ANTIMICROBIAL SCREENING OF SOME MEDICINAL PLANTS AGAINST SELECTED BACTERIAL SPECIES

Pragya Sapkota,* Sapana Bhattarai,* Anup Muni Bajracharya,* Pramesh Bahadur Lakhe, Nishant Shrestha****

*Department of Microbiology, Balkumari College, Chitwan, Nepal.
**Department of Plant Resources, Thapathali, Kathmandu, Nepal.

Abstract: Medicinal plants are used as traditional treatment for numerous human diseases. According to WHO, medicinal plants would be the best source to obtain a variety of drugs. Contrary to the synthetic drugs, antimicrobial of plant origin are not associated with many side effects and have an enormous therapeutic potential to heal many infectious diseases. Present study was carried out on the screening of some medicinal plants against selected pathogenic organisms in the period of October 2017 to January 2018. A total of nine different medicinal plants were screened and evaluated for their antimicrobial activity against 10 bacterial species. Among them, *Euphorbia hirta, Azadirachtaindica, Artemisia vulgaris* were found to be effective against gram positive bacteria (*Bacillus subtilis*, *Staphylococcus aureus*, Methicillin resistant *Staphylococcus aureus* MRSA), whereas rest six medicinal plants (*Aeglemarmelos, Justiciaadhatoda, Ficusreligiosa, Syzygiumcumini, Nyctanthes arbor* and *Meliaazedarach*) were found to be ineffective against all the microorganisms(*Bacillus subtilis, Escherichia coli, Klebsiellapneumoniae, Proteus vulgaris, Pseudomonas aerogenosa, Salmonella typhii, Shigelladysentriae, MRSA*). The minimum bactericidal concentration (MBC) of *Euphorbia hirta* against *Bacillus subtilis* and *Staphylococcus aureus* was found to be 12.5mg/ml and MBC of *Artemisia vulgaris* against *Bacillus subtilis* and MRSA was also found to be 12.5 mg/ml while of *Staphylococcus aureus* was 25mg/ml. Similarly, the MBC of *Azadirachta indica* against *Staphylococcus aureus* was 25mg/ml.

Keywords: Medicinal plants; Bacteria; Antimicrobial activity; Minimum bactericidal concentration.

1. INTRODUCTION

Medicinal plants have been used in homeopathy, ayurvedic, allopathy and traditional medicine since prehistoric period[1]. According to World Health Organization, 80% of the population in developing countries depend on traditional medicine for their primary health care and 85% of traditional medicine is derived from plant. Medicinal properties of plant are due to its active chemical constituents present in different parts of plants. Various phytochemicals like alkaloids, flavonoids, tanins, phlobatannin, terpenoid, saponin, steroid and glycosides are known to show medicinal potential[2].

Even though pharmacological industries have produced a number of new antibiotics in the last three decades, resistance to these drugs by microorganisms has increased[3]. Today's problem is the increase of bacterial resistance towards different antibiotics that specifically leading to treatment failures and is directly responsible for the current increase in morbidity and mortality associated with bacterial infections. Multi-drug resistance bacteria have emerged throughout the world causing several problems, clinical infections[3]. The increasing prevalence of multidrug resistant strains of bacteria and the recent appearance of strains with reduced susceptibility to antibiotics raises the specter of untreatable bacterial infections and adds urgency to the search for new infection-fighting strategies[4]. There is a rich local ethnobotanical bibliography in Nepalese traditional medicine; however, there is a lack of experimental scientific studies confirming the possible antibiotic properties of a great number of these medicinal plants[5]. Medicinal plants may be used to solve the problem of emerging resistance of microorganisms. Therefore, in this study we made an attempt to assess the antimicrobial properties of medicinal plants against some selected pathogenic microorganisms.

