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RESEARCH ARTICLE

Antimicrobial Activity and Phytochemical screening of Some Traditionally Used Nepalese Medicinal Plants

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ABSTRACT

Methanolic extract of fifteen Nepalese medicinal plants were subjected to phytochemical screening, antibacterial and antifungal properties. Human pathogenic bacteria *Enterococcus specie, Escherichia species, Staphylococcus species, Proteus mirabilis, Salmonella typhi* and *Salmonella paratyphi* were used to evaluate the antibacterial property while *Candida albican* was used to evaluate the antifungal property. Phytochemical screening of the extract revealed the presence of alkaloids, tannins, saponins, flavonoids, glycosides, terpenoids and steroids in different proportions among the plants. Most of the extracts exhibited the selective antibacterial activity. The extracts showed highest antibacterial activity against *Enterococcus* species (20.8±0.72 mm) followed by *Escherichia coli (*19.37±0.35) by plants *Clematis buchananiana* and *Psidium guajava* respectively. Antifungal screening of these plant extract showed that only three plants *Solanum xantocarpum, Jasminum humile* and *Aegle marmelos* were slightly active against *Candida albican.*

Keywords: Medicinal plants, Ethnopharmacology, Antimicrobial, Phytochemicals

INTRODUCTION

Medicinal plants are playing an especial role in the therapies of rural areas of Nepal because of the ease of availability, least side effects and low cost make the herbal preparations. Many plants are considered a fundamental source of potent drugs for traditional medicine since ancient period. Local herbalists and practitioner emphasized the abundance of herbal resources for immediate and long-term treatment of diseases. These plants still constitute one of the major sources of drug in modern medicine [1]. Plant parts are used in traditional medicinal practice in Nepal for curing of cough and cold, tonsillitis, headache, malarial fever, neck pain, to reduce blood pressure, chest pains, lung diseases, bronchitis, and respiratory diseases [2]. The traditional use of plants by indigenous communities hints the immense pharmacological potential to cure many diseases [3-5]. The biological diversity, traditional medication systems, and fork medicinal practices of Nepal offers immense opportunities for ethnobotanical and ethnopharmacological studies [6-10]. In Nepali traditional medicine, more than 2300 plant species [11] are used by 125 caste/ethnic communities speaking approximately 123 different languages [12].

In recent years, there is an increasing trend of intensive studies on extracts from plants to investigate the new antimicrobial agent for natural therapies. Plethora of study showed that Asian and African continents have 56% and 17% share of the worldwide distribution of therapeutic herbal plants, respectively [13]. Plants synthesize bioactive substances in the form of secondary metabolites which may be responsible for antimicrobial activity. The antimicrobial property of most plants makes them to be valuable in the ethnomedicine. In recent years, increase in antibiotic resistant strains of clinically important pathogens has drawn global attention which has urged scientists to search for potential antimicrobial herbal medicine [14]. Thus, the effectiveness of plant extracts on microorganism has been studied worldwide that can serve as source for the new antimicrobial drugs [15-17]. The present study was, therefore, aimed at evaluating the phytochemical potential and antibacterial

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activity of randomly selected some medicinal plants of Nepal.

MATEIALS AND METHODS

Plant materials and extraction

Plant materials were obtained from different parts of Kavrepalanchowk and Chitwan districts of Nepal during June-July, 2014. The plant parts were identified as per pertinent taxonomic literature and herbarium is deposited in Department of Pharmacy, Kathmandu University, Nepal. Plant materials were thoroughly washed with distilled water and dried under shade at room temperature for about two weeks. The dried plant samples were individually ground well into a fine powder in a mixture grinder. These powder samples were stored in air sealed polythene bags at room temperature before extraction. Extraction was carried out at room temperature with powder soaked in methanol. The extract was filtered, the residue was evaporated in water bath and dried extracts were then stored in a refrigerator at 4^0C[18-19] . The extracts of different plant samples were subjected to preliminary phytochemical screening for the presence of alkaloids, terpenoids, flavanoids, tannins, polyphenols, saponins, steroids, carbohydrate, cardiac glycosides using the standard method [20].

