Antibacterial Efficacy of Selected Herbal Plants against Common Pathogenic Bacteria

Kalyan Subedi[1], Pawan Gautam[1], Shiva Pariyar[1*]
[1]Department of Microbiology, Kathmandu College of Science and Technology, Kathmandu, Nepal

***Corresponding author**: Shiva Pariyar, Department of Microbiology, Kathmandu College of Science and Technology (KCST), Kathmandu, Nepal; E-mail: pariyarshiva680@gmail.com

ABSTRACT

Objectives: The purpose of this study was to ascertain the effectiveness of particular herbal plants as antibacterial agents.

Methods: Different herbal plants were collected from Asan Bazar, Kathmandu, then the samples were dried, reduced to a powder, and combined with the appropriate amounts of hexane, ethyl acetate, and methanol to produce the extract accordingly. To obtain the desired extract, filtration, incubation, and vaporization were used. *Escherichia coli, Staphylococcus aureus*, and *Bacillus spp*. were the three test organisms. The test organism was uniformly swabbed onto a Muller Hinton Agar (MHA) plate. With the use of a borer, agar wells were created on MHA plates. The extracts of about 0.1 ml were applied to those wells using sterile micro pipette, which were then incubated at 37°C for 24 hours. Each well's zone of inhibition (ZOI) was evaluated in relation to test organisms. Using the dilution approach, the minimum inhibitory concentration (MIC) was determined.

Results: *Bacillus spp*. were found to be significantly inhibited by ethyl acetate extract of *Coriandrum sativum* (Coriander) with a ZOI of 25 mm. The ZOI 14mm test revealed that ethyl acetate extract of *C. sativum* had minimum efficacy against *E. coli*. The methanolic extract of *C. sativum* was reported to have MICs of 1:16, 1:32, and 1:64 against *S. aureus*, *Bacillus* spp., and *E. coli,* respectively.

Conclusion: *C. sativum* is the most effective herbal plant against common pathogenic bacteria. Ethyl acetate extract was found to be more effective when compared to methanolic and hexane extracts. To update its medicinal value, additional research on its antibacterial characteristics may be required.

Keywords: Antibacterial efficacy, herbal plants extract, zone of inhibition, minimum inhibitory concentration.

INTRODUCTION

Medicinal herbal plants, are those that can treat infections and diseases with a natural remedy. They are in use since prehistoric times. Plants synthesize hundreds of chemicals like phytochemicals with potential biological activities. In non-industrialized communities, medicinal herbs are commonly used. It's because they are more easily accessible and less expensive than contemporary medications. To combat antibiotic resistance, focus is now being turned to biologically active components that have been isolated from plant species-used as herbal medicines because they may produce a strong source of antibacterial and antifungal, immunomodulatory and antioxidant efficacy as they have capacity to produce a number of secondary metabolites with complecated structure (Maiyo et al., 2010; Erjan & Marouj, 2019).

Date of Submission: August 22, 2024 **Date of Acceptance:** December 2, 2024
Published Online: December 31, 2024 **DOI:** https://doi.org/10.3126/tujm.v11i1.85501

An antimicrobial agent is a chemical that either eliminates or inhibits the growth of microorganisms. Antimicrobial drugs can either have a bacteriostatic or bactericidal effect (Tripathi, 2003). *Psidium guajava* leaf extract has antimicrobial effects due to variety of chemical components, including tannins, gallic acid, and phenolic compounds including quercetin, catechin, and epicatechin (Bever, 1986), which is used for skin, wounds, ulcers, diarrhea, and toothaches, and lungs infection (Heinrich *et al*., 1998). *Staphylococcus. aureus* and *Salmonella* spp were inhibited by the essential oil extract which can be a good source of possible novel anti-microbial compounds.