2. MATERIALS AND METHODS

2.1 Plant collection:

The plant material (leaves) were collected from local areas of Nawalparasi district and were identified according to various literatures like Medicinal plants of Nepal by HMG/N (1993), Ethnobotany of Nepal[6,7] and including other pertinent taxonomic literature. The list of medicinal plants, their corresponding parts used in this study, month of sample collection and location/place of the sample plants were given in Table1.

2.2 Sample processing:

The leaves of the medicinal plants were properly washed in the tap water. The rinsed leaves were then dried in shade region where there was no direct exposure of sunlight, for 4-5 days. The dried leaves of each plant were crushed to obtain powder. These powdered samples were stored in airtight polythene bags protected from sunlight until use.

2.3 Preparation of extract:

After completion of collection, drying and grinding then these plant materials were extracted by soxhlet extraction using 70% ethanol as a solvent. Then, the solvent was distilled under reduced pressure in a rotary vacuum evaporator and water bath at 65^{o}C until the extract

Author for Correspondence : Anup Muni Bajracharya, Department of Microbiology, Balkumari College, Chitwan, Nepal.
Email: anubjr@gmail.com

became dry. The percentage yield for each extract was determined and the crude extract was then transferred in a sterile vial by sterile spatula. One gram crude ethanol extract from each medicinal plant was mixed with 9ml of 70% ethanol and vortexed to make homogenous mixture of 1g/10ml i.e. 100mg/ml working suspension and stored in a refrigerator at 2-8°C.[5]

2.4 Microorganism:

The strain of microorganism used were *Bacillus subtilis* ATCC 6051, *Escherichia coli*ATCC8739 , *Enterococcusfaecalis* ATCC 29212, *Klebsiellapneumonia* ATCC700603, *Pseudomonasaeruginosa* ATCC9027, *Proteus vulgaris* ATCC 6380, *Staphylococcusaureus* ATCC6538P, *Shigelladysentriae* clinical sample, *Salmonella typhi* clinical sample and Methicillin Resistant *Staphylococcusaureus* clinical sample. The entire microorganisms were maintained in 4°C on Nutrient agar plate.

2.5 Antibacterial activity

The crude extract of medicinal plants was screened for its antibacterial property i.e, determination of zone of inhibition against tested bacteria was done by agar well diffusion method. The fresh bacterium culture comparable with turbidity standard i.e McFarland turbidity standard was prepared and swabbed on the surface of Muller-Hinton agar plates (MHA). Well of diameter 6mm were made in the inoculated MHA media plate. To evaluate the efficiency of methodology, 50µl of each working solution 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, if the plate showed zone of inhibition then minimum bactericidal concentration (MBC) was carried out by two-fold serial dilution method[5].

3. RESULTS

Considering the ethanol extracts of the examined medicinal plants, the percentage yield of 70% ethanol extracts of medicinal plants obtained by soxhlet extraction was as shown in Figure 1. The yield varied among the medicinal plants, *Euphorbia hirta* gave the highest yield (27.14%) while *Artemisia vulgaris* gave the lowest yield (8.86%).

