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Antimicrobial and Antioxidant Activity of Some Indigenous Plants of Nepal

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ABSTRACT
Seven indigenous medicinal plants, *Mallotus philippensis, Pogostemon cablin, Colebrookea oppositifolia, Mussaenda macrophylla, Celosia argentea, Pilea symmeria* and *Thysanolaena maxima,* have been investigated for their antimicrobial activity and antioxidant activity. The ethanol extract of these medicinal plants were subjected to evaluate their antibacterial properties and their antioxidant potential. The antibacterial screening against four bacteria, *Staphylococcus aureus, Klebsiella pneumoniae*, *Proteus vulgaris* and *Escherichia coli),* was done by disc diffusion method and Zone of Inhibition (ZOI) was observed. The ZOI obtained ranges from 7 to 16 mm. The Antioxidant activity of the extract was tested using scavenging activity of DPPH (1, 1-Diphenyl-2- Picrylhydrazyl) radical method. Ascorbic acid was taken as standard. IC_{50} of four extracts were obtained < 100 µg/ml whereas three had > 100 µg/ml. The overall result shows that almost all the plant extracts have interesting antibacterial activity and among seven, four had remarkable radical scavenging potential to be used as an antioxidant.

Keywords: Plant extract, antibacterial, medicinal plants, antioxidant properties, Nepal.

1. Introduction

In recent years, the number of multi –drug resistant microbial strains and the appearance of strains with reduced susceptibility to antibiotics is continuously increasing in alarming rate. This increase has been attributed to the abusive and indiscriminate use of broad-spectrum antibiotics, immunosuppressive agent, intravenous catheters, organ transplantation and ongoing epidemics of HIV infection [1, 2, 3, 4]. In addition to this problem, antibiotics are sometimes associated with adverse effects on the host including hypersensitivity, immune-suppression and allergic reactions. In this scenario, natural products from plants could be interesting alternatives since these are valuable source of medicinal agents with proven potential of treating infectious diseases and with lesser side effects compared to the synthetic drug agents [5, 6, 7]. The literature survey reveal that a number of studies have been conducted in different countries to demonstrate such efficacy of medicinal plants [8, 9, 10].

On the other hand, free radicals are known to play a crucial role in the development of tissue damage in various human diseases such as cancer, aging, neurodegenerative disease, arteriosclerosis and pathological events in living organism [11, 12]. Antioxidants may have an important role in prevention of these diseases by inhibiting or preventing the oxidation of oxidizable materials by scavenging free radicals and diminishing oxidative stress [13, 14]. The most commonly used synthetic antioxidants are butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG) and butylated hydroquinone. However, these synthetic antioxidants have possible activity as promoters of carcinogenesis. Therefore, there is a need for isolation and characterization of natural antioxidant having less or no side effects, for use in foods or medicinal materials in order to replace synthetic antioxidants. So far many plants have been claimed to pose beneficial health effects such as antioxidant properties and antimicrobial properties [15, 16] and still the potential of many plants as source for new drugs is still largely unexplored. Screening of plants for their antimicrobial activities is important for finding potential new compounds for therapeutic use. The present study was undertaken to investigate the antimicrobial and antioxidant properties of ethanolic extracts of *M. philippensis, P. cablin, C. oppositifolia, M. macrophylla, C. argentea, P. symmeria* and *T. maxima.* The findings from this work may add to the overall value of the medicinal potential of the plants.

2. Materials and Methods
2.1 Collection and processing of plants
In this study medicinal plants were selected on the basis of their medicinal importance in literature and to people, especially in Tanahun and Dhankuta districts of Nepal. Roots of *C. oppositifolia, M. macrophylla, P. symmeria*, *T. maxima*, bark of *M. philippensis,* whole plant of *C. argentea* (from Tanahun) and leaves of *P. cablin* (from Dhankuta) were collected during the winter of 2011, dried in shadow, and then powdered. All plants were authenticated by National Herbarium and Plant Laboratories, Godawari, Nepal.
Extraction was carried out by soaking 150 g of dried powdered samples in about 600 ml of ethanol (Analar grade) for 3 days. The extracts were filtered first through cotton wool, then through Whatman filter paper no. 42 (125 mm).The collected extract was dried using a rotary evaporator.

2.2 Chemicals
The chemicals used were ethanol (Merck, Germany), DPPH and Ascorbic acid (Sigma Aldrich, USA). All other chemicals used were of the highest commercially available grade.

2.3 Antibacterial screening
Inhibition of bacterial growth was tested by using the paper disc diffusion method with slight modification [17].

2.3.1 Micro organism
The micro organisms used in this study were identified strains obtained from Central Department of Microbiology, TU, Nepal. Among bacteria taken in this study, one was gram positive and three were gram negative as given below.