A family's *Acorus calamus* linn, known as Bojho in Nepali has a variety of health advantages, including anti-microbial, anti-fungal, anti-helminth, anti-tumor properties (Foster, 1999). *In vitro* tests using ampicillin, chloramphenicol, and benzoyl benzoate revealed that hexane and methanol extracts of the rhizome of *Acorus* species significantly suppressed a variety of drug-resistant cultures of *S. aureus* (Kim El Al, 1998). *In vitro* synergism was found between the respective crude extracts of A. calamus with either tetracycline or ciprofloxacin for a specific strain of extended-spectrum beta-lactamase-producing E. coli (Ahmad and Agil, 2007).

The mint family (Camiaceae) includes the annual herb known as *Ocimum basilicum* commonly called as Tulsi has antibacterial properties that can kill bacteria like *E. coli* and *S. aureus*. As it contains Linalool, methyl chavicol (Estragole), eugenol, citral, limonene, rosmarinic acid, flavonoids. It is also said that Tulsi can be used to lower fevers, treat Asthma, cough, colds, and influenza, malaria. Mixture of Tulsi and neem extracts can benefit those with blood sugar issues. Tulsi leaves act as a nerve tonic and help to improve memory. Consuming Tulsi leaves reduces tension since they have anti-stress properties.

Coriandrum sativum (Coriander) belonging to Apiaceae family, it has been used to treat sleeplessness, anxiety, improve good skin, ease flatulence, and aid digestion. Coriander seeds possess antibacterial and antioxidant properties as it has chemical constituents like Linalool, Geraniol, Pinene, Borneol, Coumarins, Flavonoids, Fatty Acids, etc. The essential oil is used in aromatherapy. Gram-negative bacteria were found to be more susceptible to the plant extract than Gram-positive bacteria, according to a study on the antibacterial efficacy of the crude methanol extract of different herbs at two different concentrations (Parekh and Chanda, 2007b).

METHODS

Herbal collection and extract preparatioon

Cross-sectional descriptive research was used in the study. This study was carried out at Microbiology laboratory of Kathmandu College of Science and Technology, Kathmandu, Nepal, between early December 2022 and April 2023. The study was proposed to examine the antibacterial efficacy of selected herbal plants. Four herbal plants were gathered from various locations in the Kathmandu Valley: *Acorus calamus* (Bojho), *O. basilicum* (Tulsi), *Psidium guajava* (Guava leaves), and *C. sativum* (Coriander), they were quickly transferred to the laboratory of the Kathmandu College of Science and Technology. The obtained plant pieces were then dried in the lab without direct sunlight for 5-7 days before being reduced to a fine powder with mortal and pistle, roughly 12 grams of fine powder were combined with 60 ml each of methanol, ethyl acetate, and hexane to produce methanolic extract, ethyl acetate extract, and hexane extract. The solution was then held at room temperature for 3 days in 12 different test tubes. The tubes were shaken hourly during the day. Whatman filter paper was used to filter the extract. With the use of vacuum evaporator, methanol, ethyl acetate, and hexane were heated to their boiling points and then evaporated from the filtrate.

Preparation of test organism

Test organisms that vary the antibacterial effectiveness of the four suggested herbal plants were tested against *Bacillus spp*., *Staphylococcus aureus*, and *Escherichia coli* in a controlled laboratory environment. In order to obtain new colonies, sufficient organisms were withdrawn from their culture. The agar plate was initially punched with a borer to a 6mm size to create agar well. Then, plant extract was applied to those wells using sterile micro pipette. Mueller Hinton Agar (MHA) was used to test the antibacterial effectiveness of plant extract. A uniform lawn of the test organism was created. Then 0.1ml plant extracts was inoculated by using sterile micro pipette. Methanol extract, ethyl acetate extract, hexane extract was inoculated onto a different MHA plate with one of the test organisms. In this way, plant extract was inoculated onto multiple plates containing test organisms. The plate was labelled well. The MHA plates were then incubated at 37°C for 24 hours after being inoculated with test organism and plant extract. The zone of inhibition (ZOI) around the well was observed on the incubated plates. Each methanolic, ethyl acetate, and hexane extract of the herbal plants was determined and recorded for its zone of inhibition. After that, the efficacy of each plant's extract against common pathogenic microorganisms was compared.