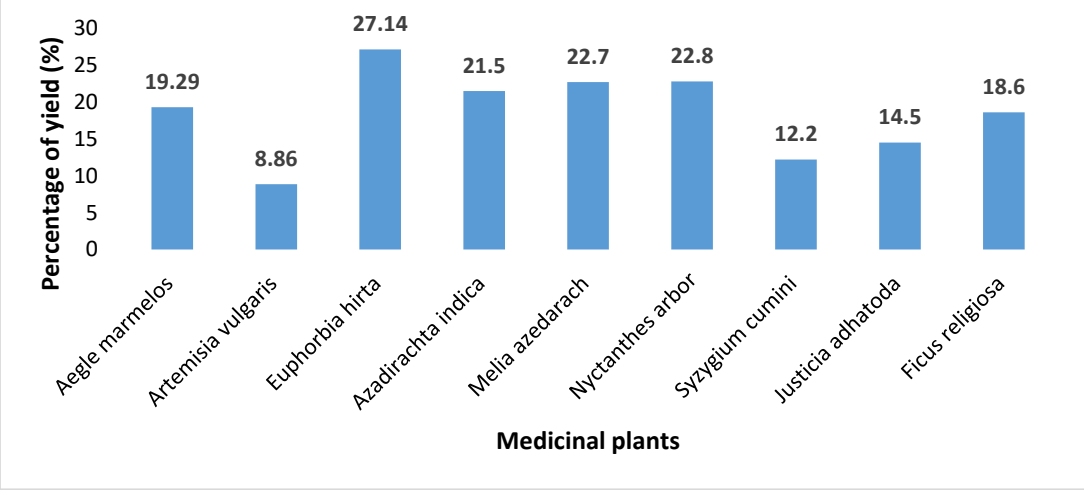

Figure: 1. Percentage yield of 70% ethanolic extracts.

Nine different medicinal plants were used for the study (Table 1). Similarly, Table 2 reflects the antibacterial activity shown by plant extracts through agar- well diffusion method. The plant extracts of *Artimisia vulgaris* and have lethal effect on *B subtilis*, *S aureus* and MRSA whereas *Euphorbia hirta* have its effect on *B subtilis, S aureus* and *Azadirachtaindica* have its lethal effect on *S aureus* and MRSA. Table 3 depicts the zone of inhibition and minimum bactericidal concentration of medicinal extracts against some pathogenic bacteria.

Table: 1. List of medicinal plants used in antimicrobial screening

List of medicinal plants							
SN	**Local name**	**Botanical Name**	**Family**	**Part used**	**Month of collection**	**Location**	**District**
1	Parijhat	*Nyctanthes arbor*	Oleaceae	leaves	September/October	Kawosoti	Nawalparasi
2	Dodhejhar	*Euphorbia hirta*	Euphorbiaceae	whole shoot	September	Kawosoti	Nawalparasi
3	Neem	*Azadirachtaindica*	Meliaceae	leaves	September	Gaindakot	Nawalparasi
4	Jamun	*Syzygiumcumini*	Myrtaceae	leaves	September/October	Kawosoti	Nawalparasi
5	Titepati	*Atrtemisis vulgaris*	Asteraceae	leaves	September/October	Kawosoti	Nawalparasi
6	Peepal	*Ficusreligiosa*	Moraceae	leaves	September/October	Gaindakot	Nawalparasi
7	Asuro	*Justicaadhatoda*	Acanthaceae	leaves	September/October	Gaindakot	Nawalparasi
8	Bakaino	*Meliaazedarach*	Meliaceae	leaves	September/October	Gaindakot	Nawalparasi
9	Bel	*Aeglemarmelos*	Rutaceae	leaves	September/October	Gaindakot	Nawalparasi

Table: 2. Antibacterial activity shown by plant extracts through Agar- well diffusion.
(+) Susceptibility (Inhibition zone >8mm) (-) Absence of susceptibility

S.N.	Medicnal plants	*B. subtilis*	*E.coli*	*E.faecalis*	*K.pneumonia*	*P.aeruginosa*	*P.vulgaris*	*S.aures*	*S.dysentriae*	*S.typhii*	*MRSA*
1	*Aeglemarmelos*	_	-	_	_	_	_	_	_	_	_
2	*Artimisia vulgaris*	+	_	_	_	_	_	+	_	_	+
3	*Eupharbiahirta*	+	_	_	_	_	_	+	_	_	_
4	*Azadirachtaindica*	_	_	_	_	_	_	+	_	_	+
5	*Meliaazedarach*	_	_	_	_	_	_	_	_	_	_
6	*Nyctanthes arbor*	_	_	_	_	_	_	_	_	_	_
7	*Syzygiumcumini*	_	_	_	_	_	_	_	_	_	_
8	*Justicaadhatoda*	_	_	_	_	_	_	_	_	_	_
9	*Ficusreligiosa*	_	_	_	_	_	_	_	_	_	_

Table: 3. Antimicrobial activity of crude ethanol extracts.