Gram positive bacteria: *Staphylococcus aureus*

Gram negative bacteria: *Escherichia coli*, *Proteus vulgaris* and *Klebsiella pneumoniae*

2.3.2. Antimicrobial assay
The antimicrobial activity of the plant extracts were carried by disc diffusion method [17]. A suspension of tested micro organisms was spread on Muller-Hilton Agar (MHA) medium. The sterile filter paper discs (6 mm in diameter) were individually impregnated with different concentration of plant extract prepared in dimethyl sulphoxide (DMSO) and then placed into the agar plates which had previously been inoculated with the tested micro organisms. The plates were subsequently incubated overnight at 37 ^{o}C. After incubation the growth inhibition rings were quantified by measuring the diameter of the zone of inhibition in mm. For control dimethyl sulphoxide (DMSO) discs were used. All tests were performed in triplicate.

2.4 Antioxidant activity
DPPH radical scavenging activity (RSA) assay
The free radical scavenging activity of samples and standard ascorbic acid solution in ethanol was determined based on their ability to react with stable 1, 1-diphenyl-2-picrylhyrazyl (DPPH) free radical [18, 19]. The plant samples at various concentrations (15-250 µg/ml) were added to a 100 µM solution of DPPH in ethanol. After incubation at 37 °C for 30 min, the absorbance of each solution was determined at 517 nm. The measurement was performed in triplicates. The antioxidant activity of the samples was expressed as IC_{50} (inhibitory concentration), which was defined as the concentration (in µg/ ml) of sample required to inhibit the formation of DPPH radicals by 50%. Ascorbic acid was used as positive control. Free radical scavenging activity was calculated by using following equation:

$$\text{\% of free radical scavenging activity} = \frac{(A_0 - A_T) \times 100}{A_0}$$

Where, A_O = Absorbance of DPPH solution and A_T = Absorbance of test or reference sample. The % scavenging was then plotted against concentrations used and from the graph IC_{50} was calculated.

2.5 Statistics
All the analysis was carried out in triplicate and the results are expressed as mean ±SD.

Table 1: List of plants screened

Family	Scientific name	Traditional uses	Traditional uses
Urticaceae	*Pilea symmeria*	Kamle	Root is used against the fractures.
Labiateae	*Pogostemon cablin*	Kalijhar	Roots are remedy for haemorrhage and antidote. Fresh leaves are styptic, bruised and applied as cataplasm to clean wound and promote healthy granulation.
Euphorbiaceae	*Mallotus philippensis*	Sindure	It is used in particular rheumatism, intestinal parasites such as tapeworm and round worms, On cuts and wounds.
Amaranthaceae	*Celosia argentea*	Sahastrajadi	It is used in diarrhea, blood diseases, Mouth sores, clearing vision.
Labiatae	*Colebrookea oppositifolia*	Dhusure	Root is used in epilepsy and leaves are applied to wounds and bruises.
Rubiaceae	*Mussaenda macrophylla*	Dhobini	Traditionally the bark of this plant is used against snake bite.
Poaceae	*Thysanolaena maxima*	Amriso	Roots are used against diarrhea, cuts, wounds etc.

3. Results and Discussion
The paper describes the antimicrobial and antioxidant activities of some ethanol extracts belonging to some indigenous medicinal plants of Nepal**.** Table (1) provides the botanical name, family, local name with their traditional therapeutic uses for the seven ethnomedicinal plants collected from Dhankuta and Tanahun districts of Nepal.

3.1 Antimicrobial
In recent years, the search for phytochemicals possessing

antimicrobial properties has been on the rise due to their potential use in the therapy of various chronic and infectious diseases. In addition, a number of antibiotics have lost their effectiveness due to the development of resistant strains, mostly through the expression of resistance genes. The results of antimicrobial screening of the ethanol extracts of all species of plants are shown in table 2. Among the plants screened, *P. symmeria, P. cablin, M. philippensis, C. argentea and C. oppositifolia* showed promising activity against tested microorganisms (Fig 1). On the contrary, *T. maxima* and *M. macrophylla* showed moderate activity against tested microorganisms. Antimicrobial activity had also been detected for *P. cablin, M. philippensis, C. argentea, C. oppositifolia, T. maxima* and *M. macrophylla* [20, 21, 22, 23]. Our results confirm the strong and moderate antimicrobial activity of the plants. To the best of our knowledge, the antimicrobial activity of *P. symmeria* described here for the first time. It showed promising activity against *E. coli* and *S. aureus.* In classifying the antibacterial activity as gram-positive or gram-negative, it would generally be expected that a much greater number would be active against gram positive than gram-negative bacteria as gram negative bacteria possess the outer protective covering capsules that help in developing resistance against different plant extracts [24]. However, in this study plant extracts are found to be active against both gram-negative bacteria and gram positive bacteria. Among all of the plant extracts tested, *P. symmeria* was found to be the most effective against gram positive bacteria *S. aureus*, a pyrogenic bacterium known to play a significant role in invasive skin diseases including superficial and deep follicular lesion and food poisoning. Similarly, *P. symmeria* was also found to be the most effective against gram negative bacteria *E. coli.* These finding results are very interesting as the microorganism *E. coli*, which is already known to be multi-resistant to drugs, was also resistant to the plant extracts tested

