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Potent free-radical-scavenging activity of bark of *Poranopsis paniculata* (roxb.) Roberly

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Abstract
In this study, we evaluated the total phenolic content, total flavonoid content, and antioxidant activity of ethanolic extract of bark of *Poranopsis paniculata* for the first time. The ethanolic extract of *P. paniculata* was phytochemically screened, total phenolic content (TPC) was determined by Folin-Ciocalteu's reagent method, total flavonoid content (TFC) was determined by aluminum chloride assay and antioxidant activity was determined by using 1,1-diphenyl-2-picrylhydrazyl assay (DPPH assay). Phytochemical screening revealed the presence of alkaloids, flavonoids, phenolics, carbohydrates, reducing sugars, terpenoids, steroids, tannins. The total phenolic content and flavonoid content are found to be 152.75±2.48 mgGAE/g and 25.7±2.09 mgQE/g respectively. The antioxidant activity of *P. paniculata* extract was expressed in terms of IC_{50} values. Interestingly, ethanolic bark extract of *P. paniculata* showed the very good antioxidant activity with IC_{50} value 13.57 µg/mL, nearly equal to that of standard ascorbic acid (IC_{50} = 11.35 µg/mL). Therefore, *P. paniculata* bark has a significant amount of vital phytoconstituents and strong antioxidant properties.

Keywords: DPPH, IC_{50}, *Poranopsis paniculata*, TFC, TPC

1. Introduction
Free radicals are the reactive chemical species, produced naturally *in vi*vo, both by normal cellular metabolism and because of disease processes or through xenobiotic activities. Free radicals have the potential to elicit many of the tissue changes associated with toxicities and disease processes but are also a consequence of such damage [1].
Major secondary metabolites such as phenolic compounds found universally in plants, and are responsible for antioxidant activity mainly due to their redox properties, which allow them to act as reducing agents, singlet oxygen quenchers, hydrogen donors, and chelating agents of metal ions [2]. Another class of compounds, flavonoids, have been proven to exhibit a broad range of biochemical and pharmacological properties such as antibacterial, antiviral, anticancer, anti-inflammation, etc. [3, 4]. In addition, flavonoids can act as free radical scavengers and terminate the radical chain reaction that occurs during the oxidation of triglycerides in food system [5, 6].
Poranopsis paniculata (Roxb.) Roberly belongs to the family Convolvulaceae is an evergreen tree available in Nepal (known as Shikari lahara), India and South Asian region. The decoction prepared from this plant is used for the treatment of fractured injury, pain, diabetics fatigue, etc. (ref). The powder paste of the stem is used to minimize severe pain in the body [7]. Despite having medicinal importance, only limited studies on phytochemical screening have been carried out. Phytochemical study on Nepalese-originated *P. paniculata* leaf has suggested limited phytoconstituents such as quinones and terpenoids while other phytochemicals were not reported. In that study, total phenolic content (TPC) and total flavonoid content (TFC) in leaf were also reported with 364.69±0.055 mgGAE/g and 88.79±0.011 mgQE/g, respectively, and low antioxidant activity with IC_{50} 475.36±0.125 µg/mL [8]. Similarly, another study from India reported that the whole plant of *P. paniculata* had 33.34 mgGAE/g total phenolic content, 59.86 mgQE/g total flavonoid content, and good antioxidant property having IC_{50} value 71.68 µg/mL [9].
To the best of our knowledge, besides these two studies, no other research has been done on *P. paniculata* until now. Thus, the present study is specifically conducted on the bark of *P. paniculata* from Nepalese soil because of its broad traditional uses. So, in this study, we have screened the phytoconstituents, TPC, TFC estimation and revealed its antioxidant activities on the bark of Nepalese origin *P. paniculata*.

2. Materials and Methods

2.1 Collection of plant materials

The barkof *P. paniculata* was collected from Chitwan national park, Chitwan, Nepal in March 2019 and identified from National Herbarium and plant laboratory, Godavari, Nepal.

2.2 Preparation of crude extract by Soxhlet extractor

The bark of the plant was air dried and finely powdered. The 40 g of a sample powder was carried in thimble and 400 mL of 50% ethanol was taken as an extracting solvent in a round bottom flask. The whole apparatus was fitted carefully, and extraction was done up to 6 hours. After extraction, the liquid extract was concentrated in a rotary evaporator under reduced pressure and thus obtained solid extract was stored in 4 ℃ until further analysis.

2.3 Phytochemical screening of plant extract

The ethanolic extract of *P. paniculata* was screened for secondary metabolites (phytoconstituents) using standard protocol [10]. The presence of phytoconstituents was denoted by '+' whereas the absence by '–'.