Determination of MIC

The extract with the highest antibacterial efficacy was diluted up to seven times (1:2, 1:4, 1:8, 1:16, 1:32, 1:64, and 1:128) for the purpose of calculating the MIC. Two MHA plates were used to drill a well with a 6mm-diameter borer. On an MHA plate, a well was created, and an extract of various dilutions was added. The plate's backside was labelled. After that, the plates were incubated for 24 hours at 37°C. The MIC was determined by examining the zone of inhibition using several dilutions (Wayne 2015).

RESULTS

Percentage yield of crude extracts of herbal plants

Table 1 shows that the methanol extract of *C. sativum* (Coriander) produced the maximum yield (44.5%), which was followed by the methanol extract of *P. guajava* (Guava leaves) (40.6%). The hexane extract of A. *calamus* (Bojho) produced the lowest yield (5.5%), followed by the hexane extract of *O. basilicum* (Tulsi) (7.2%).

Antibacterial efficacy of ethyl acetate extract of *A. calamus*, *O. basilicum*, *P. guajava*, and *C. sativum*

The maximum efficacy of ethyl acetate extract of *A. calamus* was seen against *Bacillus* spp (ZOI=10 mm) followed by *S. aureus* (5 mm) but nill with *E. coli*. The highest efficacy of *O. basilicum* extract was seen against *S. aureus* (7 mm) followed by *Bacillus* spp (3 mm). but not *E. coli*.

The maximum efficacy of *P. guajava* extract was seen against *Bacillus* spp (15 mm) followed by *S. aureus* (13 mm) but not *E. coli*. The highest efficacy of *C. sativum* extract was seen against *Bacillus* spp (25 mm) followed by *S. aureus* (15 mm) while *E. coli* was least susceptible (14 mm ZOI).

Antibacterial efficacy of methanolic extracts

The maximum efficacy of methanolic extract of *A. calamus* as seen against *S. aureus* (13 mm) followed by *Bacillus* spp (7 mm). *E. coli* was not susceptible. The maximum efficacy of *O. basilicum* extract was seen against *S. aureus* (3 mm) followed by *Bacillus* spp (1 mm) but not *E. coli*. The maximum efficacy of methanolic extract of *P. guajava* was seen against *S. aureus* (14 mm) followed by Bacillus spp (9 mm). *E. coli* was not susceptible. The maximum efficacy of methanolic extract of *C. sativum* was seen against *Bacillus* spp (13 mm) followed by *S. aureus* (12 mm). *E. coli* was least susceptible to the extract of *C. sativum* with 11 mm.

Antibacterial efficacy of hexane extracts

The maximum efficacy of hexane extract of *A. calamus* was seen against *Bacillus* spp (10 mm) followed by *S. aureus* (9 mm). *E. coli* was not susceptible. Extract of *O. basilicum* had it against *Bacillus* spp (7 mm) followed by *S. aureus* (5 mm). *E. coli* was not susceptible.

The highest efficacy of extract of *P. guajava* was seen against *Bacillus* spp (15 mm) followed by *S. aureus* (12 mm). *E. coli* was not susceptible. The maximum efficacy of *C. sativum extract* was seen against *Bacillus* spp (16 mm) followed by *E. coli* (12 mm). *S. aureus* was least susceptible with 11 mm ZOI.

MIC of different extracts of *C. sativum*

MIC of the ethyl acetate extract of *C. sativum* against *Bacillus* spp, *S. aureus and E. coli was found to be* 1:16, 1:64 and 1:4, respectively. MIC of the methanolic extract against *Bacillus* spp, *S. aureus and E. coli was found to be* 1:8, 1:32 and 1:4, respectively. Similarly, MIC of the hexane extract against *Bacillus* spp, *S. aureus and E. coli was found to be* 1:8, 1:16 and 1:4, respectively (Table 2).