Table 2: Mean Zone of inhibition (ZOI) shown by different medicinal plants against tested bacteria

Plants	Test organisms	Clear zone (mm)							
		S. aureus		*K. pneumoniae*		*P. vulgaris*		*E. coli*	
	Concentration	0.5 mg/ml	1 mg/ml	0.5 mg/ml	1 mg/ml	.5 mg/ml	1 mg/ml	.5 mg/ml	1 mg/ml
P. symmeria		9	14	8	8	9	10	10	16
P. cablin		10	11	10	10	10	12	11	15
M. philippensis		11	11	8	10	8	12	8	11
C. argentea		8	9	9	11	10	11	9	10
C. oppositifolia		7	7	9	11	9	11	8	9
M. macrophylla		8	9	8	10	8	9	8	10
T. maxima		8	8	8	9	8	8	8	8

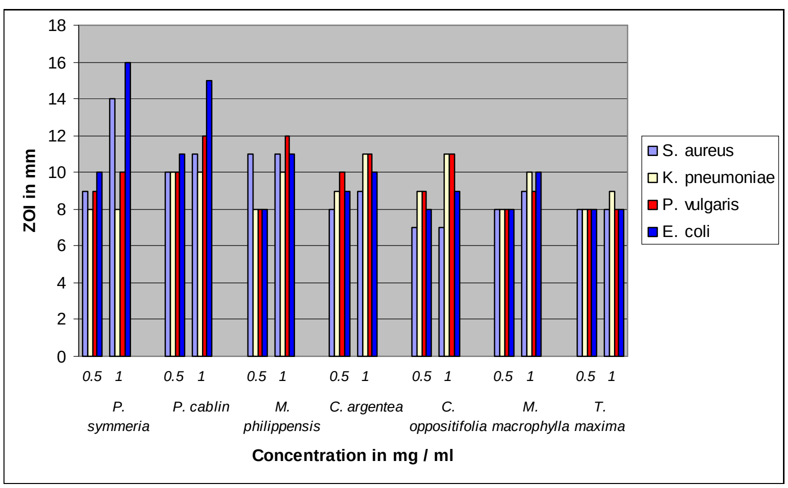

Fig 1: Plot of antibacterial activity of the ethanol extract of medicinal plants against various bacteria.

3.2 Antioxidant

DPPH radical scavenging activity (RSA) assay

The ethanol extractives of *M. philippensis, P. cablin, C. oppositifolia, M. macrophylla, C. argentea, P. symmeria* and *T. maxima,* were assessed for free radical scavenging activity and results are presented in Table-3. The IC_{50} was calculated from the graph obtained by plotting the % scavenging against concentrations used (Fig. 2). The antioxidants act either by scavenging various types of free radicals derived from oxidative processes, by preventing free radical formation through reduction precursors or by chelating metals [25, 26]. The reduction of DPPH assay has been used to detect products with antioxidant activity as free radical scavengers [27]. In this study, all the extractives were shown to possess significant DPPH radical scavenging activity.

P. cablin was found to have the highest antioxidant activity with an IC_{50} value of 32 µg/ml followed by *M. philippensis* (IC_{50} 62.5 µg/ml), *M. macrophylla* (IC_{50} 63 µg/ml) and *C. oppositifolia* (IC_{50} 68 µg/ml) respectively. The antioxidant activity of essential of oils of *P. cablin* has been reported [28, 29]. The results obtained here are in consistent with those previously reported [30, 31, 32].

On the other hand, moderate antioxidant activity was revealed by extracts of *C. argentea* (IC_{50} 195 µg/ml), *T. maxima* (IC_{50} 250 µg/ml) and *P. symmeria* (IC$_{50}$ 280 µg/ml). Antioxidant activity had also been detected for *C. argentea* [33]. The antioxidant properties of *T. maxima* and *P. symmeria* in the past has not been reported. In the present study *T. maxima* and *P. symmeria* showed the least activity and *P. cablin* showed the highest activity. All extracts, however were found to be less active than ascorbic acid (AA), a standard antioxidant drug (Fig 3). It becomes evident that the antiradical activities of all the extracts are due to the presence of phenolic compounds, especially phenolic acids and flavonoids [34]. The antioxidant activities of polyphenols were attributed to their redox properties, which allow them to act as reducing agents, hydrogen donators and singlet oxygen quenchers, as well as their metal chelating abilities [35]. On the basis of overall results, it seems that the plants having potential to act as antioxidant also have potential to act as sources of antimicrobial agent except *P. symmeria* (Table 2 and 3).