2.4 Total phenolic content determination

The total phenolic content (TPC) in the plant was quantitatively estimated by using Folin-Ciocalteu' sreagent, using the method previously described with few modifications [11]. The extract of 1 mg/mL, 10 % Folin-Ciocalteu's reagent and 7% Na_2CO_3 were prepared for phenolics content assessment. From the stock solution of extract, different concentrations *viz*. 10, 25, 50, 75, 100, 125, 150 µg/mL were made. To individual concentrations, 5 mL of 10 % Folin-Ciocalteu's reagent was treated and after 3 min, 4 mL 7% Na_2CO_3 was added to the mixture. The sample was then incubated at 40 ℃ for 30 minutes, cooled and measured the absorbance at 760 nm. For phenolic content determination, gallic acid was taken as a standard compound and from this calibration curve was made. All the results were expressed as milligram of gallic acid equivalent pergram of extract (mgGAE/g).

2.5 Total flavonoid content determination

The total flavonoid content (TFC) in the plant was determined using aluminium chloride reagent, based on the method previously described with some modifications [12]. An extract of 4 mg/mL along with 5% $NaNO_2$, 10%$AlCl_3$ and 1 M NaOH was prepared. From the stock solution of extract, different concentrations, *viz.* 0.1, 0.25, 0.50, 1.0, 1.5, 2.0mg/mL, were prepared and mixed each concentration to 4 mL distilled water in the test tube. To each tube, at zero-time, 0.3 mL of 5% $NaNO_2$ was added and after 5 min, 0.3 mL 10% $AlCl_3$ was added. Then after 6 minutes, 1 mL of 1 M NaOH and 4.4 mL of distilled water were added to the mixture to make a total volume of 10 mL. The absorbance of the colored mixture was at read 510 nm. For flavonoids determination, quercetin was taken as standard and from this calibration curve was made. All results were expressed as milligram of quercetin equivalent per gram of extract (mgQE/g).

The total phenolic and flavonoid content were calculated by using the formula.

$$C = cV/m \quad (1)$$

Where, C is the total phenolic content (mg/g) in quercetin equivalent (QE) ortotal flavonoid content (mg/g) in gallic acid equivalent (GAE), c is the concentration of gallic acid or quercetin established from calibration curve in mg/mL, V is the volume of the extract (in mL), and m is the weight of the plant extract (in gm).

2.6 Free radical scavenging potential of plant extract

The free-radical-scavenging potential of *P. paniculata* was determined by 1, 1-diphenyl-2-picrylhydrazyl (DPPH) assay as previously performed with some modifications [13]. The sample solution of 1 mg/mL was prepared as a stock solution and DPPH of 0.1mM. From the stock solution, different concentrations, such as 0, 10, 20, 30, 40, 50, 75, 100 µg/mL, were made and to each concentration, 1 mL of DPPH was added, where concentration zero was taken as control. The mixture was shaken thoroughly and incubated in the dark for 30 min. After incubation, the absorbance was measured at 517 nm, and the experiment was performed triplicate. The percentage inhibition was calculated by a given formula. Here, ascorbic acid was taken as a standard antioxidant.

The inhibition percentage was evaluated by using formula,

$$I\% = \frac{Ac - Ao}{Ac} \times 100\% \quad (2)$$

Where Ac is the absorbance of the control (1 mL methanol + 1 mL DPPH solution), A_0 is the absorbance of the sample solution, and I% is percentage inhibition.

The linear correlation coefficient (R^2) value will be evaluated from recorded absorbance. The regression equation is,

$$Y = mx + c \quad (3)$$

where, y is the absorbance, m is the slope from the calibration curve, x is the concentration, and c is the intercept.

Based on this regression equation, concentration of the extract was evaluated. The percentage inhibition was plotted against concentration and using linear equation for y=50, the radical scavenging activities of extract were expressed in terms of their IC_{50} values.

3. Results

3.1 Phytochemical analysis

The percentage yield in ethanol extract of the plant (bark) was found to be 12.52%. The phytochemical screening of PP bark extract showed the presence of alkaloids, flavonoids, steroids, saponins, terpenoids, phenolics, reducing sugars, carbohydrates, and tannins whereas volatile oils, proteins, and polysterols were not found (Table 1).

