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Antioxidant, Phytotoxic and Antimicrobial Activities of Methanolic Extract of *Bauhinia variegata* Barks

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ABSTRACT

Bauhinia variegata is well-known medicinal plant used from the ancient era to till date for their medicinal values. The methanolic extract of *Bauhinia variegata* barks was screened for phytochemical constituents, antioxidant, phytotoxic and microbial activity. The microbial activity was tested against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Salmonella typhi* at different concentration of 10, 15 and 20 mg/mL by agar well diffusion method. The plant extract showed the potent antimicrobial activity against the *Staphylococcus aureus, Salmonella typhi*, *Bacillus subtilic* and *Escherichia coli* with zone of inhibition 14 mm, 12mm, 14mm and 11mm respectively. Phytochemical analysis of the extract revealed that the antioxidant and antimicrobial activity of the plant materials is due to the presence of active secondary metabolites. In DPPH free radical scavenging assay the IC_{50} value of *Bauhinia variegata* was found to be 6.48±0.08 µg/mL, while the IC_{50} value of the reference standard ascorbic acid was 45.93µg/mL. The extract of *Bauhinia variegata,* contains high value of phenolic (156.30±0.3 mg GAE/gm) and flavonoid (16.04±1.4 mg QE/gm) content exhibited the high antioxidant activity. The *in-vitro* phytotoxic bioassay showed 65%, 40%, and 25% growth regulation at just higher conc. of 1000, 100 and 10 µg/mL with number of fronds 07, 12 and 15 respectively with respect to standard drug Paraquat of concentration 0.015µg/mL.

Keywords: medicinal plants, methanolic extract, free radical, phytotoxic, DPPH.

INTRODUCTION

Plants have been important sources of medicines since beginning of human civilization. Many herbal remedies have been employed in various medicinal systems for treatment and management of different chronic and infectious diseases (Luitel *et al*. 2014, Pandey *et al*. 2015). The majority of population in developing country, especially tribal, ethnic groups and mountain people relies on traditional medical practices. In many cases this practice is transmitted orally from generation to generation and confined to certain people (Mishra *et al*. 2013). Large proportions of world's population depend on traditional medicine because of scarcity, high cost of orthodox medicine and unpleasant side effect. Thus, in the present study we would like to collect the Nepalese medicinal plants *Bauhinia variegata* from the Syangja district of 1088 meters altitude. The plant species is deciduous tree about 10 m high. The plant is distributed throughout Nepal at 150-1800 m in open valleys with good loamy soil but generally planted on private land for fodder and as a fuel wood substitute (Nascimento *et al*. 2011). The plant species have rich in phytoconstituents with high antioxidant value and potent to microbial activity towards gram positive and gram negative bacteria. The oxidation induced by reactive oxygen species (ROS) can result in cell membrane disintegration, membrane protein damage and DNA mutation, which can further initiate or propagate the development of many diseases, such as cancer, liver injury, cardiovascular disease, tumour inflammation, hemorrhagic shock, atherosclerosis, diabetes, infertility, gastrointestinal, ulcerogenesis, asthma, rheumatism and neurodegenerative diseases (Negi *et al*. 2012). Although the body possesses such defense mechanism, as enzymes and antioxidant nutrients, which arrest the damaging properties of ROS, continuous exposure to chemicals and contaminants may lead to an increase in the amount of free radicals in the body beyond its capacity to control them, and cause irreversible oxidative damage (Divya *et al*. 2012). Both cigarette smoking and chronic inflammation are of the major causes of cancer have strong free radical components in their mechanism of action. Some research has indicated that people who smoke tend to have lower antioxidant levels than non smokers and are at an increased risk for both cancer and cardiovascular disease. In this respect polyphenolic compounds, like flavonoids and phenolic acids, found in plants have been reported to have multiple biological effects, including antioxidant

activity (Pahwa *et al*. 2011). It is generally assumed that frequent consumption of plant derived phytochemicals from vegetables, fruits, tea and herbs may contribute to shift the balance toward an adequate antioxidant status (Sawhney *et al*. 2011). In the present study, antioxidant potential of the methanol extracts of *Bauhinia variegata* with IC_{50} value was found to be 8.05±1.4 µg/mL as compared to the standard ascorbic acid 45.93 µg/mL. by using 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay.