However, only *P. cablin* and *M. philippensis* gave both strong radical scavenging abilities (Fig 3) and antimicrobial activities (Fig 1). It is therefore desirable to isolate and characterize the antioxidant agents from these two plants, *P. cablin* and *M. philippensis* and determine whether or not the same constituents are responsible for both the antimicrobial and antioxidant activities.

Table 3: Comparison of the antioxidant properties seven plants

Name of the plants	IC$_{50}$ (µg/ml)
Ascorbic acid (standard)	20
P. cablin	32
M. philippensis	62.5
M. macrophylla	63
C. oppositifolia	68
C. argentea	195
T. maxima	250
P. symmeria	280

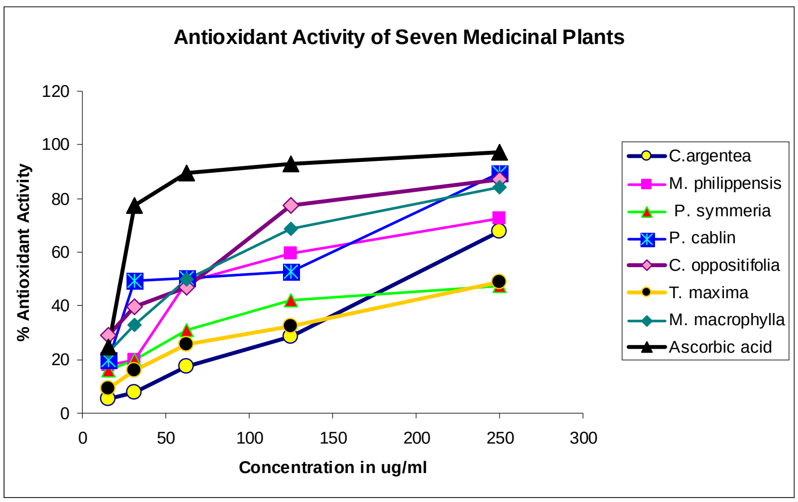

Fig 2: Antioxidant activity of seven medicinal plants

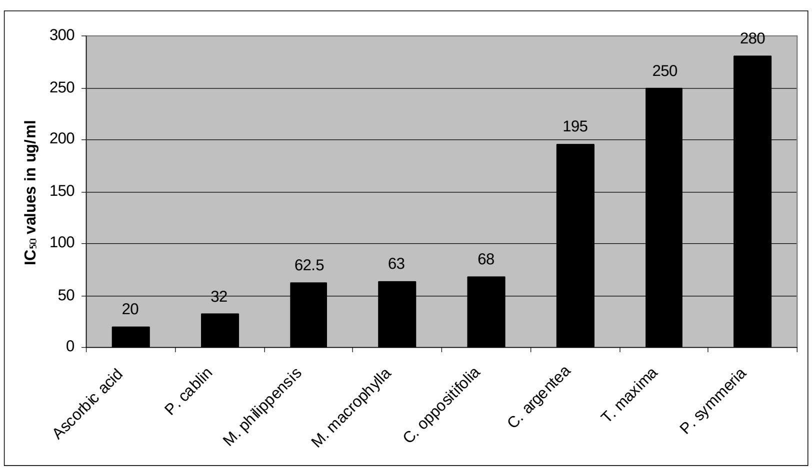

Fig 3: The IC$_{50}$ values of the plant extracts on DPPH. (Lower values indicate more powerful antioxidant capacity)

4. Conclusion

Based on the results of the present study, it can be suggested that the ethanolic extracts of *P. cablin, M. philippensis, C. oppositifolia and M. macrophylla* exhibited potential antimicrobial and antioxidant activity. The ethanolic extract of *C. argentea and T. maxima* with moderate antimicrobial activity showed weak antioxidant activity. On the other hand, *P. symmeria* with the highest antimicrobial activities showed lowest antioxidant activity.

We conclude that most of the results of this study are in good agreement with the traditional uses of the investigated plants. The study of bioassay-guided fractionation of these extracts in order to isolate and identify the compounds responsible for each of these activities, is highly desirable.

5. Conflict of interest

The authors declare that they have no conflict of interest.

6. Acknowledgements

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