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Antibacterial Property of Different Medicinal Plants: *Ocimum sanctum*, *Cinnamomum zeylanicum*, *Xanthoxylum armatum* and *Origanum majorana*

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ABSTRACT
Countries like Nepal and India have been using crude plants as medicine since Vedic period. A major part of the total population in developing countries still uses traditional folk medicine obtained from plant resources (Farnsworth 1994). With an estimation of WHO that as many as 80% of worlds population living in rural areas rely on herbal traditional medicines as their primary health care, the study on properties and uses of medicinal plants are getting growing interests. In recent years this interest to evaluate plants possessing antibacterial activity for various diseases is growing (Clark and Hufford, 1993). Based on local use of common diseases and Ethnobotanical knowledge, an attempt has been made to assess the antibacterial properties of selected medicinal plants.VIZ. *Ocimum sanctum (Tulsi)*, *Origanum majorana (Ram Tulsi)*, *Cinnamomum zeylanicum (Dalchini)*, and *Xanthoxylum armatum (Timur)*, for potential antibacterial activity against 10 medically important bacterial strains, namely *Bacillus subtilis, Bacillus cereus, Bacillus thuringiensis, Staphylococcus aureus, Pseudomonas* spp, *Proteus* spp, *Salmonella* Typhi, *Escherichia coli, Shigella dysentriae, Klebsiella pneumoniae.* The antibacterial activity of ethanol extracts was determined by agar well diffusion method. 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 *Shigella dysenteriae, Klebsiella pneumoniae* and *Salmonella typhi*. From the screening experiment, *Origanum majorana* showed the best antibacterial activity; hence this plant can be further subjected to isolation of the therapeutic antimicrobials and pharmacological evaluation. The largest zone of inhibition was obtained with X*anthoxylum armatum* against *Bacillus subtilis (23mm)* and the minimum bactericidal concentration (MBC) value of *2.5 mg/l was obtained.*

Key words: Antibacterial property, Gram positive bacteria, Gram negative bacteria, zone of inhibition, minimum bactericidal concentration

INTRODUCTION
The use of plants and plant products as medicines could be traced as far back as the beginning of human civilization. The earliest mention of medicinal use of plants in Hindu culture is founds in “Rigveda”, which is said to have been written between 4500-1600 B.C. and is supposed to be the oldest repository of human knowledge. It is Ayurveda, the foundation of medicinal science of Hindu culture, in its eight division deals with specific properties of drugs and various aspects of science of life and the art of healing. (Rastogi and Mehrotra, 2002)

Medicinal plants are a source of great economic value all over the world. Nature has bestowed on us a very rich botanical wealth and a large number of diverse types of plants grow in different parts of the country. Nepal is rich in all the 3 levels of biodiversity, namely species diversity, genetic diversity and habitat diversity. In Nepal thousands of species are known to have medicinal value and the use of different parts of several medicinal plants to cure specific ailments has been in vogue since ancient times. Herbal medicine is still the mainstay of about 75-80% of the whole population, and the major part of traditional therapy involves the use of plant extract and their active constituents (Akerele, 1993). Following the advent of modern medicine, herbal medicine suffered a set back, but during last two or three decades advances in phytochemistry and in identification of plant compounds effective against certain diseases have renewed the interest in herbal medicines (FAO 1990).

Nowadays multiple drug resistance has developed due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious disease. In addition to this problem, antibiotics are sometimes associated with adverse effects on the host including hypersensitivity, immune-suppression and allergic reactions. This situation forced scientists to search for new antimicrobial substances. Given the alarming incidence of antibiotic resistance in bacteria of medical importance, there is a constant need for new and effective therapeutic agents. Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases from medicinal plants (Agarwal *et al.*, 1996)

