Total Phenolic Compounds and Radical Scavenging Activity of Root and Bark of *Premna barbata* Wallich Plant

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Abstract- The study is related to phytochemical analysis and examination of antioxidant property of root and bark of *Premna barbata* Wallich. Qualitative analysis of the phytochemicals was carried using known protocol and the in this we have calculated the phenolic present in plant by the protocol which are reported in the reference. While the antioxidant activity was carried using the known (DPPH)assay. The phytochemical constituent's and activity discovered in root and bark fraction of the plant shows the presence of the usual and natural compounds like carbohydrates, phenols, saponins, terpenoids, alkaloids, flavonoids and other components are also present in methanolic extract. The methanolic extract of the plant is richer than the water extract. There is positive correlation between phenol content and the DPPH activity. The bark of plant shows good antioxidant activity as compare to root. The plant extract act as the good antioxidant agent for various supplement. These studies demonstrate the need to further investigate native wild plants from Uttarakhand as important sources for antioxidant compounds.

Keywords: DPPH, phytochemicals, Premna, Radical Scavenging.

INTRODUCTION

Antioxidants are considered multifunctional because of the diversity of their compositions and mechanisms, which allow them to chelate metals, inhibit different enzymes, and scavenge free radicals [1,2]. These compounds are abundant in plants, in which phenolic compounds, carotenoids, and vitamins C and E have been reported as the main components with antioxidant activities [3,4].

Since very old times, herbal medications have been used for relief of symptoms of disease [5]. Despite the great advances observed in modern medicine in recent dec-ades, plants still make an important contribution to health care. Much interest, in medicinal plants, however, emanates from their long use in folk medicines as well as their prophylactic properties, especially in developing countries. Large number of medicinal plants has been investigated for their antioxidant properties. Natural antioxidants either in the form of raw extracts or their chemical constituents are very effective to prevent the destructive processes caused by oxidative stress [6]. *Premna* species are known to have high-antioxidant capacity, such as *P. cordifolia* Roxb. [7,8], *P. esculenta* Roxb. [9], *P. integrifo- lia* [10,11], *P. microphylla* [12] and *P. serratifolia* [13].

Secondary metabolites such as flavonoids, xanthones, chalcone and other phenolic compounds with high-hydroxyl group substitution are hypothetically contributing to the high antioxidant activity of the plant. For example, two flavone glycosides from *P. latifolia* leaves significantly inhibited oxidation of DPPH (IC_{50} 22.5 and 16.0 lg/mL, respectively) [14]. Furofuran lignans and iridoid glycosides might contribute to antioxidant activity of the stem bark of *P. integrifolia* when evaluated with radical scavenging (DPPH and NO) and ferric reducing antioxidant power (FRAP) assays [15]. Al-though the toxicity profile of most medicinal plants have not been thoroughly evaluated, it is generally accepted that medicines derived from plant products are safer than their synthetic counterparts [16,17].

MATERIAL AND METHODS

Selection of Plants:

Plant material (root and bark of P. barbata Wallich) was collected from Chauras, District Tehari Garhwal, Uttrakhand. These species of the plant authentic by Prof R. D. Gaur, Department of Botany, HNB Garhwal University, India and voucher specimen (No.

GUH 8877) is deposited in ethnobotanical herbarium. Root and Bark collected was washing away with water by the several times, then shade dried for the 15 days. Then this material was dried in oven for 30°C, till constant weight was obtained. Finally, ground into fine powder. This fine powder was kept in airtight container.

Preparation of the extract

The powder plant material extracted with methanol and water in 9:1 ratio by using the soxhlet apparatus for about 90 hrs. This was cooled at room temperature then filter through it was pass through the filter paper Whatman number 1, then the remainder was the concentrate in vacuum evaporator to dryness to form a fine powder. Powder dried till constant weight was obtained and stored at room temperature.

Estimation of total phenol

Folin-cinocalteu assay using gallic acid was described for the phenolic content determination using the standard using the slight modification

Use Value (UV): value using UV data of different compoundshas high importance.They are locally calculated by UV = (ΣU/n), U speciesare reports as number and number of informants forgiven plant. 1mLof solution and folin-ciocalteu reagent (FCR) reagent 1 mL of 1 mL of 10% NaHCO3 and 8 mL distilled water gestated for 25 min.dark room using room temperature. The amount present in the plant the phenolic compounds are easilydone by simple spectrophotometryby gauging the absorbance by UV visible spectrophotometry using 780 nm in contradiction of blank water of 1 mL in tube with 1 mL of the 10 % sodium bicarbonate. Total phenol content was articulated in the Gallic acid equivalents in the given extract,this can be done using calibration curve of Gallic acid compound .there is linear calibration was shown as the 10 to 100µg/ml of the sample (r = 0.99) [18].

Antioxidant Activity using the in vitro protocol

Radical scavenging assay DPPH method was used powder roots and bark is dissolved in methanol as 9:1 and refluxed for around 90 hrs [19]. Then cooled at 30°C was subjected to rpm 20000 rpm. We prepared different concentration by various sequential weakening methods using methanol water used as various diluents and mixed with 0.16mM methanol and DPPH. Same protocol used for the ascorbic acid or vitamin C for the assessment and ration 3:1 mL mixture of methanol and DPPH as standard, water used as adverse control. Preoccupation was leisurely at 520 nm using Shimadzu UV visible spectrophotometer. The capability of scavenging DPPH in radicals was calculated as [20-23]

Scavenging effect % = [1-(optical density of sample /optical density of DPPH) X100

We have calculated the % scavenging effect is plotted by the logarithmic plot.