Infectious disease account for approximately one half of all deaths in tropical countries and they are considered a major threat to human health because of the unavailability of vaccines or limited chemotherapy. Infectious diseases caused by multi-resistant microbial strains are on the increase and fighting these diseases with natural products may be more efficacious (Dhale *et al*. 2011). The aim of this study is to investigate the *in vitro* antioxidant and antimicrobial activity of methanolic extracts of *Bauhinia variegata* barks. The most important bioactive constituents of plants are steroids, terpenoids, carotenoids, flavonoids, alkaloids, tannins and glycosides (Saha *et al*. 2011). In this study the methanolic plant extract was tested against gram positive and gram negative bacteria and showed higher antibacterial activity against *Salmonella typhi*, *Bacillus subtilic*, *Escherichia coli* and *Stayphylococcus aureus*. Phytotoxic natural products may be utilized either directly or as lead compounds for the development herbicides (Morimoto *et al*. 2009). Some wild plants are known to exhibit phytotoxicity by releasing water soluble phytotoxins (Horshy 1977, Sterling 1987). These undesired plants that complete with crop plants for nutrients and produce toxic chemicals which inhibit germination and growth of desired plant (Ghulan *et al*. 2013).

MATERIALS AND METHOD

Plant materials

The plant sample was collected from Syangja district of Nepal based on ethnobotanical uses. The plant was identified by Rita Chhetry, Research Officer, National Herbarium and Plant Resources, Ministry of Forests and Soil Conservation, Godawari, Nepal.

Extraction

The plant sample was shade dried at room temperature and powdered material was then weighed (50 g), soaked in methanol for 72h and filtered using Whatman No 40 filter paper. The filtrate obtained was concentrated under reduced pressure in a rotatory evaporator to obtain the crude extract.

Phytochemical screening

Phytochemical analysis of crude methanolic extracts of these medicinal plants was carried out based on the procedure described on the standard protocol (Edeoga *et al*. 2005).

Antimicrobial activity

Nutrient agar was added in distilled water in the ratio of 28 g/litre in appropriate size of conical flasks and boiled with continuous shaking and autoclaved at 121 ^{o}C for 30 minutes. Sterilized media was allowed to cool about 50 ^{o}C. These were distributed in the sterile Petri-plates of size of 90 mm diameter in the ratio 25 mL per plate aseptically and labeled properly. Plates were left as such for solidification.

The antibacterial of crude extract was employed against the test organisms by agar well diffusion method. Sterile Muller Hinton Agar (MHA) plates of approximately 4mm thickness were prepared. Before using the plates, they were dried under hot air oven at appropriate temperature to remove excess of moisture from the surface of the media. The fresh inoculums comparable with turbidity standard were prepared. Then a sterile cotton swab was taken out and was dipped into the prepared inoculums. The excess of inoculums was removed by pressing and rotating against the upper inside side wall of the tube above the liquid level and then swabbed carefully all over the plate. The plate was rotated through the angle of 60^{o} after each swabbing. Finally the swab was passed round the edges of the agar surface. The inoculated plates were left to dry for few minutes at room temperature with the lid closed. Then with the help of sterile cork borer no 5, wells were made in the inoculated media plates and labeled properly. So, the diameter of a well was 6 mm. Then 50 µL of the plant extract was introduced into respective well. In one well pure methanol was filled as control. The plates were then left for half an hour with the lid closed so that the extract diffused into media. The plate was incubated overnight at 37 ^{0}C. After proper incubation (18-24 hours) the plates were observed for the zone of inhibition around well (Farrukh *et al*. 2010, Nucrat *et al*. 2006, Rao *et al*. 2008).