Test Microorganism

Bacterial strains; *Escherichia coli, Klebsiella pneumonia, Enterococcus species, Salmonella typhi, Salmonella paratyphi*, *Proteus mirabilis* and *Staphylococcus aureus* and fungal strain; *Candida albicans* were obtained from Department of Microbiology, Kathmandu University Teaching Hospital, Dhulikhel, Nepal.

Antimicrobial Screening

Disc diffusion method was used for the antimicrobial susceptibility testing. Then the stock culture of each microorganism was sub-cultured onto nutrient broth at 37^0C overnight before experiment. The cultures were adjusted to a suspension density equal to 0.5 McFarland turbidity standards, which has an approximate cell density of 1.5×106 cfu/ml [18]. Inoculums suspension of the bacteria and fungi were spread on the surface media. The filter paper disc impregnated with different concentrations of plant extracts were placed on test organism –seeded plates. Blank disc impregnated with methanol followed by drying off was used as negative control. Standard ciprofloxacin (30mcg/disc) were used as approximate control for the sensitivity of tested microorganisms. The antibacterial assay plates were incubated under normal atmospheric conditions at 37^0C for 24 hours. The experiments were performed in triplicate as per the procedure given in the literature and mean values and their standard deviations are presented in tables. The zone of inhibition was measured for all the plates.

The antifungal activity was tested by disc diffusion method [21].The blood agar plates were inoculated with the fungal culture. Methanol was used to dissolve the extract and was completely evaporated before application on test organism seeded plates. Blank disc impregnated with methanol followed by drying off was used as negative control and Amphoterricin B (10 mcg/disc) was used as positive control. The activity was determined after 3 days of incubation at 28^0C.The diameters of the zones of inhibition were measured in mm.

RESULTS AND DISCUSSION

We collected fifteen medicinal plant species from Kavre and Chitiwan districts of Nepal (**Table 1**). These locally used medicinal plants were found to treat more than four dozen different ailments. The majority of plant species have more affinity towards the Ayurvedic system of medicine.

Table 1: Plants collected based on ethnobotanical uses from the different parts of Nepal

Scientific Name (Code)	Local Name (collected place)	Parts Used	Traditional Use
Solanum xantocarpum	Kanthakari (Kavre)	Fruits	diabetics, rheumatism, catarrhal, stone in the bladder, abortion, and gonorrhea.
Artemisia vulgaris	Titepati (Kavre)	Leaves	fever, scabies, skin irritation, and natural pesticide
Clematis buchananiana	Abijalo (Chitwan)	Whole	immunity, cooling effect, headache
Trigonella foenum-graecum	Methi (Kavre)	Leaves	skin problems, wounds, blood sugar regulation.
Nephrolepis cordifolia	Paniamala (Kavre)	Fruits	asthma, immunity and biliousness
Myrica esculenta	Kafal (Kavre)	Leaves	bleeding during meansuration, cough and cold.
Jasminum humile	Jaai (Kavre)	Leaves	treatment of ringworm, infestation, stomach disorders
Gaultheria fragrantissima	Dhasingre (Kavre)	Leaves	wound healing, anti-inflammatory and analgesic
Choerospondias axillaris	Lapsi (Kavre)	Leaves	parturition, postpartum recovery and infant healthcare
Lycopodium clavatum	Nagbeli (Kavre)	plant	immunity, rheumatism, high blood pressure.
Azadirachta indica	Neem leaf (chitwan)	Leaves	leprosy, intestinal worms, skin ulcers, blood purifier, natural pesticide
Aegle marmelos	Bel (Chitwan)	Flower	cure dysentery, diarrhea, hepatitis, dyspepsia and diabetes
Psidium guajava	Amba (Chitwan)	Leaves	diarrhea, dysentery, gastroenteritis, ulcers, bacteria, hemorrhagemia
Artocarpus heterophyllus	Rukh Katahar (Chitwan)	Leaves	anti-inflammatory, antibacterial, vegetable

Eupatorium adenophorum	Banmara (Chitwan)	Aerial	wound healing, stop bleeding from wound

Phytochemical Screening of the Plant

The research work was carried out on the fifteen selected medicinal plants which shows that phytochemical constituent's i.e., alkaloids, tannins, saponins, flavonoids, glycosides, terpenoids and steroids are either present or absent in these plants and the results were summarized in (**Table 2).** It was investigated that alkaloids, tannins and flavanoids are most dominant among the several plants while steroids are found rarely distributed. Phytochemical screening of the plants revealed some differences in the constituents among the plants tested. Plants *C. buchananiana* and *P. guajava*, which are potential in antimicrobial property, were found rich in alkaloids, tannin and flavanoids.