EC50 value (mg/µl) for 50 % extract of the plant and DPPH concentration

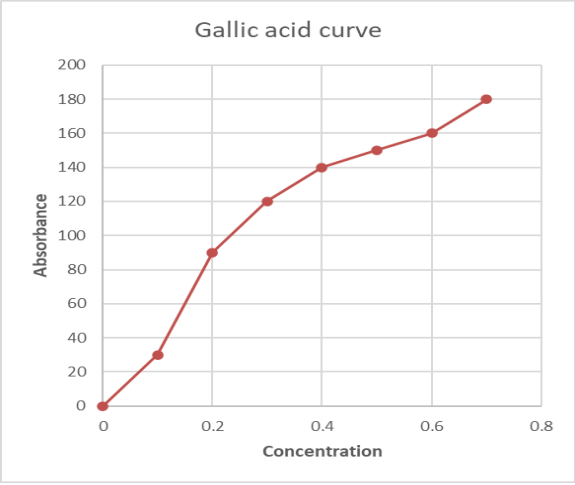
RESULT AND DISCUSSION

Folin-Ciocalteu Reagent (FCR) Method for the detection of the phenol

Most of time the for the phenolic compounds having the OH radicals, these having the foraging activity and work as the with the antioxidants. Total phenolic concentration was used for the quick scanning of the antioxidant activity. The total phenolic content was articulated as gallic acid equivalent (mg of GAE/gm sample) by equation based on the calibration curve

Y = 0.007 X - 0.036 R2 = 0.997

S. No	Concentration	Absorbance
1	0	0
2	0.1	30
3	0.2	90
4	0.3	120
5	0.4	140
6	0.5	150
7	0.6	170
8	0.7	180



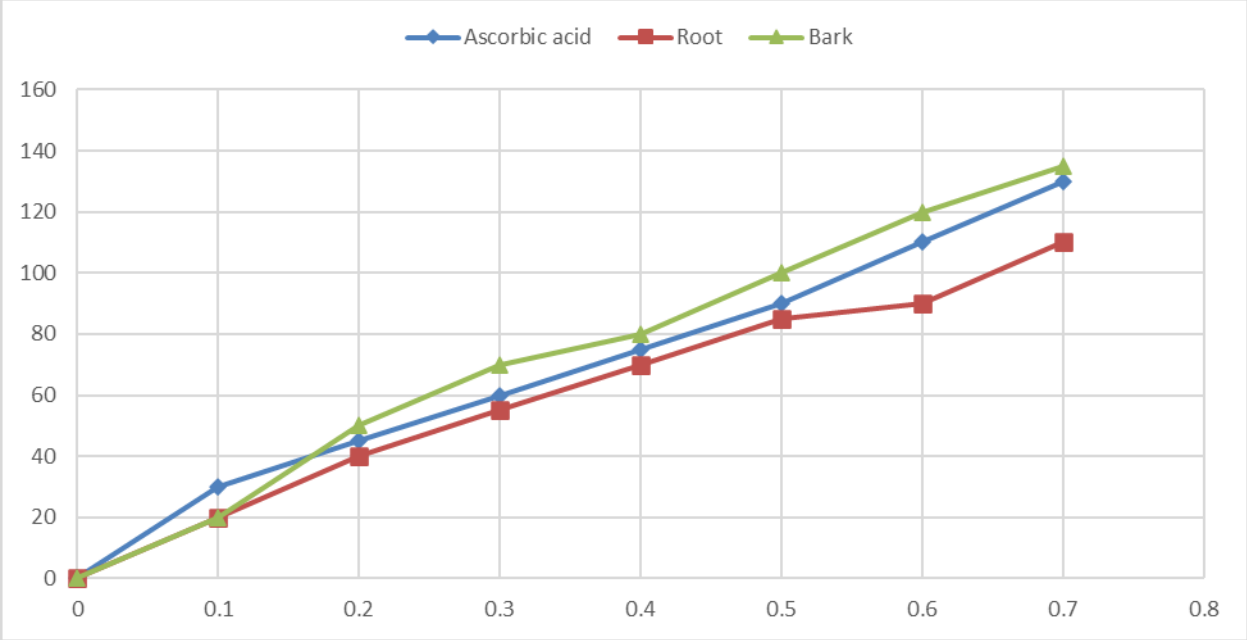
The total phenolic content plant was phenolic content for plant Root for Methanol Water the total phenol content in mg 2.45 and 2.30.

Bark Methanol Water Total phenol content 1.40 and 1.25.

DPPH Antioxidant Activity:

Yellow colour was obtained when powder(antioxidant) was added to the DPPH. the antioxidant activity and antioxidant capacity of extract [EC 50] was compared with the ascorbic acid as positive control

S. No	Concentration	Ascorbic Acid	Root	Bark
1	0	0	0	0
2	0.1	30	20	20
3	0.2	45	40	50
4	0.3	60	55	70
5	0.4	75	70	80
6	0.5	90	85	100
7	0.6	110	90	120
8	0.7	130	110	135



EC50 Value

EC50 Value: The EC 50 values are inversely related to the antioxidant activity

The DPPH radicle scavenging activity is usually quantified in the term of the inhibition percentage of the preformed free radicles by the antioxidants while the EC(50) (concentration required to obtain a 50% antioxidant effect) is used to express the antioxidant capacity compare to other extract's.

CONCLUSION

The bark parts of the plant show the highest activity as compared to the root of the of plant *P. barbata* Wallich act as moderate antioxidating agent. This has been suggested that both root and bark of the plant have good threptic agent and used for the preventing the ageing and oxidative stress. This plant can act as good antioxidant activity.

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