Table 1: Percentage yield of crude extract of herbal plants prepared by maceration and percolation

S. N.	Herbal Plant	Solvent used for Extraction	% Yield	Concentration (mg/ml)
1	*Acorus calamus* (Bojho)	Ethyl acetate	19.7	10
		Methanol	12.9	7
		Hexane	5.5	9
2	*Ocimum basilicum* (Tulsi)	Ethyl acetate	23.4	5
		Methanol	7.2	8
		Hexane	9.8	7
3	*Psidium guajava* (Guava Leaves)	Ethyl acetate	34.1	5
		Methanol	40.6	6
		Hexane	28.7	8
4	*Coriandrum sativum* (Coriander)	Ethyl acetate	30.3	4
		Methanol	44.5	7
		Hexane	29.9	6

Table 2: Minimum Inhibitory Concentration of ethyl acetate, methanolic and hexane extracts of *C. sativum* (Coriander) against common pathogenic bacteria

Extract	Concentration	Zone of Inhibition (ZOI) in mm		
		***Bacillus* spp**	***S. aureus***	***E. coli***
	1:128	-	-	-
	1:64	-	1	-
	1:32	-	4	-
Ethyl acetate	1:16	3	7	-
	1:8	8	10	-
	1:4	11	13	10
	1:2	14	19	15
	1:128	-	-	-
	1:64	-	-	-
	1:32	-	7	-
Methanolic	1:16	-	9	-
	1:8	9	10	-
	1:4	11	12	12
	1:2	18	16	14
	1:128	-	-	-
	1:64	-	-	-
	1:32	-		-
Hexane	1:16		4	-
	1:8	3	7	-
	1:4	6	10	5
	1:2	8	13	7

DISCUSSION

Four herbal plants were gathered from various locations in the Kathmandu Valley: *Acorus calamus* (Bojho), *Ocimum basilicum* (Tulsi), *Psidium guajava* (Guava leaves), and *Coriandrum sativum* (Coriander). These herbal plants were chosen based on their efficacy for treating illnesses including cough, fever, wounds, diarrhea, common cold, sore throat, etc., as described in literature and history.

After selecting the preferred plants, gathering the necessary pieces, washing them, placing them in plastic bags, and labelling them appropriately, they were quickly transferred to the laboratory of the Kathmandu College of Science and Technology to be used in future procedures. The plant pieces were sometimes kept at 4°C when quick processing was not possible.

As drying in direct sunlight may result in the loss of some active chemicals, samples were left to dry in the shade.

Large plants were chopped before drying and then submitted to grinding to facilitate full and simple extraction of the active compounds. Large plants that are extracted without grinding result in a poor percentage yield on extraction.

According to the literature that was studied, there is no particular method for extracting chemicals from plants. For the quick and effective extraction of chemicals from the plants, we used the maceration and percolation procedure. Compounds from the plant sample were extracted using a variety of solvents. Methanol, ethanol, and other alcoholic solvents were frequently used. Ironically, water, the universal solvent, is rarely used since it is less effective for non-polar organic compounds and requires high temperatures or advanced tools to remove the water from a solution. A plant's crude extract can include a variety of compounds with significantly different chemical characteristics and polarities. In order to identify the active compounds, it is important to first arrange the compounds into groups with similar polarity.

Three solvents, namely hexane, ethyl acetate, and methanol, were utilized in this work in order of increasing polarity to produce hexane, ethyl acetate, and methanol extracts (extracts) using the maceration and percolation method. In order of increasing polarity, Thapa (2006) and Baidya (2001) extracted chemicals from the plant using hexane, chloroform, n-butanol, and an aqueous solvent. Our study's goal is supported by this research.

It was discovered that for the same plant, continuous extraction with several solvents, namely hexane, ethyl acetate, and methanol, in ascending order of polarity, provided varying percentage yields. In comparison to ethyl acetate and methanol solvents, hexane solvent requires less time to finish the extraction.