Table 1: Phytochemical analysis of ethanolic extract of *P. paniculata*

S. No.	Name of phytoconstituents	Inference
1	Volatile oils	-
2	Alkaloids	+
3	Flavonoids	+
4	Steroids	+
5	Saponins	+
6	Terpenoids	+
7	Phenolics	+
8	Reducing sugars	+
9	Polysterols	-
10	Proteins	-
11	Carbohydrates	+
12	Tannins	+

Where, (+) indicates the presence and (-) indicates the absence of phytochemicals

3.2 Total phenolic content

The difference absorbance values found at different concentrations of gallic acid were used to draw the calibration curve which is given below and the linear equations from calibration curves were used to calculate total phenolic and flavonoid content.

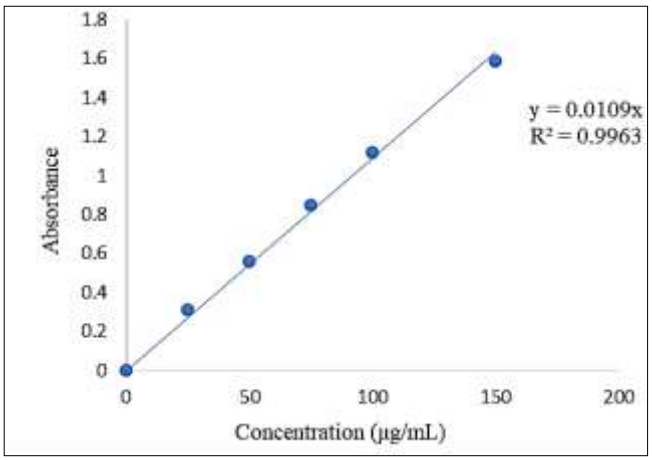

Fig 1: Calibration curve (absorbance vs concentration) from standard gallic acid

The total phenolic content (TPC) of ethanolic extract of *P. paniculata* (mg gallic acid equivalent per g dry extract) was calculated by using formula (equation 1) and tabulated below.

Table 2: Total phenolic content (TPC) in *P. paniculata*bark extract

Sample name	TPC (mgGAE/g)
P. paniculata ethanolic extract	152.75±2.48

The total phenolic content in bark of *P. paniculata*was found to be, quantitatively152.75±2.48 mgGAE/g sample.

3.3 Total flavonoid content

The difference absorbance values obtained at various concentrations of quercetin were used to draw the standard calibration curve which is presented below.

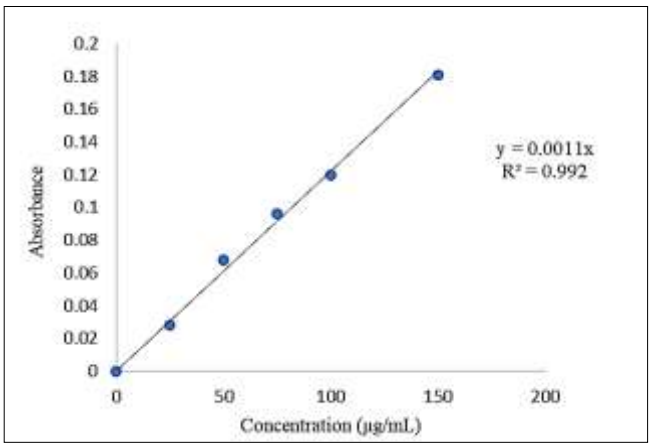

Fig 2: Calibration curve (absorbance vs concentration) from standard quercetin

The total flavonoid content (TFC) of ethanolic extract of *P. paniculatav* (mg quercetin equivalent per g dry extract) was calculated by using formula (equation 1) and tabulated below.

Table 3: Total flavonoid content (TFC) in PP extract

Sample name	TFC (mgQE/g)
P. paniculata ethanolic extract	25.7±2.09

From the aluminium chloride colorimetric method, quantitatively, the total flavonoid content in the ethanolic extract of PP was found to be25.7±2.09 mgQE/g sample.

3.4 Free radical scavenging potential

The comparison of percentage inhibition at various concentrations between extract and ascorbic acid as standard is shown in the graph below.

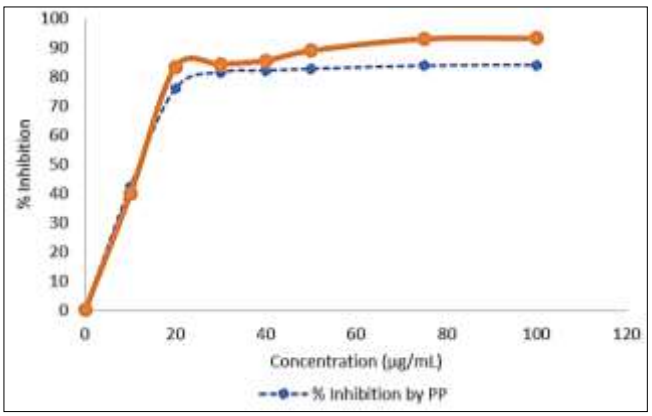

Fig 3: A plot of percentage of inhibition vs concentration of *P. paniculata*'s ethanolic extract and ascorbic acid

The IC_{50} value of ethanolic bark extract and ascorbic acid is demonstrated below.

