayurveda and among the different ethnic groups of Nepal for common disorder. Therefore, extracts of the following 9 medicinal plants were tested for their potential activity against microbial pathogens: *Acorus calamus, Aegle marmelos, Woodfordia fruticosa, Tinospora cordifolia, Asparagus racemosus, Terminalia chebula, Terminalia bellirica, Mimosa pudica* and *Holarrhena antidysenterica.*

Methodology

This study was conducted from June 2007 to July 2008, partly in the Central Department of Microbiology, T.U and partly in the Central Department of Chemistry, T.U., Kirtipur. The extraction part of the study was carried out at the Central Department of Chemistry and antibacterial activity of the plants was carried out in laboratory of Central Department of Microbiology.

Collection of medicinal plants

The selected nine medicinal plants were collected from different parts of Nepal. They were identified according to the description given on different books viz: Flora of Kathmandu valley (1986), Flora of British India (1992) and other pertinent taxonomic literature. Collected samples were then chopped into small fragments and dried. Then samples were ground to obtain fine powder.

Table 1. List of medicinal plants used in the evaluation of antimicrobial activities

Medicinal plants	Family	Part(s) used	Place of collection	Traditional uses
Acorus calamus	Araceae	Rhizome	Kirtipur	Remitted fevers, cough, bronchitis, sore throat, diuretic, epilepsy, helminthiasis (Baral & Kurmi 2006, Rajbhandari *et al.* 1995)
Aegle marmelos	Rutaceae	Fruit	Surkhet	Diarrhea, dysentery, laxative, digestive (Baral & kurmi 2006)
Asparagus racemosus	Liliaceae	Tuberous root	Chitwan	Diarrhea, dysentery, leucorrhoea, uterine disorder, ulcerated tongue, jaundice, fever, galactogogue, diuretic (Joshi 2006)
Mimosa pudica	Leguminosae	Root	Nawalparasi	Diarrhea, dysentery, leucorrhea, hydrocele, piles, fistula, wound infection (Joshi 2006)
Terminalia bellirica	Combretaceae	Fruit	Dhankuta	Diarrhea, leprosy, fever, headache, dyspepsia, biliousness, dropsy, laxative, tonic (HMG/N 1993)
Terminalia chebula	Combretaceae	Fruit	Dhankuta	Cough, bronchitis, ulcers, wound infection, swelling, skin and eye diseases, chronic and recurrent fever, diuretic, digestive, ulceration of gum, laxative (Joshi 2006, HMG/N 1993)
Tinospora cordifolia	Menispermaceae	Stem	Chitwan	Fever, skin diseases, leprosy, splenopathy, , jaundice, cough, asthma, uropathy, gonorrhea, gout, tonic, expectorant, immunomodulator (Baral & Kurmi 2006, Joshi 2006)
Woodfordia fruticosa	Lythraceae	Flower	Kirtipur	Antibacterial, diarrhea, dysentery, helminthiasis, leprosy , ulcers, hepatopathy, haemorrhoids, skin diseases, foul ulcers, uterine sedative (Baral & Kurmi 2002 , Rajbhandari *et al.* 1995)
Holarrhena antidysenterica	Apocynaceae	Seed	Kavre	Diarrhea , dysentery, eczema, piles, leprosy, helminthiasis , febrifuge, carminative, astringent (IUCN 2000)

Extraction of plant materials

Finely powdered plant parts were subjected to continuous extraction from three different solvents viz hexane, ethylacetate and methanol in the order of increasing polarity, by using soxhlet apparatus to obtain crude extract viz hexane extract, ethylacetate extract and methanol extract respectively. After complete extraction, the solvent will be totally removed

by using rotary evaporator and these extracts were separately assayed for their antibacterial activity.

Prepation of stock/working solution

Stock solution (100 mg/ml) of each crude extract was made by dissolving 1 g of extract in 10 ml of dimethyl sulphoxide (DMSO) in clean and capped test tubes, which were sealed and stored in refrigerator (2-8°C) until use.

Collection of standard culture

The hexane, ethylacetate and methanol extracts of medicinal plants were screened against a total of 10 bacterial strains: *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 20063), *Klebsiella oxytoca, Proteus mirabilis* (ATCC 49132), *Proteus vulgaris, Pseudomonas aeruginosa* (ATCC 27853), *Salmonella typhi, Salmonella paratyphi* and *Shigella dysenteriae.*

Antibacterial activity assay

The antibacterial activity of crude extract of medicinal plants was screened against the test organism by agar well diffusion method as given by Dingle *et al.* (1953). The bacterial isolates were first grown in a nutrient broth and standardized to 0.5 Mc Farland (10^6 CFU/ml). Inoculums containing 10^6 CFU/ml of bacteria were