S.N.	Test organisms	Antimicrobial activity					
1.	*S. aureus*	***Artemisia vulgaris***		***Euphorbia hirta***		***Azadirachtaindica***	
		ZOI (mm)	MBC(mg/ml)	ZOI (mm)	MBC(mg/ml)	ZOI(mm)	MBC(mg/ml)
		18.60	25	14.84	12.5	14.4	25
2.	MRSA	14	12.5	_	_	20.47	25
3.	*B. subtilis*	9.6	12.5	14.89	12.5	_	_

The maximum zone of inhibition was observed in case of Methicillin resistant *Staphylococcus aureus* (MRSA) (20.47mm) due to the action of *Azadirachtaindica* and minimum was against *Bacillus subtilis* (9.6mm) shown by *Artemisia vulgaris*. The minimum bactericidal concentration (MBC) of the three plants against *Staphylococcus aureus*, MRSA, *Bacillus subtilis* were found to between12.5-25 mg/ml.

4. DISCUSSION

The results bring to light that each medicinal plant shows various 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 well, concentration of plant extracts and size of inoculums can be fixed and standardized during experiment. However, intrinsic factors such as nature of medicinal plants including its components, solubility and diffusing property are predetermined[5].

The results from Table 2 suggests that only three of the medicinal plant extract viz. *Artemisia vulgaris, Euphorbia hirta* and *Azadirachtaindica* had activity against three different microorganisms, *Staphylococcus aureus*, MRSA and *Bacillus subtilis*. This inhibitory effect shown in the study refers that extracts having high antimicrobial activity against gram positive bacteria doesn't necessarily have activity against gram negative bacteria. Antibacterial activity due to *Azadirachtaindica* was previously reported[8].Similarly, activity due to *Euphorbia hirta* was also reported[9]. The antibacterial activity of medicinal plants against gram-positive bacteria was studied in different studies. For instance, I.E. Cock studied the antibacterial effects of 39 methanol extracts of 25 Australian herbs against two gram-positive bacteria of *Bacillus cereus* and *Bacillus subtilis* and two gram-negative bacteria of *Pseudomonas aeruginosa* and *Aeromonashydrophila*, the disk diffusion method was used. The results showed the sensitivity of gram-positive bacteria[10]. In addition, Joshi *et al* mentioned the antibacterial effects of ten different medicinal plant extracts of Nepal. The plant extracts were more active against Gram-positive bacteria than against Gram-negative bacteria. The most susceptible bacteria were *B. subtilis*, followed by *S. aureus*, while the most resistant bacteria were *E.coli*, followed by *Shigelladysenteriae*, *Klebsiellapneumoniae* and *Salmonella typhi*.[11].

Ten important human pathogenic bacteria were investigated and the well method was used. Extracts were more effective in gram-positive bacteria compared to gram-negative bacteria. The most sensitive bacteria were *Bacillus subtilis* and *Staphylococcus aureus* and the most resistant bacteria were *E. coli, Shigelladysenteriae,*

Klebsiellapneumoniae and *Salmonella typhimurium*[11].In fact, gram-positive bacteria are more sensitive to herbal extracts than gram-negative bacteria. This may be because of inherent tolerance of gram negative and the nature and composition of herbs. According to the studies, the cell wall of gram-positive bacteria compared with gram-negative bacteria, are more sensitive to many of antibiotics, antimicrobial chemical compounds[12] and even many herbal drugs[13]. Lipopolysaccharides layer and periplasmic space of gram-negative bacteria are the reasons of relative resistance of gram-negative bacteria.

5. CONCLUSIONS

The plant extracts have great potential as antimicrobial compounds against microorganisms. Our research study revealed that plants like *Artemisia vulgaris, Euphorbia hirta* and *Azadirachtaindica* have great therapeutic potential against some diseases. Further researches can be done to discover major group of phytochemical compounds that open the possibility of finding new clinically effective antibacterial compounds.

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