Antimicrobials of plant origin have enormous therapeutic potential. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials. The beneficial medicinal effects of plant materials typically result from the combinations of secondary products present in the plant. In plants, these compounds are mostly secondary metabolites such as alkaloids, steroids, tannins, and phenol compounds, flavonoids , steroids, resins fatty acids gums which are capable of producing definite physiological action on body. Compounds extracted from different parts of the plants can be used to cure diarrhea, dysentery, cough, cold, cholera, fever bronchitis etc. Decoction or powder of *Xanthoxylum armatum* (Rutaceae) can be taken orally with warm water to treat constipation, stomach pain, toothache and cold (Joshi and Edington,1990; Manadhar,1987). The main objective of the research is to screen and evaluate antibacterial activity of crude ethanol extract and to find out minimum bactericidal concentration (MBC) against these extracts both gram positive as well as gram negative bacteria (Pokhrel, 2000)

MATERIALS AND METHODS

Collection of samples

The medicinal plants used for the experiment were *Ocimum sanctum*, *Origanum majorana*, *Cinnamomum zeylanicum*, and *Xanthoxylum armatum*. Medicinal plant were identified according to various literatures, Medicinal plants of Nepal by HMG/N (1976) and including other pertinent taxonomic literature .Collected plants were washed thoroughly and chopped into small pieces shade dried and grinded into powdered form. Clean and dry separating funnel was taken. 75 gm of ground material and 250 ml of 95% dehydrated ethanol was filled in to the separating funnel and filtered using standard filter paper for 48 hours. The filtrate was dried using rotatory vacuum evaporator. Extract thus obtained was heated at 72°C in a water bath shaker and the volume of the crude extract was reduced to 90% of its volume.

Collection of test Organism and Preparation of Stock Culture

Test organisms were received from Department of Microbiology, National College and reconfirmed by gram staining and sub culturing in appropriate selective media.

Gram positive	*Gram negative*
Staphylococcus aureus	*Klebsiella pneumoniae*
Bacillus subtilis	*Proteus* spp
Bacillus cereus,	*Escherichia coli,*
Bacillus thuringiensis	*Pseudomonas* spp,
	Salmonella Typhi,
	Shigella dysenteriae

Preparation of standard culture inoculum of test organism

Three or four isolated colonies were inoculated in the 2 ml nutrient broth and incubated till the growth in the broth was equivalent with Mac-Farland standard (0.5%) as recommended by WHO.

Determination of Zone of Inhibition (ZOI)

The freshly prepared inoculum was swabbed all over the surface of the MHA plate using sterile cotton swab. Five wells of 6mm diameter were bored in the medium with the help of sterile cork-borer having 6mm diameter and were labeled properly and fifty micro-liters of the working suspension/solution of different medicinal plant extract and same volume of extraction solvent for control was filled in the wells with the help of micropipette. Plates were left for some time till the extract diffuse in the medium with the lid closed and incubated at 37°C for 24 hour and measured using scale and mean were recorded after incubation, plates were observed for zone of inhibition.

Determination of Minimum Bactericidal Concentration (MBC)

Freshly prepared nutrient broth was used as diluents. Crude extract was diluted by two fold serial dilution method. 50µl of the standard culture inoculums was added to each test tube except the negative control tube. All tubes were incubated at 37°C for 24 hours. The tube content was subculture in fresh nutrient agar separately and minimum bactericidal concentration was determined as that showing no growth.

RESULTS

Among all the pathogens, all gram positive bacteria were inhibited by all four plant extract. All Gram negative bacteria i.e. *Pseudomonas* spp, *Proteus* spp, *Escherichia coli*, *Shigella dysenteriae, Klebsiella pneumonia* were found to be resistant to all of the 4 extracts, Exceptionally *Salmonella* Typhi showed zone of inhibition against extract of *Ocimum sanctum*. Following table shows the antimicrobial effect of different plant extracts.

Table 1: Observation of Antimicrobial Property of Alcoholic Extracts of Different Medicinal Plants against Different Pathogenic Microorganisms.

