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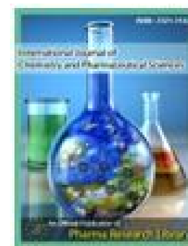

RESEAECH ARTICLE

Studies on Flavonoid, Polyphenol Content and Antioxidant Activity of Medicinal and Poisonous Plants from Nepal

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ABSTRACT
Antioxidants are compounds that protect cells against the damaging effects of reactive oxygen species. The higher antioxidant property of plant species is mainly due the active constituents like phenol and flavonoids present in them. Nepal is rich in biodiversity containing more than fifteen hundred high valuable medicinal plant. So, the ethanol extract of fourteen species of medicinal plants collected from Eastern and Central part of Nepal were evaluated for their Polyphenol, flavonoids and antioxident activity. Among them *Rhododendron setosum* showed highest flavonoid content and *Senna fistula* contain highest polyphenol content with value155.0 mg QE/g and 105.3 mg GAE/g respectively. Similarly *Ophihlossum reticulatum* showed the highest antioxidant activity with IC_{50} value of 10.73 μg/mL.
Keywords: Medicinal plants, Antioxidant, Polyphonols and Flavonoids

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1. Introduction

Antioxidants are compounds that protect cells against the damaging effects of reactive oxygen species, such as singlet oxygen, superoxide, peroxyl radicals, hydroxyl radicals and peroxy nitrite. An imbalance between antioxidants and reactive oxygen species results in oxidative stress, leading to cellular damage. Oxidative stress has been linked to cancer, aging, atherosclerosis, ischemic injury, and inflammation and neurodegenerative diseases [1]. Antioxidants play an

important role as health protecting factor. Scientific evidence suggests that antioxidants reduce the risk for chronic diseases including cancer and heart disease. Primary sources of naturally occurring antioxidants are whole grains, fruits and vegetables [2]. Plant sourced antioxidants like vitamin C, vitamin E, carotenes, phenolic acids, flavonoids etc. have been recognized as having the potential to reduce disease risk [3]. Flavonoids may help provide protection against these diseases by contributing, along with antioxidant vitamins and enzymes, to the total antioxidant defense system of the human body. Epidemiological studies have shown that flavonoid intake is inversely related to mortality from coronary heart disease and to the incidence of heart attacks [4]. In this study, to evaluate the antioxidant activity of medicinal plants from eastern region of Nepal have been selected, because they were not studied for this purpose.

Polyphenols exhibit a wide range of biological effects as a consequence of their antioxidant properties. Similarly Polyphenols easily protect cells against the damaging effects of reactive oxygen species. The Plant phenolics are multifunctional and can act as reducing agents (free radical terminators), metal chelators and singlet oxygen quenchers. Large numbers of medicinal plants have been investigated for their anti oxidant activities [5]. Because the synthetic antioxidants, e.g. butylated hydroxyl anisole (BHA), butylated hydroxyl toluene (BHT) etc., may have carcinogenic and other harmful effects on the lungs and livers of human beings [6]. So the exploration of naturally occurring potential and non-toxic antioxidants can replace the harmful synthetic antioxidants. Nepal is rich in diversity of medicinal and aromatic plant (MAP), and the research is aimed to screen the antioxidant activity of plant extract whose ethno pharmacological information is available. There are different techniques for the measuring the antioxidant activity *in-vitro* and in-vivo. DPPH assay have been used in our study. The method is based on the reduction of ethanolic-DPPH solution because of the presence of antioxidant substances having hydrogen donating groups (RH) such as phenolics and flavonoids compounds due to the formation of non radical DPPH-H form the primary reaction which takes place is the formation of free radical and the reduced form of DPPH. Oxidised form of DPPH free radical with an odd electron shows higher absorption, when free radical form of DPPH get electron from a free radical-scavenging antioxidant, it reduced to DPPH-H as a result absorption decreases with color change on sample [7].