Table 1 shows that the methanol extract of *Coriandrum sativum* (Coriander) produced the maximum yield (44.5%), which was followed by the methanol extract of *Psidium guajava* (Guava leaves) (40.6%). The hexane extract of Acorus *calamus* (Bojho) produced the lowest yield (5.5%), followed by the hexane extract of *Ocimum basilicum* (Tulsi) (7.2%).

In this study, it was discovered that the ethyl acetate extract of *Acorus calamus* (Bojho) was highly effective against *Bacillus* spp. (10mm), while *Escherichia coli* (0mm) showed no efficacy. Maximum efficacy was shown against *Staphylococcus aureus* (13mm) in the case of the methanolic extract of *Acorus calamus* (Bojho), whereas no efficacy was observed in the case of *Escherichia coli* (0mm). Maximum efficacy was shown against *Bacillus* spp. (10mm) in the case of the hexane extract of *Acorus calamus* (Bojho), whereas no efficacy was observed in the case of *Escherichia coli* (0mm). Researchers Abascal and Yamal found that "acorenone, from *Acorus calamus* rhizome, strongly inhibited a strain of *S. aureus*" in their 2002 study (Souwalak *et al*. 2005) reported that β-asarone from methanol extract of it exhibited inhibitory effect against *Staphylococcus aureus*. In our study also, methanolic fraction of *Acorus calamus* exhibited inhibitory effect against *Staphylococcus aureus*. Only two bacterial species; *Bacillus* spp. and *S. aureus* were shown to be inhibited by *A. calamus* during this study. Against *E. coli*, it was ineffective. Only two bacteria are inhibited by the ethanol, methanol, and hexane fraction (Ahmad, 2001), the result is comparable.

In this study, *Staphylococcus aureus* (7mm) was found to be highly sensitive to the ethyl acetate extract of *Ocimum basilicum* (Tulsi), whereas no efficacy was observed in the case of *Escherichia coli* (0mm). Maximum efficacy was shown against *Staphylococcus aureus* (3mm) in the case of the methanolic extract of *Ocimum basilicum* (Tulsi), whereas no efficacy was observed in the case of *Escherichia coli* (0mm). Maximum efficacy was shown against *Bacillus* spp. (10mm) in the case of the hexane extract of *Ocimum basilicum* (Tulsi), whereas the no efficacy was observed in the case of *Escherichia coli* (0mm). According to J. Vuliana and Group's study published in the "Asian Journal of Plant Science" in 2023, an ethanol extract of *Ocimum basilicum* (Tulsi) was able to stop *E. coli* from growing despite the fact that it is a gram-negative bacterium. Ethanol extract of *Ocimum basilicum* (Tulsi) was able to suppress the growth of *E. coli* at concentrations of 40%, 60%, and 80%. The National Library of Medicine (2008) states that a methanolic extract of *Ocimum basilicum* (Tulsi) is effective against *S. aureus* and *Bacillus* spp. Similar to this, our research demonstrates that *Ocimum basilicum* (Tulsi) extract in methanol, ethyl acetate, and hexane was efficient against *S. aureus* and *Bacillus* spp. According to our research, a methanolic extract of *Ocimum basilicum* (Tulsi) was able to demonstrate an inhibitory effect against *Bacillus* spp. with 1mm ZOI and *S. aureus* with 3mm ZOI. Similar to methanolic extract. Hexane and ethyl acetate extracts were also effective against both bacteria, but none of the extracts were effective against E. coli, a gram-negative bacterium.

In this study, it became clear that the ethyl acetate extract of *Psidium guajava* (Guava leaves) was extremely effective against *Bacillus* spp. (15mm), while *Escherichia coli* (0mm) showed no efficacy. Maximum efficacy was shown against *Staphylococcus aureus* (14mm) in the case of the methanolic extract of *Psidium guajava* (Guava leaves), while no efficacy was observed in the case of *Escherichia coli* (0mm). Maximum efficacy was shown against *Bacillus* spp. (15mm) in the case of the hexane extract of *Psidium guajava* (Guava leaves), whereas the no efficacy was observed in the case of *Escherichia coli* (0mm). According to the findings of a study conducted by Bipul Biswas et al.