Table 2: Phytocheical screening results of mehanolic extracts of different plants from Kavrepalanchowk and Chitwan district of Nepal

Plants	Alkaloids	Tannins	saponins	Flavanoids	glycosides	terpenoids	steroids
A. vulgaris	+	+	+	+	--	+	--
C. buchananiana	+	+	--	+	--	--	--
T. foenum-graecum	+	+	+	+	+	+	--
N. cordifolia	+	--	--	--	--	+	+
M. esculenta	--	+	--	+	--	+	--
S. xantocarpum	+	--	--	--	+	--	+
J. humile	--	+	--	+	--	--	--
G. fragrantissima	+	--	+	--	--	+	--
C. axillaris	--	+	+	+	+	--	--
L. clavatum	--	--	--	+	--	+	--
A. indica	+	+	+	--	--	+	--
E. adenophorum	+	+	+	--	--	+	--
A. marmelos	--	+	+	+	+	+	--
P. guajava	+	+	--	+	--	+	--
A. heterophyllus	+	--	+	+	--	--	+

+ = indicates presence of phytochemicals and -- = indicates absence of phytochemicals

Antimicrobial property

The pharmacological property of the plant cannot be ascertained by the result of phytochemical studies only. Therefore the antibacterial and antifungal activity against pathogenic bacteria and fungi was also evaluated in this study. The present investigation shows the efficacy of all the extracts against the selected pathogenic bacteria (**Table 3, 4 & 5**) and fungi (**Table 6**).

The methanolic extract of plants *C. buchananiana* and *P. guajava* showed highest antibacterial activity against *Enterococcus* species (20.8±0.72 mm) followed by *Escherichia coli* **(**19.37±0.35) respectively. The maximum zone of inhibition was observed at the concentration of 20 %. Varying degree of antibacterial activity by extracts against various tested bacterial species has been reported. Studies have shown that *Clematis* species have various compounds including triterpenes, flavonoids, lignins, coumarins, alkaloids, organic acids, macrocyclic compounds and poly phenols extensively. Among these compounds, the triterpenes, saponins, flavonoids and lignins are found more abundantly than other groups[22]. Similarities can be observed in the results of this study that the ethanolic extract of *C. papuasica* against bacteria such as Staphylococcus, Enterococcus and E. coli had considerably antimicrobial effects, but Pseudomonas and Klebsiella were not sensitive to these extracts[23]. Similarly, previous research has found no antibacterial activity of ethanolic extracts of guava against *E. coli* [24]; however, found guava sprout extracts were effective against inhibiting *E. coli* [25]. Here, *T.foenum-graecum, S. xantocarpum, J. humile* however, showed selective antibacterial activity, inhibiting growth of only one microorganism. Out of fifteen plants only *P. guajava* and *C.axillaris* showed the strong activity against *Salmonella typhii, Escherichia coli* and *Proteus mirabilis* at small concentration (5%). Some of the medicinal plants *N. cordifolia, G. fragrantissima, L. clavatum,* and *A. heterophyllus* did not show any response against tested bacteria.