S. N.	Test organism	Name of the plants			
		Cinnamom	Timur	Origanum	Tulsi
1	*Staphylococcus aureus*	+	+	+	+
2	*Bacillus subtilis*	+	+	+	+
3	*Bacillus cereus*	+	+	+	+
4	*Bacillus thuringiensis*	+	+	+	+
5	*Pseudomonas* spp	-	-	-	-
6	*Proteus* spp	-	-	-	-
7	*Escherichia coli*	-	-	-	-
8	*Salmonella* Typhi	-	-	-	+
9	*Shigella dysenteriae*	-	-	-	-
10	*Klebsiella pneumoniae*	-	-	-	-

Note: The positive sign indicates that ethanol extract of particular medicinal plant can inhibit the growth of microorganisms and thus zone of inhibition was produced which indicates that the plant has antimicrobial activity. The negative sign indicates that ethanol extract of particular medicinal plant cannot inhibit the growth of micro organism and cannot produce zone of inhibition. It indicates that plants have no antimicrobial property.

EVALUATION OF ANTIMICROBIAL ACTIVITY

Evaluation of antimicrobial activities was performed by agar well diffusion method and two fold broth dilution method. Diameter of the zone of inhibition (ZOI) was measured for the estimation of potency of the antimicrobial substance. Similarly, two fold broth dilution methods were applied for the determination of minimum bactericidal concentration (MBC). The zone of inhibition, minimum bactericidal concentration of ethanol extract of the different medicinal plants, which are able to produce zone of inhibition during screening process (as indicated in Table: 2) are shown in the following Table.

Note: During determination of zone of inhibition, the diameter of the well was 6 mm and the height of the well was 4 mm and the concentration of loaded extract in each well was50µl.

Table 2: Zone of inhibition and minimum bacterial concentration (MBC) of different organisms

S.N.	ORGANISMS	PLANTS	ZONE OF INHIBITION (mm)	MINIMUM BACTERICIDAL CONCENTRATION (MBC) mg/ml
1.	*Staphylococcus aureus*	*Cinnamomum zeylanicum*	10	10
		Xanthoxylum armatum	7	2.5
		Origanum majorana	12	2.5
		Ocimum sanctum	1	>10
2.	*Bacillus subtilis*	*Cinnamomum zeylanicum*	11	>10
		Xanthoxylum armatum	23	>10
		Origanum majorana	14	2.5
		Ocimum sanctum	5	5
3.	*Bacillus cereus*	*Cinnamomum zeylanicum*	10	>10
		Xanthoxylum armatum	6	10
		Origanum majorana	10	>10
		Ocimum sanctum	3	>10
4.	*Bacillus thuringiensis*	*Cinnamomum zeylanicum*	8	2.5
		Xanthoxylum armatum	1	2.5
		Origanum majorana	8	5
		Ocimum sanctum	12	>10
5.	*Salmonelle* Typhi	*Ocimum sanctum*	6	2.5