2. Materials and Methods

Preparation of the plant extract

The plant materials were collected from the eastern part of the Nepal. The plants were shade dried and powered. 100 g of powder of each plant were extracted by maceration in ethanol (200mL) for 24 hrs with frequent agitation. The mixture was filtered through clean muslin cloth followed by double filtration with filter paper, repeating the same process 2/3 times for complete extraction and the filtrate was concentrated by Rotary Evaporator.

Total Flavonoid Content Determination

Total flavonoid content of the extract was determined according to colorimetric method [8,9]. Briefly 0.5 ml of each extract (50 mg/mL) was separately mixed with 1.5 ml of ethanol, 0.1ml of Aluminum trichloride ($AlCl_3$, 10%) Subsequently add 0.1mL of 1M potassium acetate and 2.8 distilled water into each bottles and the reaction mixture was allowed to stand for 30 min. then absorbance was measured in 415 nm with UV-visible spectrophotometer. Quercetin was used for constructing the standard curve (10 to 50 μg/mL; $y = 60.87x + 3.020$, $R^2 = 0.987$) and the total flavonoid compounds concentration in the extract was expressed as milligrams of quercetin equivalent per gram of dry weight (mgQE/g) of extract.

Total Polyphenolic Content Determination

The total polyphenolic content of the extract were measured using Folin-Ciocalteu reagent [9]. Briefly 0.5 mL of each extract (5 mg/mL) was separately mixed with Folin-Ciocalteau's reagent (5 ml, 1:10 v/v diluted with distilled water) and aqueous sodium carbonate (Na_2CO_3, 4mL, 1M) solution. Then the mixture was allowed to stand for 15 min at room temperature. The absorbance of the reaction mixture was measured at 765 nm using spectrophotometer. Gallic acid was used for constructing the standard curve (10 to 80 μg/ml; $y = 274x-17.59$, $R^2 = 0.912$) and the total polyphenolic compounds concentration in the extract was expressed as milligrams of gallic acid equivalent per gram of dry weight (mgGAE/g) of extract.

Antioxidant Activity

The free radical scavenging activity was measured by using DPPH assay**.** Quantitative measurements were carried out according to the method described by Braca *et al.* [10]. Different concentration of test samples (7.81, 15.61, 31.25, 62.5, 125, 250, 500 and 1000 μg/ml) were prepared while the concentration of DPPH was 1mM in the reaction mixture. These reaction mixtures were taken in eppendorf tubes and incubation at 37°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 ethanol treated control group; ascorbic acid was used as positive control. Measurement was performed at least in triplicate. The percentage of the DPPH free radical was calculated using the following equation:

DPPH scavenging effect (%) = ((A0- A1)/ A0) x 100

Where A0 was the absorbance of the control and A1 was the absorbance in the presence of the extracts of different sample. The actual decrease in absorption induced by the test was compared with the positive controls. The IC_{50} (concentration providing 50% inhibition) values were calculated use the dose inhibition curve in linear range by plotting the extract concentration versus the corresponding scavenging effect by using standard protocol.

3. Results and Discussion

Total Flavonoids and Polyphenol Contents

For total flavonoids content, quercetin was used for constructing the standard curve. The absorption was taken at 415 nm by UV-visible spectrophotometer with different

concentration from 10 to 80 µg/mL then the graph was plotted absorption versus concentration for the linear equation value. Similarly, for total polyphenol content, gallic acid was used for the constructing the standard curve. The absorption was taken at 765 nm using UV-visible spectrophotometer then graph was plotted absorption versus concentration of gallic acid for the linear equation value. The flavonoid contents of the extracts in terms of quercetin equivalent (the standard curve y = 274x-17.59, R^2 = 0.912) were between 18.80 to 155.0 mg QE/g. The highest total flavonoid levels have been detected in ''*Rhododendron setosum*''. Similarly, polyphenols content were calculated in mg gallic acid equivalent/gram (Table 2).