Table 3: Antibacterial activity of methanolic extracts of different plants against human pathogenic bacteria. Concentration of exexracts5%, 10%, 15% and20% (w/v in methanol). Chloramphenicol (30mcg/disc) as Standard [24±1] and Nitrofurantoin (30 mcg/disc) as Standard [29.16±0.64]

Plant extracts	***Escherichia coli* (chloramphenicol as std.)**				***Enterococcus* species (Nitrofurantoin as std.)**			
	concentrations				**concentrations**			
	5%	10%	15%	20%	5%	10%	15%	20%
A.vulgaris	8.83±0.77	9.93±0.49	11.7±0.75	11.66±0.64	9.7±0.56	10.6±0.62	12.53±0.42	12.7±0.36
C. buchananiana	8.3±0.26	8.87±0.42	9.8±0.53	9.8±0.7	18.23±0.77	18.47±0.72	18.73±0.64	20.8±0.72

T.foenum-graecum	-	-	-	-	-	-	-	-
N. cordifolia	-	-	-	-	-	-	-	-
M. esculenta	7.93±0.31	7.93±0.5	8.6±0.6	8.47±0.47	9.43±0.25	9.4±0.53	9.067±0.9	9.067±0.42
S.xantocarpum	-	-	-	-	-	-	-	-
J. humile	-	-	-	-	-	-	-	-
G.fragrantissima	-	-	-	-	-	-	-	-
C.axillaris	12.9±0.46	13.8±0.72±	13.87±0.84	14.97±0.35	-	-	-	-
L.clavatum	-	-	-	-	-	-	-	-
A.indica	-	-	-	-	-	-	-	
E. adenophorum	-	-	-	-	8.1±0.17	8.53±0.15	8.63±1	8.4±1.23
A. marmelos	-	-	-	-				
P. guajava	11.93±0.57	12.9±0.1	16±0.61	19.37±0.35	10.5±0.65	11.27±0.93	12.07±0.47	12.03±0.58
A. heterophyllus	-	-	-	-	-	-	-	-

Table 4: Antibacterial activity of methanolic extracts of different plants against human pathogenic bacteria. Concentration of exexracts5%, 10% , 15% and20% (w/v in methanol). Nitrofurantoin (30 mcg/disc) as Standard [23.2±0.95]. and Nitrofurantoin (30 mcg/disc) as Standard [23.8±0.56]

Plant extracts	***Proteus mirabilis* (nitrofurantoin as std.)**				***Staphylococcus aureus* (nitrofurantoin as std)**			
	concentrations				**concentrations**			
	5%	10%	15%	20%	5%	10%	15%	20%
A.vulgaris	8.07±0.15	8.3±0.26	7.83±0.21	8.07±0.50	8.87±0.66	9.4±0.26	9.33±0.32	9.3±0.44
C. buchananiana	-	-	-	-	9.37±0.21	9.63±0.60	9.87±0.76	9.9±0.1
T.foenum graecum	-	-	-	-	10.17±0.15	10.07±0.47	10.23±0.30	10.3±0.53
N. cordifolia	-	-	-	-	-	-	-	-
M. esculenta	9.13±0.15	9.27±0.15	9.3±0.26	9.43±0.15	10.37±0.15	10.37±0.87	10.47±0.66	10.73±0.76
S.xantocarpum	-	-	-	-	7.4±0.44	7.13±0.15	7.5±0.4	7.6±0.44
J. humile	7.03±0.6	7.23±0.35	7.17±0.31	7.13±0.32	11.2±1.05	11.53±0.42	11.63±1.34	11.9±0.78
G.fragrantissima	-	-	-	-	-	-	-	-
C.axillaris	12.13±0.15	12.5±0.1	12.43±0.38	12.53±0.47	10.13±0.21	9.97±0.45	9.67±0.58	10.3±0.66
L.clavatum	-	-	-	-	-	-	-	-
A.indica	-	-	-	-	11.93±0.15	11.97±0.25	12.1±0.35	12.13±0.25
E. adenophorum	-	-	-	-	-	-	-	-
A. marmelos	-	-	-	-	9.23±0.15	8.7±0.44	9.23±0.15	9.8±0.62
P. guajava	15.23±0.15	15.5±0.56	15.6±1.47	16.13±0.15	8.63±0.35	8.5±0.7	8±0.1	8.93±0.90
A. heterophyllus	-	-	-	-	-	-	-	-

Table 5: Antibacterial activity of methanolic extracts of different plants against human pathogenic bacteria. Concentration of exexracts 5%, 10%, 15% and 20% (w/v in methanol). Nitrofurantoin (30mcg/disc) as Standard [15.73±0.55] and ciprofloxacin (30 mcg/disc) as Standard [18.47±0.67]

