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ANTIOXIDANT AND ANTIBACTERIAL ACTIVITY OF METHANOLIC EXTRACT OF *MACHILUS ODORATISSIMA*

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ABSTRACT
The methanol extract of the bark of *Machilus odoratissima* was subjected to investigate its antioxidant and antibacterial properties. The phytochemical screening demonstrated the presence of different types of compound like terpenoids, tannins, deoxy sugar, saponins and phenolic compounds. The methanol extract of the plant was tested for antioxidant activity using scavenging activity of DPPH(1,1-diphenyl-2-picrylhydrazil) radical method and antibacterial activity against *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli* & *Pseudomonas aeruginosa* bacteria using cup plate method. The extract exhibited high free radical scavenging activity. IC_{50} was found to be 3.37µg/ml. Antibacterial activity was observed against *S. aureus* in dose dependent manner.

Keywords: Antibacterial, antioxidant, cup-plate, DPPH, lauraceae, *Machilus odoratissima*, Nepal

INTRODUCTION
Plants, as the source of medicine, have been playing an important role in the health services around the globe [1]. About three quarters of the world's population relies on plant and their extracts for health care [2]. A good number of our population particularly those living in rural areas depend largely on herbal remedies for the treatment of different types of diseases. It indicates the importance of the individual plants in the health care system.

Although, Nepal is a small country, it is rich in biodiversity due to its geographical features and has many plants with medicinal and aromatic values. Some of them are used in traditional medicine and some are still not explored scientifically for their medicinal values. So, the present study of plant *Machilus odoratissima* was selected for biological scrutiny. Literature survey of this plant revealed not much information about its medicinal uses; however other species of this plant have been reported to have antibacterial and antioxidant properties [3, 4, 5]. It is likely that when bioactive compounds are found in one species, more species of the same genus may contain active compounds of the similar nature.

M. odoratissima of the lauraceae family, locally called as Kaulo, is evergreen tree with grey & rough bark, green leaves and yellowish green flower. It is found in sub-tropical and lower part of Himalayas at altitude of 2100-2300 m.

MATERIALS & METHODS

The bark of the *M. odoratissima* was collected in the month of October – November, 2009, from Phulchowki Hill of Kathmandu Valley, Nepal. It was duly identified as *M. odoratissima* in National Herbarium, Godawari, Nepal.

Extraction of Plant

The bark of *M. odoratissima* was cleaned with distilled water, air dried, powered and 100 gm of the powdered plant material was extracted by maceration using methanol. The methanol extract was then defatted by mixing with petroleum ether and removing the petroleum ether fraction. Finally, the methanol portion was evaporated by vacuum evaporator (RICON) at temperature below 40°C to obtain the dried powder.

Phytochemical Screening

The phytochemical screening of methanol extract was done to identify the main groups of chemical constituents present in methanol extracts of *M. odoratissima* by their color reaction [6, 7].

Antioxidant Activity by DPPH Scavenging

1,1-Diphenyl-2-picryl-hydrazyl (DPPH) is free radical but stable [8, 9]. DPPH solution is initially violet in color which fades when antioxidants donate hydrogen [10]. The change in color is monitored by spectrophotometer and DPPH free radical scavenging activity is calculated [11]. The method described by Molyneux [12] was used. Stock solution of 100 µM DPPH in methanol was made. Test sample of the extract were made at 1.0, 2.5, 5.0, 7.5, 10.0 & 20.0 µg/ml in methanol. Similarly, reference samples of ascorbic acid were made at similar concentration. Two milliliters of 100 µM DPPH was mixed with 2.0 ml of methanol and kept in dark for 30 minutes. Similarly, 2.0 ml 100µM DPPH was mixed with different concentration of 2.0 ml test sample and 2.0 ml ascorbic acid and kept in dark. The absorbance was measured at 517 nm by spectrophotometer (UV 1800 – SHIMADZU) after 30 minutes and % scavenging was calculated by the equation:

$$\textbf{Percent Scavenging} = \left(\frac{Ao - \mathbf{AT}}{Ao}\right) \times 100\%,$$

where, A_O = Absorbance of DPPH solution and A_T = Absorbance of test or reference sample. The % scavenging was then plotted against concentration and regression equation was obtained to calculate IC_{50} (micromolar concentration required to inhibit DPPH radical formation by 50%) values. The experiments were conducted in triplicate.