Antioxidant activity

DPPH radical scavenging effect: It is well known that antioxidants can seize the free radical chain of oxidation and form stable free radicals, which would not initiate or propagate further oxidation. 1,1-diphenyl-2-picrylhydrazyl (DPPH) has been used extensively as a free radical to evaluate reducing substances. In this model it was observed that the test extract (in dose of 7.18, 15.62, 31.25, 62.50 and 125 µg/ml) significantly scavenged by DPPH free radical with respect to standard ascorbic acid 0.57%, 24.54%, 70.74%, 95.17% and 96.36% respectively. Similarly other plants sample was scavenge by DPPH free radicals shown in figure 1 and 2.

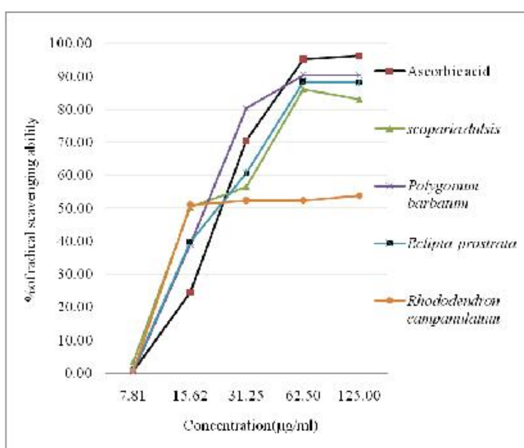

Figure 1: DPPH % radical scavenging ability of four active plants with respect to standard ascorbic acid at different concentrations (7.81-125 µg/mL)

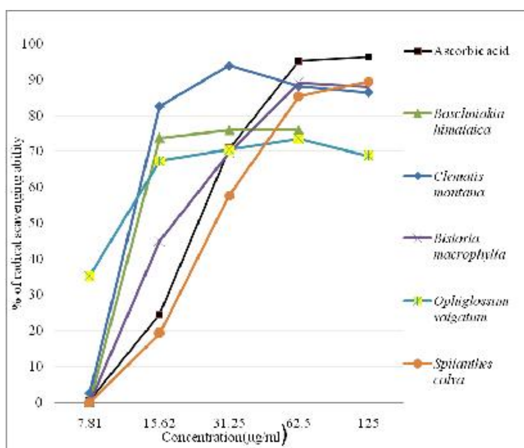

Figure 2: DPPH % radical scavenging ability of five active plants with respect to standard ascorbic acid at different concentrations (7.81-125 µg/mL)

IC_{50} Value for DPPH Radical Scavenging Activity

The DPPH radical-scavenging capacity in the studies was reported after 30 minutes reaction time for all samples evaluated. The parameter used to measure the radical scavenging activity of extracts and fractions evaluated is IC_{50} value, defined as the concentration of antioxidant required for 50% scavenging of DPPH radicals in this specified time period. The smaller IC_{50} value, the higher antioxidant activity of the plant extract/fraction. The IC_{50} value of various plant extracts and positive controls were shown in figure 3.

