Antimicrobial and cytotoxic properties of selected medicinal plants from Kavrepalanchowk, Nepal

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Antibacterial properties of Nepalese plant species - *Myrica esculenta, Mahonia nepaulensis, Madhuca longifolia* and *Schima wallichii* were evaluated on human pathogenic microorganisms: *Salmonella typhi, Staphylococcus aureus* and *Escherichia coli*. The plants were found to possess phytochemicals- saponins, glacosides, flavonoids, tannins and alkaloids. Methanolic extract from these plants showed antimicrobial activity against tested organisms. Similarly, Brine shrimp lethality test of extracts showed the LC50 values of 15.4 ppm, 136.4 ppm, 76.9 ppm and 76.0 ppm for *M. esculenta, M. nepaulensis, M. longifolia* and *S. wallichii* respectively, which suggests that these plants are less toxic to human consumption for drug purpose.

Key words: Nepalese plants, phytochemicals, antimicrobial and cytotoxic activities, human pathogens

The history of medicine and medicinal plants in Nepal can be traced back to the Vedic Period where Nepal Himalaya has been mentioned as a sacred heaven of potent medicinal and aromatic plants. The original "Sanskrit Nighantu," written on palm leaves in Newari script and Sanskrit verses during Mandeva Era 301 (879 AD), is said to be the oldest of these books. The usage of the plants as subsistence for folk therapies was largely influenced from traditional medicinal health care systems mainly for treating or preventing various ailments. A major part of total population in developing countries uses folklore medicine obtained from plant resources (Fabricant and Farnsworth, 2001). Medicinal plant usage forms the backbone in many rural communities for treating ailments with varying severity (Wyk *et al.*, 1997). The utilization of medicinal plant remedies in preventing or curing various ailments were the sole source of ensuring human welfare until the development of chemistry and organic compound synthesis in the 19th century (Kong *et al.*, 2003).

The research on biologically active compounds from natural sources has always been of great interest for scientists looking for new sources of useful drugs against infectious diseases. In recent years, interest to evaluate plants possessing antibacterial activity for various microbes is growing. This is because of the increase in the resistance by microorganisms to new antibiotics as compared to the past. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs which are utilized as therapeutic agents (Gislene *et al.*, 2000). Plant parts such as fruits, leaves, stems and barks are used in traditional medicinal practice for curing cough and cold, tonsillitis, headache, malarial fever and neck pain; to reduce blood pressure, chest pains, lung diseases, bronchitis and respiratory diseases; and for treatment of animals affected by different kinds of insects, scabies and wounds (Gyawali *et al.*, 2008). Several medicinal plants have been screened to identify the possible natural antibacterial agents from medicinal plants (Gyawali *et al.*, 2013, 2014). *Myrica esculenta* Buch–Ham ex D. Don (Myricaceae) bark is useful for cough, asthma, sinusitis and chronic bronchitis, diarrhea and dysentery (Baral and Kurmi, 2006; Watanabe *et al.*, 2005). *Mahonia nepaulensis* DC (Berberidaceae) bark juice is traditionally used in many communities to treat infections and wounds. The barks of *Madhuca longifolia* (J. Koeing ex L) J.F. Macbr (Sapotaceae) are said to possess antibacterial

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activity, bleeding and spongy gums. The barks of *Schima wallichii* (DC) Korth (Theaceae) are used as an antiseptic for cuts and wounds, vermicide, mechanical irritant and to cure gonorrhea. The barks juice is given to animals infested with liver flukes. Decoction of barks is good for fever and effective against head lice (Gurung, 2002).

Due to specific climatic and geographical conditions of the Himalaya, medicinal plants offer greater possibilities of having novel antimicrobial property with large quantities of the active compounds. Based on the ethno-pharmacological information, the authors have recently documented several medicinal plants found in the various geographical locations of Nepal so as to evaluate the phytochemical profiles and efficacy of traditional medicines. All these conditions were taken into account while conducting the research aimed to assess phytochemical and biological properties of Nepalese medicinal plants.

Materials and methods

Study area

The plant materials were collected from 1400 to 1600 m altitudes within Khopasi Village Development Committee of Kavrepalanchowk District of Central Nepal.

Plant materials

Some barks of *M. esculenta*, *M. longifolia*, *M. nepaulensis* and *S. wallichii* were collected as samples for the purpose of this study. Each sample was pulverized by using home blender, and the powdered sample was initially soaked in methanol for 7 days with occasional shaking. After 7 days, the mixture was filtered, and the process was repeated two times more. The filtrates were combined and the methanol was evaporated from the extracts.