Table 2. Antibacterial activities of extracts of medicinal plants against tested bacteria

S. No.	Plants	Extracts	ZOI (mm) shown by plants against tested bacteria									
			E. coli (ATCC 25922)	*S. aureus* (ATCC 25923	*K. pneumoniae* (ATCC 20063)	*K.oxytoca*	*S. Typhi*	*S. paratyphi*	*P. aeruginosa* (ATCC 49132)	*P. mirabilis* (ATCC 49132)	*P. vulgaris*	*S. dysenteriae*
1.	*A.calamus*	He	-	13	-	-	-	-	-	-	-	-
		Ea	13	18	-	-	-	-	-	-	-	17
		Me	-	12	-	-	-	-	-	-	-	-
2	*H. antidysentrica*	He	11	-	-	-	-		-	-	-	-
		Ea	-	-	-	-	-	-	-	11	-	17
		Me	-	12	-	10	-	-	11	-	-	-
3	*W. fructicosa*	He	-	-	-	-	-	-	-	-	-	14
		Ea	26	20	-	-	15	14	-	-	-	26
		Me	-	16	12	-	-	-	-	-	-	23
4	*T. bellerica*	He	-	-	-	-	-	-	-	-	-	-
		Ea	31	18	-	13	18	-	15	-	-	17
		Me	21	19	-	-	-	-	-	-	-	19
5	*T. chebula*	He	-	11	-	-	-	-	-	-	-	-
		Ea	13	19	-	12	-	18	-	-	-	21
		Me	-	21	-	-	-	-	-	-	-	22
6	*T. cordifolia*	He	-	-	-	-	-	-	13	-	-	-
		Ea	-	-	-	-	-	-	-	-	-	-
		Me	-	-	-	14	-	-	-	13	-	-
7	*M. pudica*	He	-	10	-	-	-	-	-	-	-	-
		Ea	-	19	-	-	-	-	-	-	-	-
		Me	-	21	-	16	-	17	-	-	-	-
8	*A. racemosus*	He	-	-	-	-	-	-	-	-	-	-
		Ea	-	13	-	-	-	-	-	-	-	-
		Me	-	-	-	-	-	-	-	13	-	-
9	*A. marmelos*	He	-	13	-	-	-	-	-	-	-	-
		Ea	-	16	12	-	-	-	-	-	-	18
		Me	-	12	12	-	-	-		-	-	20

Note: He = Hexane, Ea = Ethylacetate, Me = Methanol

spread on the solid Muller-Hinton Agar (MHA) plates with a sterile cotton swabs moistened with the bacterial suspension. Wells were made in plates using sterile cork borer (8 mm diameter). The working suspension/ solution (100µl) of different medicinal plant extracts (100mg/ml) and same volume of DMSO as a control were filled in the wells with the help of micropipettes. The plates were left for sometime so that the extracts diffused into media and they were incubated at 37°C for 24 hours. After overnight incubation, the plates were observed for zone of inhibition (ZOI) and diameter of ZOI were scaled and were recorded. The triplicate assay was performed in case of the presence of Zone of inhibition. Then the mean of ZOI was recorded.

Determination of minimum bactericidal concentration (MBC)

The crude extracts of different fractions of medicinal plants, which showed antibacterial activity were then subjected to two fold serial dilution method as described by Baron *et al*. (1994) to determine minimum bactericidal concentration (MBC).

Results and Discussion

In this study, 27 extracts obtained by continuous extraction from 9 different plants using hexane, ethylacetate and methanol solvents in the order of increasing polarity were studied for their antimicrobial

Table 3. Minimum bactericidal concentration (MBC) shown by plants against tested bacteria