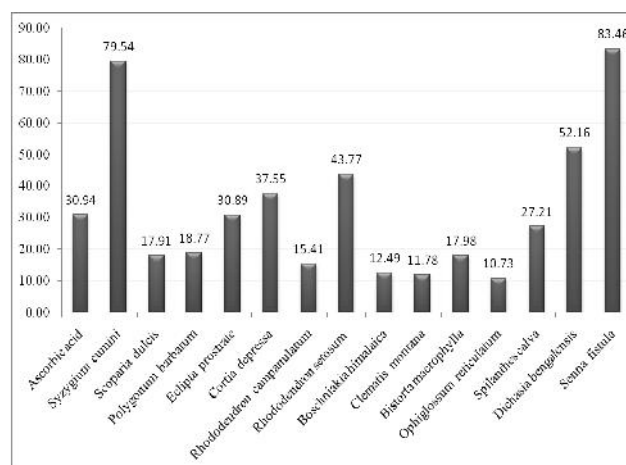

Figure 3: IC_{50} Values of 14 plant species comprising with Ascorbic acid

The standard IC_{50} value of ascorbic acid was calculated that 30.94 µg/mL. Among fourteen selected medicinal and poisonous plants species, *Scoparia dulcis, Polygonum barbatum*, *Eclipta prostrate, Rhododendron campanulatum, Boschniakia himalaica, Clematis montana, Bistorta macrophylla, Ophiglossum reticulatum* and *Spilanthes calva* shows lower values (17.91, 18.77, 30.89, 15.41, 12.49, 11.78, 17.98, 10.73 and 27.21µg/mL respectively) than standard ascorbic acid. It means, these plants are active towards antioxidant activity. Whereas *Syzygium cumini, Cortia depressa, Rhododendron setosum, Dichasia bengalensis* and *Senna fistula* shows higher values (79.54, 37.55, 43.77, 52.16 and 83.46 µg/mLrespectively) than standard ascorbic acid and shows low antioxidant activity. It is concluded that *Ophiglossum reticulatum* (IC_{50} 10.73 µg/ml) is most active and more potent than standard ascorbic acid.

This study determined that ethanolic extract of fourteen randomly selected ethnic medicinal and poisonous plant species showed better antioxidant potential by DPPH radical scavenging method. More than 64% plants (9 plants) showed good antioxidant activities with compare to standard ascorbic acid. It also indicated that most of the higher value of polyphenols and flavonoids contain plant shows higher antioxidant activity.

Table 1: List of the medicinal plants used in this study

Plant name	**Family**	**Parts used**	**Local name**	**Therapeutic applications**
Syzygium cumini	Myrtaceae	Bark	Jamun	Diarrhea, dysentery
Scoparia dulcis	Scrophulariaceae	Arial part	Chini Jhar	Diabetes, Jundish, fever
Polygonum barbatum	Polygonaceae	Arial part	Pirre	Fish poisoning
Eclipta prostrate	Asteraceae	Whole plant	Bhringaraj	Cuts, Wounds
Cortia depressa	Umbelliferae	Whole plant	Ban dhania	Pressure, rheumatism, sedative and stomachache
Rhododendron campanulatum	Ericaceae	Thalamus	Nilo Chimal	Household goods.
Rhododendron setosum	Ericaceae	Arial part	Jhuse sunpati	incense purpose, antibacterial
Boschniakia himalaica	Orobanchaceae	Whole plant	Besegano	Antifungal
Clematis montana	Ranunculaceae	Arial part	Junge Lahara	Ear disorders
Bistorta macrophylla	Polygonaceae	Whole plant		Root uses in Anorexia
Ophiglossum reticulatum	Ophioglossaceae	Whole plant	Jibre sag	Vegetable
Spilanthes calva	Asteraceae	Arial part	Parpare jhar	Fever, tonic, natural herbal tooth powder
Dichasia bengalensis	Asclepiadaceae	Whole plant		Antiinflametory, fever
Senna fistula	Fabaceae	Pods	Rajbrikchha	Tonic, fever

Table 2: Total flavonoids and polyphenol Contents of the plant extracts

Plants Name	**Flavonoid contain (mg QE/g)**	**Polyphenol content (mg GAE/g)**
Syzygium cumini	51.11	64.08
Scoparia dulcis	37.85	18.41
Polygonum barbatum	57.19	4.07

Eclipta prostrate	95.70	102.50
Cortia depressa	12.52	4.07
Rhododendron campanulatum	62.67	8.08
Rhododendron setosum	155.00	30.22
Boschniakia himalaica	16.50	5.07
Clematis Montana	41.60	39.9
Bistorta macrophylla	24.10	3.79
Ophiglossum reticulatum	29.86	30.56
Spilanthes calva	20.90	91.51
Dichasia bengalensis	18.80	41.55
Senna fistula	24.57	105.30

4. Conclusion

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