Phytochemical screening

The crude methanolic extracts of the barks of different plants were screened to detect the presence of phytochemicals, as per the Standard Screening Procedure (Trease and Evans,1996).

Antimicrobial assays

In this study, strains of human pathogenic microorganisms *viz*. *Salmonella typhi*, *Staphylococcus aureus* and *Escherichia coli*, collected from Dhulikhel Hospital, Kathmandu University and Teaching Hospital, were used to investigate the antimicrobial potential of the extracts with the help of disk diffusion method. Required amount of bark extract of all four plants were dissolved in methanol to give methanolic extract solution of concentrations of 0%, 3%, 6% and 9%; sterile, 6 mm diameter grade I Whatmans filter paper discs were impregnated with the already prepared methanolic solution. Ciprofloxacin (30 µg) was used as positive control while Methanol (30 µl) was used as negative control. Triplicates of each extract were impregnated in 6 mm diameter disc. To evaporate the methanolic residue from discs, they were placed over the water bath for 1 hour. Each microorganism, at a concentration of 1.5 × 106 cells/ml (adjusted to the 0.5 McFarland turbidity standards), was inoculated on the surface of the respective media. After holding the plates at room temperature for one hour to allow diffusion of the test samples into the agar, they were incubated at 37^{o}C for 24 hours. The results thus obtained were recorded by measuring the zones of growth inhibition around the disc, and presented as the arithmetic average. Inhibition zone values were corrected i.e. the disk diameter was subtracted from the value of the inhibition zone. Overall, cultured microorganisms with zone of inhibition equal to or greater than 7 mm were considered susceptible to the samples tested.

Brine shrimp lethality test

Methanolic extract of the samples were evaluated for lethality to Brine shrimp larvae (*A. salina* Leach) according to the published procedure (Meyer *et al.*, 1982). Brine shrimp eggs were suspended for 48 hours in a conical flask containing 300 ml. of artificial seawater for hatching (the eggs). The flasks were well aerated with the aid of an air pump, and kept in a water bath at 29–30°C. The extracts were dissolved in 1% aqueous DMSO, and then in sea water to obtain a concentration of 500 ppm, 250 ppm, 100 ppm, 50 ppm, 25 ppm and 10 ppm. An aliquot of each concentration (1 ml) was transferred, in triplicate, into clean sterile vials with pipette, and aerated seawater (9 ml) was added. Ten Brine shrimp nauplii were transferred to each vial. Thymol 1% aqueous solution and 1% DMSO in seawater were used as positive and negative controls, respectively. After 24 hours, the numbers of survivors were counted, and the mortality percentage was calculated. The lethal

concentration for 50% mortality after 24 hours of expo-sure, the chronic LC_{50} and 95% confidence intervals were determined using the Probit method (Finney, 1971) as the measure of toxicity of the extract or fractions. The extracts were considered as toxic unless the LC_{50} was found to be less than 10 ug/ml (Setzer *et al.*, 2001).

Results and discussion

The randomly selected barks of the plants subject to phytochemical screenings were found to contain different compounds *viz*. alkaloids, tannin, saponins, flavonoids and glycosides. The methanolic extract of *M. esculenta* barks exhibited antimicrobial activity against all the test-microorganisms. The methanolic extract of *M. esculenta* barks exhibited the highest activity against *E. coli* with the mean growth inhibition zones of 10.33±1.52 mm, 12.33±1.15 mm and 15±1 mm at 3%, 6% and 9% concentrations respectively (Table 1). Similarly, it exhibited the highest significant antibacterial activity against *Salmonella typhi* with the mean inhibition zone of 9.33±0.57 mm at 9% concentration.

The methanolic extract of *M. nepaulensis* barks exhibited maximum effect against *Staphylococcus aureus* with the mean growth inhibition zones of 10.33±1.52 mm and 12.67±1.53 mm at 6% and 9% concentrations respectively (Table 1). The methanolic extract of *M. nepaulensis* barks showed a broad spectrum of antimicrobial activity which might be due to the presence of alkaloids and flavonoids.

The methanolic extract of the *S. wallichii* barks exhibited highest activity against *S. aureus* and *E. coli* with 8.67±0.57 mm and 10.33±0.57 mm mean growth inhibition zones respectively at 9% concentration (Table 1). The hydroalcoholic extract of the *S. wallichii* bark has been found to exhibit the highest sensitivity against *E. coli* while the least activity against the selected Gram positive strains (Dewanjee *et al.*, 2008). *S. aureus*, being Gram positive, supports our results.