Antibacterial Activity

Cup-plate technique according to Okeke [13] was used in antibiotic assay with modifications. Muller-Hinton agar plates of 4 mm thickness were prepared and cup of uniform diameter of 5 mm were bored. Antibacterial screening of methanolic extract dissolved in 15% dimethyl sulphoxide (DMSO) was done at concentration of 1.6, 3.2 and 6.4 mg/cup keeping gentamicin 10 µg/cup as standard and DMSO 15% as control against two gram positive bacteria - *Staphylococcus aureus, Enterococcus faecalis,* and two gram negative bacteria - *Escherichia coli, Pseudomonas aeruginosa,* by inoculating bacterial broth of 0.5 McFarland standard turbidity in Muller-Hinton agar [14, 15]. The agar plates were then kept overnight at 2°C to

allow diffusion of extract. The plates were then incubated at 37°C and zones of inhibition were measured after 24 hours. All the bacteria isolates were collected from patients visiting Tribhuvan University Teaching Hospital, Kathmandu.

Data Analysis

Data were analyzed using MS-Excel 2010 and all the numerical results were expressed in arithmetic mean.

RESULTS

Phytochemical Screening

The extractive value of the methanol extract was 14.52% on dry basis. The methanol extract of the plant revealed the following phytochemicals (Table 1).

Table 1. Different group of phytochemicals constituents present in methanolic extract of *M. odoratissima* ('+' indicates presence and '-' indicates absence)

SN	Test Compound	Result
1	Alkaloid	-
2	Glycoside	-
3	Terpenoid	+
4	Steroid	-
5	Tannin	+
6	Flavone Aglycone	-
7	Flavonoid	-
8	Deoxy Sugar	+
9	Saponin	+
10	Coumarin	-
11	Phenolic Compounds	+
12	Reducing Sugar	-

Antioxidant Activity by DPPH Scavenging

The extract was found to have antioxidant property in dose dependent manner as shown in Fig.1. The IC_{50} value of extract was found to be 3.37 µg/ml and that of ascorbic acid was 2.27µg/ml (12.89µM) from regression equation in Fig.2.

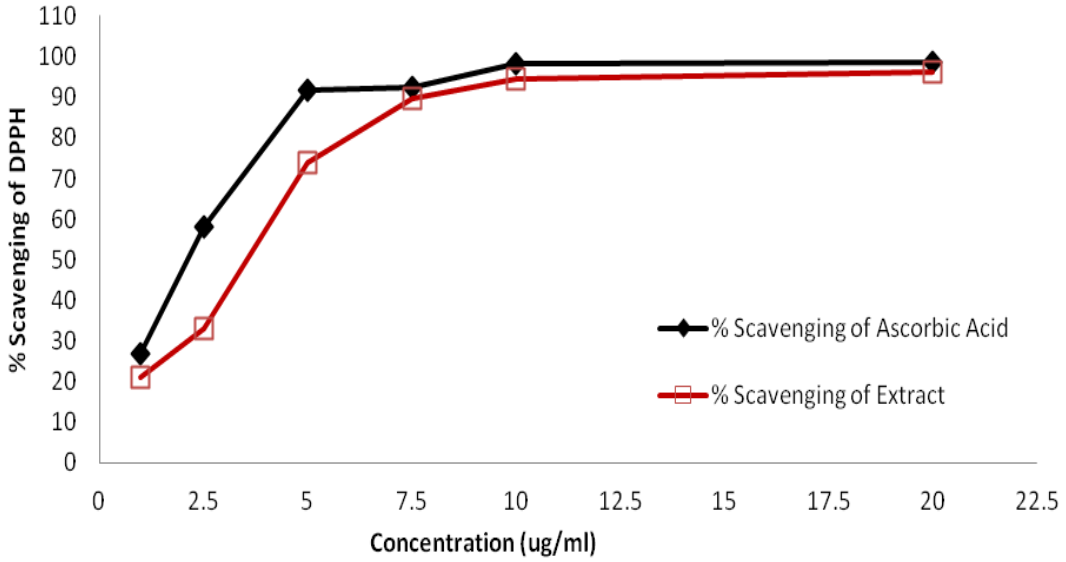

Fig .1: Comparison of % Scavenging of DPPH by Ascorbic acid and Cold Methanolic Extract at different concentration