Plant extracts	***Salmonella typhii* (nitrofurantoin as std.)**				***Salmonella paratyphi* (ciprofloxacin as std.)**			
	concentrations				**concentrations**			
	5%	10%	15%	20%	5%	10%	15%	20%
A.vulgaris	9.4±0.20	9.57±0.42	9.57±0.32	9.87±0.15	-	-	-	-
C. buchananiana	-	-	-	-	-	-	-	-
T.foenum graecum	-	-	-	-	-	-	-	-
N. cordifolia	-	-	-	-	-	-	-	-
M. esculenta	9.33±0.49	9.67±0.55	10.33±0.55	9.97±0.35	8.17±0.21	8.27±0.55	8.47±0.32	9.13±0.21
S.xantocarpum	-	-	-	-				
J. humile	-	-	-	-				
G.fragrantissima	-	-	-	-				
C.axillaris	12.1±0.26	12.27±0.76	12.13±0.71	12.9±1	10.33±0.25	10.37±0.38	10.8±0.36	11.8±0.56
L.clavatum	-	-	-	-				
A.indica	-	-	-	-	7.87±0.25	7.93±0.25	8.07±0.15	8.5±0.26
E. adenophorum	-	-	-	-	-	-	-	-
A. marmelos	8.17±0.21	8.37±0.11	8.53±0.55	8.5±0.46	-	-	-	-
P. guajava	12.6±0.46	12.77±0.71	13.27±0.55	13.8±0.75	14.8±0.26	14.8±0.4	15.03±0.76	15.5±0.98
A. heterophyllus	-	-	-	-				

Medicinal plants contain many natural products, which are abundant source of bioactive compounds. Plants are source for development of new antimicrobial agents [26]. The high antibacterial activity in the methanolic extract may be due to the presence of tannins, flavonoids, and terpenoids. These medicinally bioactive components exert antimicrobial action through different mechanism. Tannins cause inhibition in the cell wall synthesis by forming irreversible complexes with prolene rich protein [27]. Flavonoids which have been found to be effective antimicrobial substances against a wide array of microorganisms in vitro are known to be synthesized in response to microbial infection by plants. They have the ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls [28] .

Antifungal screening of these plant extract showed that only three plants *Solanum xantocarpum, Jasminum humile* and *Aegle marmelos* were slightly active against *Candida albican.*

Table 6: Screening of antifungal assay of methanolic extract of plants. Amphotericin B (10µg per disc) as standard (16.07±0.78)

Plant extracts	*Candida albumins* concentrations			
	5%	10%	15%	20%
A.vulgaris				
C. buchananiana	-	-	-	-
T.foenum-graecum	-	-	-	-
N. cordifolia	-	-	-	-
M. esculenta	-	-	-	-
S. xantocarpum	11.3±0.36	11.43±0.38	12.03±0.15	11.97±0.06
J. humile	-	-	-	-
G. fragrantissima	-	-	-	-
C. axillaris	-	-	-	-
L. clavatum	-	-	-	-
A. indica	-	-	-	-
E. adenophorum	6.73±0.47	6.7±0.61	7±0.95	7.27±0.21
A. marmelos	12.9±0.26	13.03±0.47	12.57±0.38	13.03±0.76
P. guajava	-	-	-	-
A. heterophyllus	-	-	-	-

CONCLUSION

In an attempt to finding new antimicrobial medicinal plant from our wealthy culture of plants we found that the varying degree of antibacterial activity of plants against various tested bacterial species. But the methanolic extract of plants *C. buchananiana* and *P. guajava* showed highest antibacterial activity against *Enterococcus* species (20.8±0.72 mm) followed by *Escherichia coli* (19.37±0.35) respectively at 20% of concentration. Antifungal screening of these plant extract showed that only three plants *Solanum xantocarpum*, *Jasminum humile* and *Aegle marmelos* were slightly active against *Candida albican.* The results of this research are encouraging, and these plants could be a source of new antibiotic compounds. Further work is needed to isolate the secondary metabolites from the extracts studied in order to test specific antimicrobial activity.

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