Antioxidant activity test (DPPH radical scavenging assay)

The free radical scavenging activity was measured by using DPPH assay. Different concentration of test samples (5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 µg/mL) were prepared while the concentration of DPPH was 0.2mM in the reaction mixture. These reaction mixtures were taken in Eppendorf tubes and incubation at 37 ^{0}C for 30 min. Discolorations were measured at 517 nm using a UV-Visible Spectrophotometer. Percent radical

scavenging activity by sample treatment was determined by comparison with methanol treated control group; ascorbic acid was used as positive control. Measurement was performed at least in triplicate. The percentage scavenging of the DPPH free radical was calculated using the following equation (Yerra *et al*. 2008, Jondaity *et al*. 2005, Pani *et al*. 2011).

$$\% \text{ Scavenging} = \frac{\text{Abs. control - Abs. sample}}{\text{Abs.control}} \times 100$$

The inhibition curve was plotted for the triplicate experiments and represented as percentage of mean inhibition ± standard deviation and the IC_{50} values was obtained.

In-vitro Phytotoxicity bioassay

E-Medium was prepared by mixing various constituents in 1000 ml distilled water and pH was adjusted between 6.0 to 7.0 by adding KOH pellets (Stock solution). Working E-medium was prepared by mixing 100 mL of stock solution and 900 ml of distills water. 30 mg for crude extract was dissolved in 1.5 ml of solvent methanol serving as stock solution. Three flasks were inoculated with 10, 100 and 1000 µL of solution pipette from the stock solution for 10, 100 and 1000 µg/ml solvent was allowed to evaporate overnight. 20 mL of working E. medium was added and then plant of Lemna minor, each containing a rosette of two to three fronds, to each flask. (total 20 fronds). Other flasks supplemented with E-medium and reference (standard drug) plant growth inhibitors and promoters serving as negative and positive controls, respectively. The flaks were placed in growth cabinet for seven days. Plants were examined daily during incubation. Number of fronds per flasks were counted and recorded on day seven. Results were analyzed as growth regulation in percentage, calculated with reference to the negative control (Rehmanullah *et al*. 2014).

RESULTS AND DISCUSSION

Phytochemiccal screening result showed that, plant extract was rich source of secondary metabolites like polyphenols, alkaloids, flavonoids, steroids, and tannin except, reducing sugars, terpenoid, glycosides and saponins.

Table 1. Phytochemical analysis of plant extracts

Polyphenols	+	Reducing sugar	+
Steroids	-	Tannin	+
Alkaloids	+	Cardiac glycoside	+
Flavonoids	+	Anthraquinone	+
Terpenoids	-	Carotenoids	+
Glycosides	-	Saponin	+

Key: + = Present - = Absent

The plant extracts containing secondary metabolites are known to be biologically active and therefore, aid the antimicrobial activities through different mechanism. The antibacterial activity of plant bark extract was assayed *in-vitro* by agar disc diffusion and agar well diffusion method against four bacterial species. Steroidal compounds present in plant extract are of importance due to their relationship with various anabolic hormones including sex hormones. Flavonoids and their constituents exhibited a wide range of biological activities like antimicrobial, anti-inflammatory and antioxidant properties. It is concluded that the plant extract is a potential source of active antimicrobial agents against the tested bacterial species. The inhibition of these plant extracts against pathogenic bacterial strains can introduce the plant as a potential candidate for drug development for treatment of ailments caused by these pathogens.

Table 2. Antimicrobial activity of plant extract

Zone of inhibition (ZOI), mm					
Plant extract	Concentration	*Stayphylococcus aureus*	*Bacillus subtilic*	*Salmonella typhi*	*E.Coli*
Bauhinia variegata	10 mg/mL	12	11	10	10
	15 mg/mL	14	12	10	11
	20 mg/mL	14	14	11	11

Table 3. In-vitro Phytotoxic bioassay

Name of the plant	Conc. of Plant extract (µg/mL)	No. of fronds		% Growth Regulation	Conc. of Standard Drug (µg/mL)
Lemna minor	1000	07	20	65	0.015
	100	12		40	
	10	15		25	
Keys: Std. Drug Paraquat Incubation Condition= (28^{o}±1C^{o})					