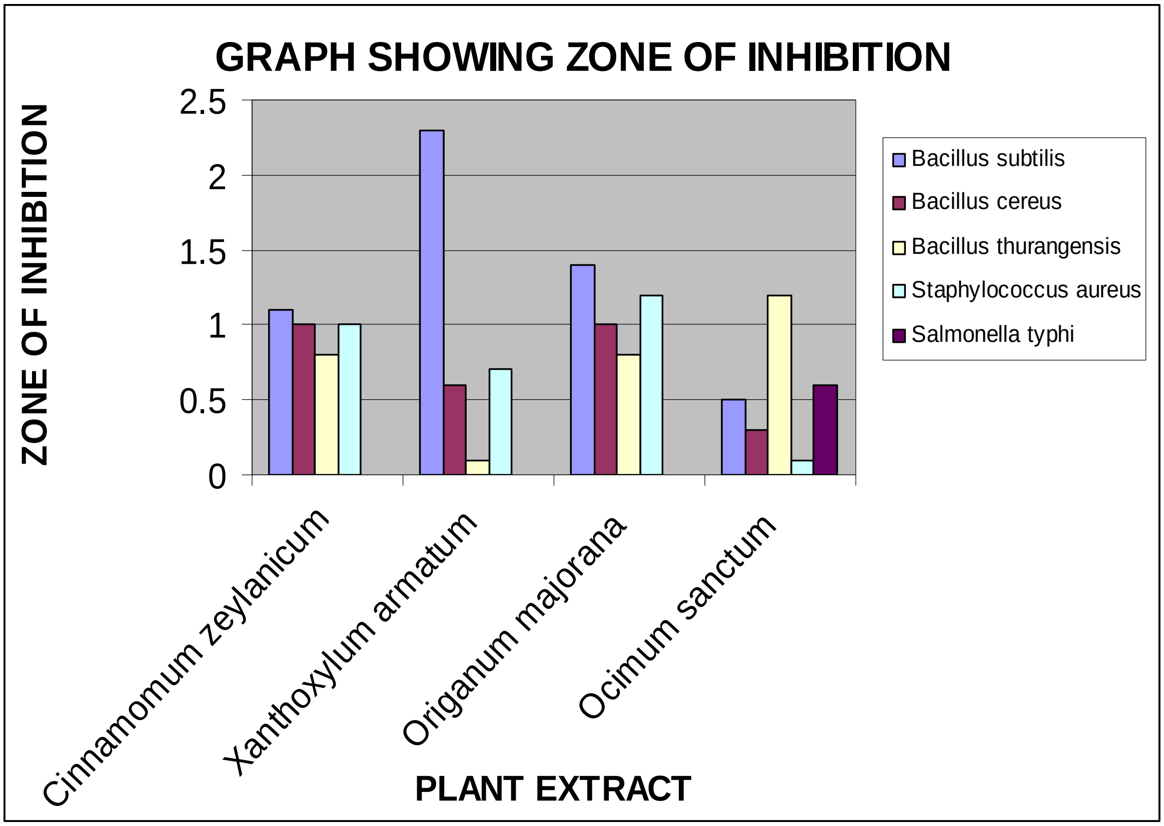

Figure 1: Graph showing zone of inhibition (cm) produced by different medicinal plants.

DISCUSSION

Plant essential oils and extracts have been used for many thousands of years, in food preservation, pharmaceuticals, alternative medicine and natural therapies. It is necessary to investigate those plants scientifically which have been used in traditional medicine to improve the quality of healthcare. Plant extracts are potential sources of novel antimicrobial compounds especially against bacterial pathogens. *In vitro* studies in this work showed that the plant extracts inhibited bacterial growth but their effectiveness varied. The antimicrobial activity of many plant extracts has been previously reviewed and classified as strong, medium or weak

The medicinal plants like cinnamon, timur, tulsi and origanum are being used traditionally for the treatment of inflammation, cough, toothache, antiseptics expectorant, and some fungal infection like candidaisis. The antibacterial activity has been attributed to the presence of some active constituents in the extracts. Studies suggested that the antibacterial activity of cinnamon was probably due to their major component, cinnamaldehyde and their properties could be multiple. Cinnamaldehyde is a natural antioxidant and the animal studies suggest that an extract of cinnamon bark taken orally may help prevent stomach ulcer. Cinnamaldehyde was completely inhibiting both sensitive and resistant stain of *Helicobacter pylori*. An important characteristic of plant extracts and their components is their hydrophobicity, which enable them to partition the lipids of the bacterial cell membrane and mitochondria, disturbing the cell structures and rendering them more permeable. Extensive leakage from bacterial cells or the exit of critical molecules and ions will lead to death. (Rastogi and Mehrotra, 2002)

The inhibition produced by the plant extracts against particular organism depends upon various extrinsic and intrinsic parameters. Duee to variable diffusability in agar medium, the antibacterial property may not demonstrate as ZOI commensurate to its efficacy. Therefore MBC value has also been computed in this study. MBC is the lowest concentration of antibacterial substance required to produce a sterile culture (Cheesbrough, 1993).