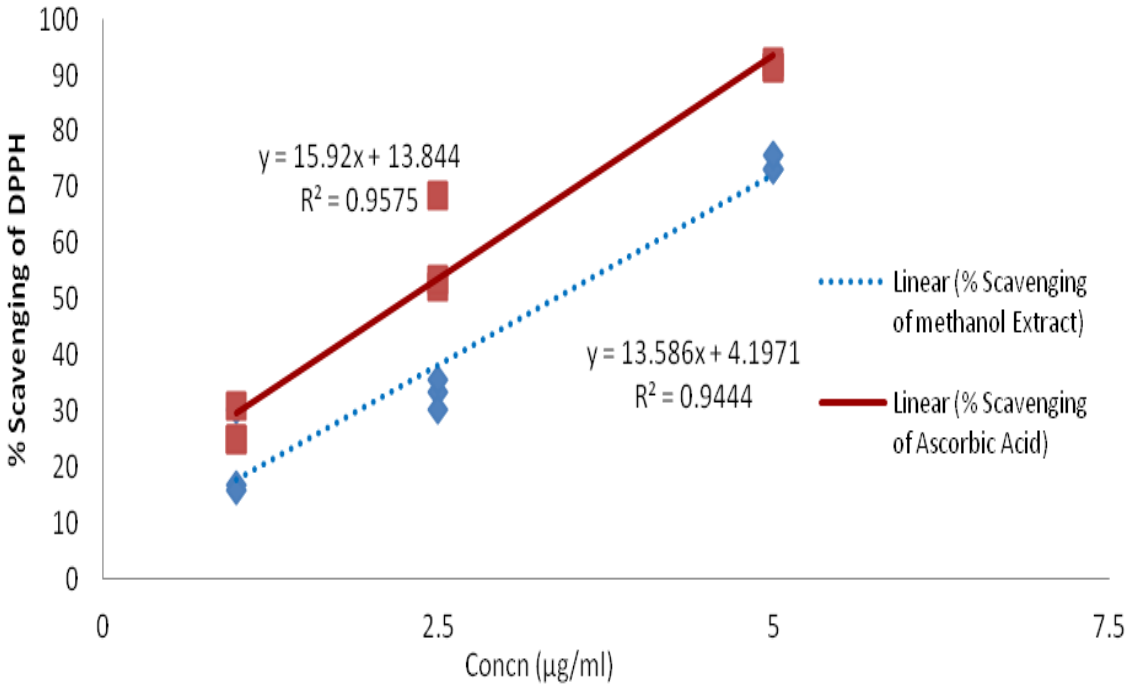

Fig. 2: Regression equation of % DPPH Scavenging Vs Concencration

Antibacterial Activity

The extract was found to be active in dose dependent manner against *S. aureus* as shown in Fig.3 but did not exhibit any activity against *E. faecalis, E. coli & P. aeruginosa*.