S. No.	Plants	Extracts	MBC (mg/ml) shown by plants against tested bacteria									
			E. coli (ATCC 25922)	*S. aureus* (ATCC 25923	*K. pneumoniae* (ATCC 20063)	*K.oxytoca*	*S. Typhi*	*S. paratyphi*	*P. aeruginosa* (ATCC 49132)	*P. mirabilis*	*P. vulgaris*	*Sh. dysenteriae*
1.	*A.calamus*	He	-	25	-	-	-	-	-	-	-	-
		Ea	50	12.5	-	-	-	-	-	-	-	25
		Me	-	25	-	-	-	-	-	-	-	-
2	*H. antidysentrica*	He	25	-	-	-	-		-	-	-	-
		Ea	-	-	-	-	-	-	-	50	-	6.25
		Me	-	50	-	25	-	-	25	-	-	-
3	*W. fructicosa*	He	-	-	-	-	-	-	-	-	-	50
		Ea	12.5	12.5	-	-	25	25	-	-	-	3.12
		Me	-	25	50	-	-	-	-	-	-	6.25
4	*T. bellerica*	He	-	-	-	-	-	-	-	-	-	-
		Ea	3.12	6.25	-	25	6.25	-	50	-	-	25
		Me	12.5	12.5	-	-	-	-	-	-	-	12.5
5	*T. chebula*	He	-	>50	-	-	-	-	-	-	-	-
		Ea	25	12.5	-	50	-	12.5	-	-	-	12.5
		Me	-	6.25	-	-	-	-	-	-	-	12.5
6	*T. cordifolia*	He	-	-	-	-	-	-	50	-	-	-
		Ea	-	-	-	-	-	-	-	-	-	-
		Me	-	-	-	25	-	-	-	25	-	-
7	*M. pudica*	He	-	50	-	-	-	-	-	-	-	-
		Ea	-	25	-	-	-	-	-	-	-	-
		Me	-	25	-	25	-	50	-	-	-	-
8	*A. racemosus*	He	-	-	-	-	-	-	-	-	-	-
		Ea	-	50	-	-	-	-	-	-	-	-
		Me	-	-	-	-	-	-	-	50	-	-
9	*A. marmelos*	He	-	25	-	-	-	-	-	-	-	-
		Ea	-	25	50	-	-	-	-	-	-	3.12
		Me	-	25	50	-	-	-		-	-	6.25

Note: He = Hexane, Ea = Ethylacetate, Me = Methanol

literature and folklore for diseases like cough, fever, wounds, diarrhea, dysentery, etc.

The antibacterial activities of hexane, ethylacetate and methanol extracts of nine selected plants showed that four plant extracts (44%) showed activity against at least five or more tested bacteria and five plant extracts (56%) were active against three or less than three tested bacteria. None of the tested plant extracts was active against all the tested bacteria. *H. antidysenterica, T. bellerica* and *W. fructicosa* showed relatively broad spectrum antibacterial activity (inhibited 6 out of 10 bacterial species) while *A. racemosus* showed least antibacterial activity (inhibited only 2 species). The highest ZOI (31 mm) was shown by ethylacetate fraction of *T. bellirica* against *E. coli* (Table 2).

The results showed that *A. calamus* inhibited 3 bacterial species viz. *E. coli, S. dysenteriae* and *S. aureus*. According to *Souwalak et al.* (2005) *A. calamus* was effective against *S. aureus* which supported with the result of this study. *A. racemosus* inhibited only *P. mirabilis* and *S. aureus*. This is supported by the result obtained by Baidya (2001). *H. antidysenterica* showed relatively broad spectrum activity, which inhibited 6 bacterial species. Baidya (2001) showed similar results with ethanol extracts. Methanol fraction of *M. pudica* inhibited *S. aureus, K. oxytoca* and *S. paratyphi*, which didnot support the study of Bajracharya (2007), where none of the tested bacteria was inhibited by *M. pudica*. Ethylacetate extracts of *T. bellirica* inhibited the growth of 6 bacterial species, methanol extract inhibited 3 bacterial species while hexane fraction did not inhibit any tested bacterial species, which was similar to the result showed by Ahmad (1998). The result showed that the active compounds might be polar compounds. Table 2 further showed that *T. chebula* inhibited 5 tested bacterial species, which further showed that methanol and ethylacetate extracts showed more inhibitory effect than hexane extracts. *W. fructicosa* was effective against 6 tested bacteria and the most effective extracts were found to be ethylacetate extracts against *E. coli* and *S. dysenteriae*. *A. marmelos* inhibited 3 tested bacterial species and all the three extracts showed antibacterial activity with *S. aureus*.

Further study showed that *S. aureus* was the most susceptible bacteria being sensitive to 18 extracts from 8 medicinal plants. Among tested Gram negative bacteria, *S. dysenteriae* was the most susceptible bacteria being sensitive to 11 extracts from 6 medicinal plants and *P. vulagaris* was the most resistant bacteria to all the selected plants. Further results showed that hexane extracts from 7 plants, ethylacetate extracts from 8 plants and methanol extracts from all plants showed antimicrobial activity against the tested bacterial species.

Table 3 showed the MBC of different effective extracts. The most bactericidal and lowest MBC of 3.12 mg/ml was shown by ethylacetate extract of *T. bellirica* against *E. coli* and ethylacetate extract of *W. fructicosa* against *S. dysenteriae*. Most of the MBC value of other plant extracts were in the ranges of 6.25 to 25 mg/ml. Least potent i.e. highest MBC value (>50 mg/ml) was shown by hexane extract of *T. chebula* against *S. aureus*.

The extracts from medicinal plants showing large ZOI and small MBC value, may contain those compounds, which are able to inhibit or kill the microbial populations of tested bacteria.

From this study it can be concluded that the selected medicinal plants had antibacterial activity against common pathogenic bacterial species.

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