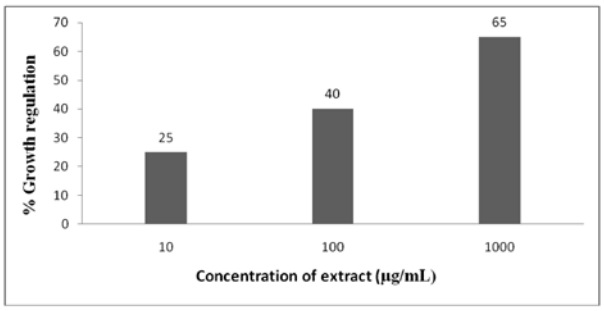

Fig. 1. Phytotoxic activity of different concentration of Bauhinia variegata

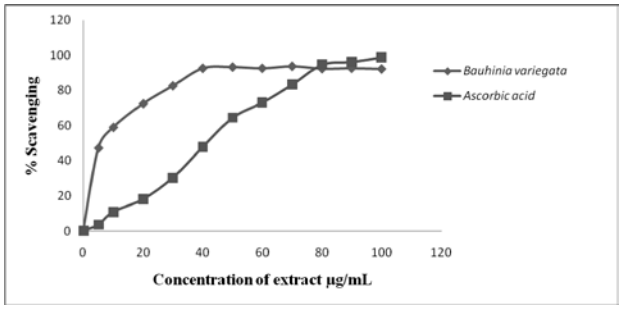

Fig. 2. Percentage scavenging of DPPH free radical with different concentration of plant extracts

Antioxidants may offer resistance against oxidative stress by scavenging the free radicals inhibiting lipid peroxidation etc. Antioxidants can act by converting the unpaired electrons to paired ones. The dose dependent inhibition of DPPH radical indicates that plant extract causes reduction of DPPH radical in a stoichiometric manner. The present study was carried out to analyze the antioxidant activity of the methanolic plant extracts. The plant extract of *Bauhinia variegata* is the source of potent antioxidant with the strongest DPPH radical scavenging activity (IC_{50} = 6.48±0.08 µg/mL) whereas standard ascorbic acid has IC_{50} of 45.93 µg/mL. The result showed significant total phenolic and flavonoid content in plant extract. The extract of *Bauhinia variegata,* contain high value of phenolic (156.30±0.3 mg GAE/gm) and flavonoid content (14.04±1.4 mg QE/gm) exhibited the greatest antioxidant activity.

Bioactivity studies of two species of mimosa species showed prominent antioxidant activity. methanol extract of the bark of *Machilus odoratissima* exhibited high free radicals scavenging activity (Amit *et al*. 2012). In our study, the antioxidant capacity of this medicinal plant extract could be compared with the results of previously studied plant extract. The antioxidant capacities in mg ascorbic acid per gram for plants were *Thymus vulgaris* (0.6±0.3), *Lavandula vera* (0.6±0.4), *Rosmarinus officinalis* (0.5±0.1), *Origanum dictamnus* (0.2±0.2), *Sideritis cretica* (0.8±0.1), *Salvia officinalis* (0.4±0.1) and *Origanum vulgare* (0.3±0.1). The result suggested that the plants extract studied in this work is the potent source of antioxidants in comparison to previous results (Zorica *et al*. 2009, Rehmanullah *et al*. 2014).

Table 4. DPPH scavenging (IC_{50}) value, total flavonoid and total phenolics content

Total flavonoid content	14.57±1.4 mg QE/gm
Total phenolic content	156.30 ±0.3 mg GAE/gm
DPPH scavenging (IC_{50})	6.48±0.08 µg/mL

CONCLUSIONS

DPPH scavenging activity showed that the methanolic extract possesses the antioxidant property. The plant extract is the potent antioxidant with DPPH scavenging IC_{50}= 6.48±0.08 µg/ mL with respect to the standard ascorbic acid of IC_{50} of 45.93 µg/mL. Phenolic compounds and flavonoids have been reported to be associated with antioxidative action in biological system, acting as scavengers of singlet oxygen and free radicals. Antimicrobial assay of different concentration showed their activity towards tested organisms and found to be most biologically active. Among the tested organisms, the plant extract showed the highest ZOI (14±0.1 mm) for *Staphylococcus aureus*. Similarly the plant extract showed the potent activity towards the organism like *Bacillus subtilic, Salmonella typhi* and *Escherichia coli* with ZOI 14±0 mm, 11±0 mm and 11±0 mm respectively. The sample showed moderate phytotoxic activity 65%, 40% and 25% growth regulation with number of fronds 07, 12 and 15 respectively which is comparable to the standard drug paraquat with number of fronds 20 at a concentration of 0.015 µg/ mL.

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