(2013), *Psidium guajava* (Guava leaves) extracts in methanol and ethyl acetate demonstrated inhibitory effects against gram-positive bacteria but not gram-negative bacteria. The antibacterial activity of the methanol extract against *Bacillus* spp. and *S. aureus* was marginally higher at 10 mg/50l, with ZOI values of 8.27 and 12.3 mm, respectively. Similar to this, we discovered in the research we conducted that *Psidium guajava* (Guava leaves) extracts in methanol, ethyl acetate, and hexane exhibited inhibitory effects against gram-positive bacteria but not against gram-negative bacteria. We used *E. coli*, a gram-negative bacterium, against which the extract from guava leaves was not effective. ZOI was found at 0mm against *E. coli*, indicating that the guava leaf extract had no inhibitory effect.

In this study, it has been discovered that the ethyl acetate extract of *Coriandrum sativum* (Coriander) was highly effective against *Bacillus* spp. (25mm), while *Escherichia coli* (14mm) showed the least efficacy. The maximum efficiency was shown against *Bacillus* spp. (13mm) in the case of the methanolic extract of *Coriandrum sativum* (Coriander), whereas the least efficacy was observed in the case of *Escherichia coli* (11mm). Maximum efficacy was observed against *Bacillus* spp. (16mm) in the case of hexane extract of *Coriandrum sativum* (Coriander), whereas least efficacy was observed in the case of *Staphylococcus aureus* (11mm). According to the research conducted by Anita Verma and her team from the College of Basic Science and Humanities in Patnanagar on November 5, 2018, all methanolic extracts of *Coriander sativum* (Coriander) demonstrated an inhibitory effect against *E. coli* and *B. subtilis*. Similar to this, our research led us to the conclusion that an extract of *Coriander sativum* (Coriander) in methanol, ethyl acetate, and hexane was effective against *E. coli*, *S. aureus*, and *Bacillus* spp. *Coriander sativum* (coriander) demonstrated the best reaction against *E. coli*, while the other plant extracts, we utilized in our study had no effect at all against gram-negative bacteria.

This study examined the minimal inhibitory concentration of *Coriandrum sativum* (Coriander) against a number of common pathogenic bacteria. In an ethyl acetate extract of *Coriandrum sativum* (Coriander), *Staphylococcus aureus* was found to be sensitive at the greatest dilution (1:32). *Staphylococcus aureus* was discovered to be sensitive in the case of the methanolic extract of *Coriandrum sativum* (Coriander) at the greatest dilution (1:64). Similar results were reported for *Staphylococcus aureus* in the case of the hexane extract of *Coriandrum sativum* (Coriander), where the greatest dilution (1:16) was shown to be sensitive.

Three bacterial isolates and a small number of herbal plants were used in the study, which only concentrated on pathogenic organisms. From the start to the end of the study, tests were conducted using crude extract without verifying its purity. To identify specific antibacterial properties, present in the extracts of these plants, more analysis is required. In order to evaluate the potential of these plant extracts in the treatment of infectious diseases, clinical trials should be performed.

Conclusion

According to the study, *Coriandrum sativum* (coriander) is the most effective herbal plant against common pathogenic bacteria. Ethyl acetate extract was found to be more effective when compared to methanolic and hexane extracts. As a result, ethyl acetate was discovered to be a better solvent than methanol and hexane. To update its medicinal value, additional research on its antibacterial characteristics may be required.

ACKNOWLEDGEMENTS

We would like to express our sincere gratitude to all the faculty members of Department of Microbiology, Kathmandu College of Science and Technology, Kathmandu, Nepal.

CONFLICT OF INTEREST

The authors declared no conflict of interest.

REFEREBCES

Abascal K and Yamal E (2002) Herbs and drug resistance: Part I- herbs and microbial resistance to antibiotics. *Alternative and Complementary Therapies* **8**(2): 237-241.

Ahmad I and Beg AZ (2001) Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multi-drug resistant human pathogens. J *Ethnopharmacol* **74**: 113-123.