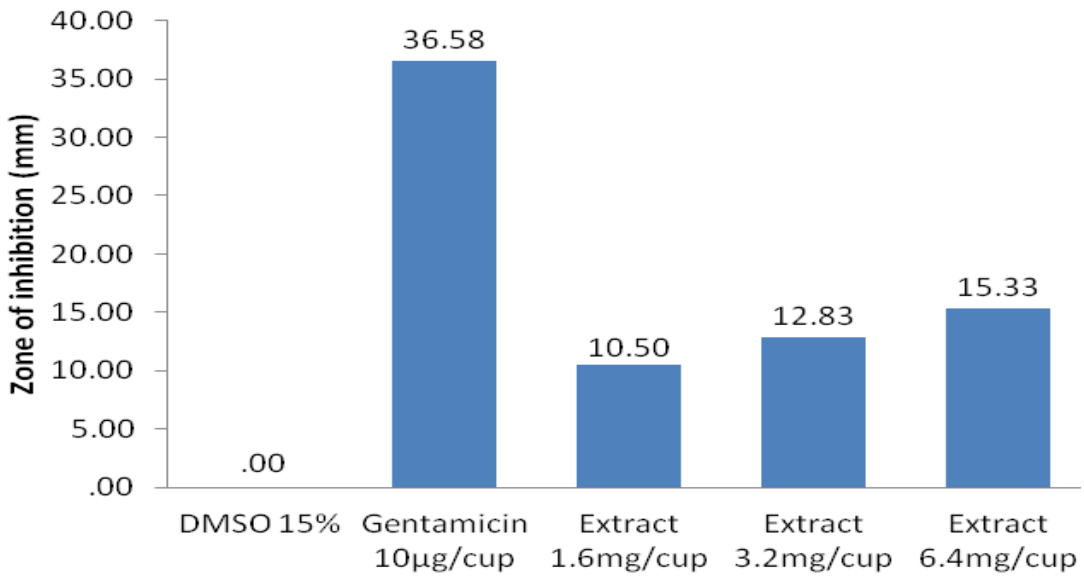

Fig. 3. Zone of inhbition of methanol extract against *S. aureus*.

DISCUSSION

Plants and their products have been used for many years for human health. There are still many plants which have various medicinal values but still not explored and used. Plants contain many novel compounds with medicinal values which need scientific exploration. The free radicals are produced in aerobic cells due to consumption of oxygen in cell growth [9, 16]. Free radicals cause decrease in membrane fluidity, loss of enzyme receptor activity and damage to membrane protein leading to death [17]. These free radicals are involved in different disorders like ageing, cancer, cardiovascular disease, diabetes, rheumatoid arthritis, epilepsy & degradation of essential fatty acids [9, 16, 18]. Antioxidant helps in treatment of above disorders. As methanol extract of this plant showed the dose dependent antioxidant activity comparable to ascorbic acid, antioxidant agent might be developed from this plant for the treatment of above disorders associated with free radicals. Phenolic compounds containing free hydrogen are largely responsible for antioxidant activity [19, 20], thus the phenolic compounds of *M. odoratissima* can be referred to be responsible for the antioxidant activity.

Every antibacterial drug comes with an active period beyond which the resistance emerges against it. Bacteria are becoming increasingly resistant to conventional antibiotics [21], and resistance is emerging at alarming rate [22]. Thus the alarming increase in resistance to antibacterial has created desperate need for the search of new antibacterial [22, 23]. The cup-plate method has shown that the methanolic extract of the bark of *M. odoratissima* is active against *S. aureus* (gm +ve) and not against *E. faecalis* (gm +ve), *E. coli & P. aeruginosa* (gm –ve). Literature has shown that terpenoids and phenolic compounds show most of the antibacterial activities and *S. aureus* are specifically more susceptible to phenolic compounds [24]. Our study shows that the methanolic extract of bark of *M. odoratissima* has both terpenoids and phenolic compounds, and also possess antibacterial activity against *S. aureus* and not against other tested species. Thus, the activity against *S. aureus* may be due to either phenolic compounds or terpenoids or combination of both. The activity of extract up to 6.4 mg/cup is not equal to that of 10 µg/cup of gentamicin, which may be because the antimicrobial compound may be present in

very few amounts in the crude extract. The pure compound could be a good lead antibacterial compound which on further modification can be safe and effective antibiotics. The *S. aureus* species used was tested resistant to erythromycin in Kirby-Bauer disk diffusion test. Thus, this plant could also be useful for the future research in antibiotics against macrolide resistant *S. aureus.* This study shows similar result to the previous work by Oliver [25], which showed that phenolic compounds of Hazel leaves have both antioxidant and antibacterial property.

Our findings suggest that there are still many plants in Nepal which are not traditionally used but possess medicinal values. So, scientific studies also need to be focused on plants which are not traditionally used. As *M. odoratissima* showed the antioxidant and antibacterial activities, a detailed biological & phytochemical study is needed to find out the chemical constituent responsible for their activities.

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