Table 4: IC_{50} values of plant extract and ascorbic acid

Sample name	IC_{50} (µg/mL)
Ethanolic extract of *P. paniculata*	13.57
ascorbic acid	11.35

From DPPH assay, the antioxidant potential of *P. paniculata* extract was expressed in IC_{50} and compared to that of standard ascorbic acid and found to have strong antioxidant potential with IC_{50} value 13.57µg/mL whereas the ascorbic acid has IC_{50} value 11.35µg/mL. Lower the value of IC_{50} indicates greater antioxidant potential. Higher amount of phenolics and flavonoids compounds in *P. paniculata* could be responsible for the highest antioxidant activity.

4. Discussion

In the current study, ethanolic extract of *P. paniculata* bark was assessed for their phytoconstituents screening, evaluation of total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activities by following standard protocols. The presence of bioactive components in the medicinal plants and herbs maybe responsible for the biological activities of those plants. Many research studies worldwide have already proven that phytochemicals such as alkaloids, polyphenols, carbohydrates, tannins, steroids, and many other classes are correlated to different biological activities: antioxidant activities, antibacterial activities, antiviral activities, anticancer activities, anti-inflammatory properties, anti-ageing properties, etc. Specifically, higher free-radical-scavenging properties witnessed by medicinal plants is due to synergistic effects of phenolic and flavonoid compounds.

Until now, the phytochemicals, TPC, TFC and antioxidant potential in the bark of *P. paniculata* has not been studied. Therefore, in this study, phytochemical analysis, phenolics and flavonoids content estimation along with antioxidant activity of the ethanolic bark extract of *P. paniculata* was

studied using their corresponding method as described in the method section.

In Table 1, the phytochemical screening of ethanolic extract of *P. paniculata* bark showed the presence of secondary metabolites such as alkaloids, flavonoids, phenolics, carbohydrates, tannins, steroids, reducing sugars, terpenoids, tannins and other classes such as volatile oils, proteins, polysterols were reported absent. And these classes of phytoconstituents have been shown to have biological activities as previously described [14–16]. Folin-Ciocalteu's reagent method, which is a very reliable method for quantitative evaluation of total phenolic compounds in plant extract, showed TPC present in PP bark is in very good amount (TPC is 152.75±2.48 mgGAE/g) as shown in Table 2. Moreover, the total flavonoid content estimated by aluminium chloride colorimetric assay in this plant is much lower than phenolics content (TFC is 25.7±2.09 mgQE/g).The currently reported figures are not in harmony to previous reports as the previous studies were performed on leaf and whole plant. Furthermore, in Table 4, the free radical scavenging activity was articulated as the efficient concentration required for 50% of DPPH free radical reduction (IC_{50}) found from a plot of graph of percentage inhibition against the concentration of the *P. paniculata* bark extract. The *P. paniculata* extract showed the very good antioxidant activity (IC_{50}=13.57µg/mL), which is almost equal to that of standard ascorbic acid (IC_{50}= 11.35µg/mL). In antioxidant activities also, previous reports are not identical. This variation might be due to the current study being performed on a specific part of *P. paniculata* (bark), and other factors such as method of experiment, time of sample collection, altitude, pH of soil, etc. may affect its polyphenols content and antioxidant activities.

The polyphenols present in *P. paniculata* may act as reducing agents and antioxidants by the hydrogen-donating ability of their abundant hydroxyl groups. Therefore, these polyphenols may possibly be responsible for the noted antioxidant activity. On this plant, more studies and investigations are recommended to assess its biological activities as well as isolate the active compounds.

5. Conclusions

This experiment demonstrated that the ethanolic extract of the bark of *P. paniculata* has a significant amount of phenolic and flavonoid class of compounds. Moreover, it has very strong antioxidant activity. The antioxidant activity of the plant extract might be credited to the presence of polyphenolic compounds. And more studies are required to determine the active antioxidant compounds of this plant and its other biological activities.

6. Acknowledgments

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7. Conflict of interest

There is no conflict of interest.

8. References

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