Akerele O (1993) Summary of WHO Guide lines for the assessment of herbal medicines. Herbal Gram **28**: 13-28.

Bajarachra AM (2007): Study of drinking water quality in the Kathmandu metropolitan area and evaluation of the antibacterial properties of some medicinal plants against isolated enteric bacteria M.Sc. Thesis, Central Department of Microbiology, Tribhuvan University, Kirtipur, Kathmandu, Nepal.

Baral SR and Kurmi PR (2006): A Compendium of Medicinal Plants in Nepal IUCN, *The World Conservation Union Mass Printing Press*, Chhauni, Kathmandu, pp. 52–392.

Baron EJ, Peterson LR, and Finegold SM (1994) Bailey and Scott's diagnostic Microbiology, 9th Edition, *Mosby Year Book*, Inc., USA, pp. 166–177.

Bhatta L. (1998): Study of the Antimicrobial Properties of *Punica granatum* M.Sc. Thesis, Central Department of Microbiology, Kirtipur, Kathmandu, Nepal.

Chakraborty P. (2000) A textbook of microbiology 1st edition. *Reprinted by Vikas Publishing House Pvt. Ltd.*

Cheesbrough M. (1993), *Medical Laboratory Manual for Tropical Countries*, Vol. II, ELBS, pp. 196–200.

Cheesbrough M. (2002). District laboratory practices in tropical countries. *Cambridge University Press*, U.K**. 2**: 124–130.

Cowan MM (1999) Plant products as antimicrobial agents *Clinical Microbiology Reviews* **12**(4): 564-582.

Devkota KP, Acharya R, Baral MP, and Adhikari RP (1999) Antimicrobial activities of some herbal plants used in traditional medicine in Nepal Proceedings of the 3rd National Conference on Science and Technology, March 8–11, Vol. II, 1311–1317.

Dixit SN and Tripati SC (1982): antifungal antibiotics from higher plants Recent Advances in the Biology of Microorganisms **2**: 519–523.

DPR (2007), Medicinal Plants of Nepal, Bulletin of the Department of Plant Resources No. 28: Medicinal Plants of Nepal (Revised) Government of Nepal Ministry of Forest and Soil Conservation, Department of Plant Resources (DPR), Kathmandu, Nepal.

ICS-UNIDO (2006), South-East Asian (SEA) regional workshop on "extraction technologies for medicinal and aromatic plants," Lucknow, India, pp. 1–2.

Joshi AR and Joshi SD (2001): Genetic heritage of medicinal and aromatic plants of the Nepal Himalayas Buddha Acadenmic Publishers and Distributors Pvt. Ltd., Kathmandu, Nepal, pp. 239.

Kokoska L., Polesny Z., Rada V., Nepovim A., and Vanek T. (2002). Screening of some Siberian medicinal plants for antimicrobial activity. *Journal of Ethnopharmacology* **82**: 51–53.

Machado TB, Pinto AV, Pinto MCFR, Leal ICR, Silva MG, Amaral ACF, Kuster RM, and Netto KR (2003) *In vitro* activity of Brazilian medicinal plants, naturally occurring naphthoquinones, and their analoques against methicilin-resistant *S. aureus International Journal of Antimicrobiology* **21**: 279–284.

Mahoto RB and Chaudhary RP (2005). Ethnomedicinal study and antibacterial activities of selected plants in Palpa district, Nepal. Scientific World **3**(3): 5-8

Malla SB and Shakya PR (1984/85): Medicinal plants of Nepal, nature's paradise Edited. by J.C. Majpuria. White Lotus Ltd., Bangkok, pp. 261-297.

Manandhar NP (1980): Medicinal Plants of the Nepal Himalaya Ratna Pustak Bhandar, Kathmandu, Nepal, pp. 85.

Mathabe MC, Nikolova RV, Lall N, and Nyazema NZ (2006). Antibacterial activities of medicinal plants used for the treatment of diarrhea in Limpopo Province, South Africa. *Journal of Ethnopharmacology* **105**: 286-293.