The methanolic extract of *M. longifolia* bark also revealed its antibacterial property. It exhibited maximum effect with the mean growth inhibition zone of 12.67±2.08 mm and 15±1 mm against *E. coli* at 6% and 9% concentrations respectively (Table 1). The barks of *M. longifolia* chiefly contain Oleanolic acid, caprylate, lepeolacetate, α-amyrin acetate, erythrodiolmonocaprylate, betulinic acid and α-spinasterol compounds (Khare, 2007). The antimicrobial property of *M. longifolia* bark found in our study could be due to the effects of lepeolacetate, α-amyrin acetate, erythrodiolmonocaprylate, betulinic acid, α-spinasterol and/or other methanol soluble constituents. Lupeol acetate belongs to lupane type triterpenes, and was reported to have antimicrobial activity (Prachayasittikul *et al.*, 2010).

Based on the Brine shrimp bioassay analysis, the LC_{50} value of the bark of *M. esculenta* plant was found to be 15.5 ppm (Table 2). Similarly, the LC_{50} values of the barks of *M. nepaulensis, M. longifolia* and *S. wallichii* plants were found to be

Table 1: Mean growth inhibition zones (mm) of the barks of different plants

Plant name/ Std. drug	*Staphylococcus aureus*			*Escherichia coli*			*Salmonella typhi*		
	Concentration of extract			Concentration of extract			Concentration of extract		
	3%	6%	9%	3%	6%	9%	3%	6%	9%
Myrica esculenta	5±0	5±1	7.33±1.52	10.33±1.52	12.33±1.15	15±1	4.33±1.15	6.67±0.57	9.33±0.57
Madhuca longifolia	5.67±1.15	7.33±0.57	9.33±0.57	8±2	12.67±2.08	15±1	6±1	7.33±1.15	8.67±1.52
Mahonia nepaulensis	7±3	10.33±1.52	12.67±1.52	0	0	7.67±0.57	0	2.33±1.15	5±1
Schima wallichii	3±1	5.33±0.57	8.67±0.57	2. 67±0.57	6.67±0.57	10.33±0.57	0	0	0
Ciprofloxacin, 30 mcg		25.67±2.83			35.67±1.10			18.29±1.98	
Methanol, 30 µl		0			0			0	

Note: The mean growth inhibition zones (mm) are presented as the mean ± SD in the above table.

Table 2: Brine shrimp bioassay results of methanolic extract of some Nepalese medicinal plant barks

Plant	Lethal concentration, LC_{50} (ppm)	95% Confidence interval	
		Lower limit (ppm)	Upper limit (ppm)
Myrica esculenta	15.452	3.388	70.631
Mahonia nepaulensis	136.458	6.324	490.907
Madhuca longifolia	76.913	5.272	1119.438
Schima wallichii	76.032	2.864	2013.724

Table 3 : Phytochemical screening of different plant barks (+ = presence, -- = absent)

Plants	Saponins	Glycosides	Tannins	Alkaloids	Flavonoids
Myrica esculenta	++	++	++	--	++
Madhuca longifolia	++	++	++	--	++
Mahonia nepaulensis	--	--	--	++	++
Schima wallichii	++	++	--	--	++

136.5 ppm, 76.9 ppm and 76.0 ppm respectively. This indicates that the bark of *M. esculenta* plant is moderately toxic, the barks of *M. longifolia* and *S. wallichii* plants are mildly toxic and the bark of *M. nepaulensis* plant is non-toxic. For the purpose, the results were compared with the available previous results: highly toxic plants (having LC_{50}<1.0 ppm); toxic plants (having LC_{50} of 1.0–10.0 ppm); moderately toxic plants (having LC_{50} of 10.0–30.0 ppm); mildly toxic plants (having LC_{50} of 30 -100 ppm) and non–toxic plants (having LC_{50} > 100 ppm) (Meyer *et al.*, 1982).

The barks of all the selected plants *viz*. *M. esculenta*, *M. nepaulensis*, *M. longifolia* and *S. wallichii* were found to possess flavonoids, an important antimicrobial phytochemical (Table 3). The barks of *M. esculenta* and *M. longifolia* plants were found to possess saponins, glycosides and tannins too. Similarly, the bark of *M. nepaulensis* plant was found to possess alkaloids too while that of *S. wallichii* was found to possess saponins and glycosides too.

Conclusion

The analysis of randomly selected Nepalese medicinal plants indicated that most of the plants are rich in antimicrobial phytochemicals. Methanolic extracts from the barks of these plants showed antimicrobial activity against human pathogenic microorganisms. So, attention to these medicinal plants can be drawn for herbo-therapeutic treatment or antimicrobial plant principles in the case of infection by selected microorganisms.

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