According to the antibacterial assay done for screening purpose, all the gram positive microorganisms viz., *Staphylococcus aureus, Bacillus subtilis, Bacilus cereus* and *Bacillus thuringiensis* were the most susceptible bacteria to all plant extracts, whereas only one of the gram negative microorganisms, i.e. *Salmonella* Typhi was susceptible to one of the plant extracts. On the contrary, the rest of the gram negative microorganisms *viz Pseudomonas* species, *Proteus* species, *Escherichia coli*, *Shigella dysenteriae* and *Klebsiella pneumoniae* were the most resistant microorganisms. These observations are likely to be the result of the differences in cell wall structure between Gram-positive and Gram-negative bacteria, with Gram-negative outer membrane acting as a barrier to many environmental substances including antibiotics.(Burt,2004)

These findings support the traditional knowledge of local users and it is a preliminary, scientific, validation for the use of these plants for antibacterial activity to promote proper conservation and sustainable use of such plant resources. Awareness of local community should be enhanced incorporating the traditional knowledge with scientific findings. In conclusion, the results of the present study support the folkloric usage of the studied plants and suggest that some of the plant extracts possess compounds with antimicrobial properties that can be further explored for antimicrobial activity. This antibacterial study of the plant extracts demonstrated that folk medicine can be as effective as modern medicine to combat pathogenic microorganisms. The millenarian use of these plants in folk medicine suggests that they represent an economic and safe alternative to treat infectious diseases

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REFERENCES

1. Agrawal P, Rai V, Singh R, B.1996. Randomized, placebo-controlled, single-blind trial of holy basil leaves in patients with noninsulin-dependent diabetes mellitus. *International Journal of Clinical Pharmacology and Therapeutics*, 1996, 34:406-409.

2. Akerele, O.1993.Summary of WHO Guidelines for the Assessment of Herbal Medicines Herbal Gram 22:13-28

3. Burt, S. 2004. Essential oils: Their antibacterial properties and potential applications in foods-a review, international journal of food microbiology 94.

4. Cheesbrough, M. 1993. Medicinal laboratory manual for tropical countries, ELBS reprinted Edition.

5. Clark, A.M., Hufford C.D., 1993 Discco and development of novel prototype antibiotics for opportunistic infections related to the acquired immunodeficiency

syndrome. In: human medical agents from plants.american chemical society(ACS Symposium series 534), Washington,D.C., pp 228-241

6. FAO 1993. Medicinal and Aromatic plant in Asia. Bangkok, Thailand RAPA, Publication.

7. Fransworth , N.R 1994. The role of medicinal plants in drug development. In: Krogsgaard-Larsen, S., Brogger-Christense, S., Kofod, H. (Eds.), Natural Products and Drug Development. Munksgaard, Copenhagen

8. HMG/N 1976. Medicinal plants of Nepal .Ministry of forest and soil conservation. Department of plant Resources Kathmandu Nepal

9. Joshi, A.R., Edington, J.M. 1990. The use of medicinal plants by two village communities in the Central Development Region of Nepal. Economic Botany. 44 (1): 71-83.

10. Manandhar, N.P. 1987. Traditional medicinal plants used by tribals of Lamjung District, Nepal. International Journal of Crude Drug Research. 25 (4): 236-240.

11. Rastogi, R.P.; Mehrotra, B.N. 2002. Glossary of Indian Medicinal Plants. National Institute of science communication, New Delhi, India.

12. Pokhrel, N. R., 2000. “Screening and evaluation of the antimicrobial activity of some medicinal plants of Nepal and isolation of pure antimicrobial compound from *Bauhinia variegata*.” A Dissertation Submitted to Central Department of Microbiology, Tribhuwan University, Kathmandu.