Mazumber R, Bhattacharya S, Mazumber A, Pattnik K, Tiwary PM, and Chaudhary S (2005). Antidiarrhoeal Evaluation of *Aegle marmelos* (Correa) Linn. Root Extract. *Phytotherapy Research* **20**(1): 82–84.

Miranda D., Pereira L., Sirsat SM., Antarkar DS., and Vaidya AB. (1995) *In vitro* action of selected medicinal plants against microorganisms involved in human gastrointestinal infections *Journal of Research in Ayurveda and Siddha* **14**(3–4): 149–159.

Mustafa NK, Tanira MOM, Dar FK, and Sanze HN (1999): Antimicrobial activity of *Acacia nilotica* subsp. nilotica fruit extracts *Pharmacy and pharmacology communications*, **5**(9), 583-586.

Nair R. and Chanda S. (2007). *In vitro* antimicrobial activity of *Psidium guajava* leaf extracts against clinically important pathogenic microbial strains. *Brazilian Journal of Microbiology* **38**: 452-458.

Parajuli S. (2001) Medicinal plants used in cuts and wounds in Kaski district (Western Nepal) and their antibacterial activities M.Sc. thesis, Central Department of Botany, Tribhuvan University, Kirtipur, Kathmandu, Nepal.

Parekh J. and Chanda S. (2007a): *In vitro* screening of the antibacterial activity of aqueous and alcoholic extracts of various Indian plant species against selected pathogens from Enterobacteriaceae. *African Journal of Microbial Research*, **1**(6), 092-099.

Parekh J. and Chanda S. (2007 b): *In vitro* antibacterial activity of the crude methanol extract of *Woodfordia fruticosa* Kurz flower (Lythraceae). *Brazilian Journal of Microbiolog*y **38**: 204–207.

Perez C., Paul M., and Bazerque P. (1990): Antibiotic assay by agar-well diffusion method.

Acta Biomedicine Experimentalis **15**: 113–115.

Radha D. (2004) Evaluation of the antimicrobial activities of some medicinal plants M.Sc. Thesis, Central Department of Microbiology, Tribhuvan University, Kirtipur, Kathmandu, Nepal.

Ram A.J., Bhakshu L.M., and Raju RRV (2004) *In vitro* antimicrobial activity of certain medicinal plants from the Eastern Ghats, India, used for skin diseases *Journal of Ethnopharmacology* **90**: 353–357.

Recio MC (1983): A review of some antimicrobial compounds isolated from medicinal plants reported in the literature from 1978 to 1988 *Phytotherapy Research* **3**: 1445–1453.

Rijal K. (1994) Preliminary study on some medicinal plants and essential oils for their antimicrobial activities. Proceedings of the National Conference on Science and Technology RONAST, Kathmandu, Nepal, pp. 390–393.

Souwalak P., Nongyao P., Vatcharin R., and Metto O. (2005). Antimicrobial activities of the crude methanol extracts of *Acorus calamus* Linn. Songklanakarin. *Journal of Science and Technology* **27**(2): 517–523.

Taylor RSL, Edel F, Manandhar NP and Towers GHN (1996) Antimicrobial activities of Southern Nepalese medicinal plants. Journal of Taylor RSL, Edel F, Manandhar NP, and

Tripathi KD (2003), "Essentials of Medical Pharmacology." 5th Edition, *Jaypee Brothers Medical Publishers (P) Ltd*., New Delhi, pp. 623-630.

Vijaya K. and Ananthan S. (1994): Effect of three medicinal plants on enterpathogens: an *In vitro* study Biomedicine **14**: 21–23.

WHO (1991): Basic laboratory procedures in clinical bacteriology World Health Organization, Geneva.

Zaiba IA, Beg AZ and Mahmood Z (1998) Antimicrobial potency of selected medicinal plants with special interest in activity against phytopathogenic fungi. *India Veterinary Medical Journal* **24**(4): 299-30.