



STUDIES ON ANTIOXIDANT POTENTIAL, PHYTOCHEMICAL PROPERTIES AND TOXICITY OF FOUR POPULAR MEDICINAL PLANTS OF BANGLADESH

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Abstract

Medicinal plant extract has long been used successfully in 'unani' or 'ayurvedic' medicine. Medicinal plant extract contains bioactive molecules and activity of these molecules may help to mitigate, eradicate or cure diseases. In the advent for the search for new medicinally important bioactive molecule, the current paper deals with the anti-oxidative, cytotoxic and phytochemical analysis of *Scoparia dulcis* leaf and root, *Curculigo orchioides* root, *Pandanus fascicularis* root and *Baccaurea sapida* leaf extract. Aqueous and methanolic extracts were made for each of the extracts where they possess significant antioxidative properties. High activities were seen in *P. fascicularis* and *S. dulcis* plant extract where IC₅₀ values were 21.87 µg/ml and 173.36 µg/ml respectively. In toxicity test, only *P. fascicularis* extracts showed lethality in a dose-dependent manner where the LD₅₀ value was 25.64 µg/ml. By the phytochemical analysis, it was found that each of these plant species possesses glycosides, protein, carbohydrates, alkaloid, flavonoid etc. which are pharmacologically active biomolecules. These important properties of those plants showed an indication that these plants can further be tested for the utilization in therapeutic purpose or in cosmetic industry.

Key words: Antioxidant, cytotoxicity, extracts, phytochemicals, plant

Introduction

A medicinal plant is a plant that is used in maintaining health, to be administered for a specific condition or both whether in modern or in traditional medicine (Smith-Hall et al. 2012, Ahn 2017). Plants have been utilized as medicines for thousands of years (Samuelsson 2009). These medicines initially took the form of crude drugs such as tinctures, teas, poultices, powders, and other herbal formulations (Balick and Cox 1996). However, with the advent of chemically or artificially synthesized drugs, these medicinal plants as herbal formulations declined popularity day by day. But the consequence of severe side effects of the synthetic drugs, modern drug discovery is retuning towards the nature and medicinal plants serve as gold mine for bioactive lead drug molecule (Rates 2001). Medicinal plants have been the single most productive source of leads for the development of drugs. A statistics shows that in recent years there were over a 100 new products in clinical development, particularly as anti-cancer and anti-infective agents (Harvey 2008). According to WHO, there are 21,000 medicinal plants possessing potentiality that can be used to reduce the human disease (WHO 2000).

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In Bangladesh, more than 500 plants have so far been enlisted as medicinal plant (Ghani 1998, Nishat et al. 2002). Around 400 government nursery and some private organization produce and sale medicinal plants and plant based products in Bangladesh. As the demand of medicinal plants and plant based products are increasing day by day; their cultivation has been started in different district in our country. A great example of this exploration is "Natore Owshodhi gram". This village is located at the Laxmipur Kholabaria Union under the Natore sadar upazilla District. Farmers of this village and nearby villages cultivate several medicinally important plant species as cash crops (Sharmin 2004). Despite the medicinal value of these plants very few studies were conducted regarding their phytochemical properties, antioxidant potential and cytotoxicity. Therefore, in the present study antioxidant potential, phytochemical properties and cytotoxic activity were examined for *Scoparia dulcis* leaf and root, *Curculigo orchioides* root, *Pandanus fascicularis* root and *Baccaurea sapida* leaf. As the four medicinally important plant species are the major focus paper, it will be rationale to provide a brief description of these plants below:

Baccaurea sapida

B. Sapida (Latkan) is a delicious fruit normally grown in Bangladesh, Nepal, India, Myanmar, China, Thailand and Peninsular Malaysia (Alam 2004). Latkan fruits are yellow, velvety, 2-3 cm in diameter with leathery pericarp. Seeds are arrilus, three in number, embedded in pale rose colored delicious pulp. *B. sapida* fruits are used as food and in treatment of bacterial infectious diseases such as diarrhea, dysentery, skin infection (Jain 1986, Mann et al. 2008, Deb and Bhowmick 2013)

Pandanus fascicularis

Pandanus roots are used in unani and other folk medicine. Roots, leaves, fruits etc are used as anthelmintic, tonic, in treatment of liver disorders (Jothimani et al. 2011). Root extract also have anti-inflammatory and analgesic activity. In treatment of diabetes, *Pandanus* roots are used. Roots are widely used in treatment of osteoarthritis and skin diseases like leprosy. In the Ayurvedic system of medicine, the roots of *P. fascicularis* are used in 'Prameha' and employed for their hypoglycemic action (Madhavan et al. 2008, Ayyanar and Ignacimuthu 2011, Jothimni et al. 2012).

Curculigo orchioides

This plant is endangered species and it is native to Indian sub-continent including Bangladesh. This plant contains curculigoside A, B, C and D and curculigine A and D can also be found. This plant is used for diseases of the urogenital system in both males and females (Chauhan et al. 2007). It is also prescribed in treatment of piles, jaundice, gonorrhea, asthma, and diarrhea (Chauhan et al. 2010). This plant extract is present in several herbal formulations for gynecological problems and sexual weakness of males (Duraipandiyani et al. 2006).

Scoparia dulcis

S. dulcis is a weed in many parts of Bangladesh but its use as traditional medicine has led to overexploitation of this plant (Mollik et al. 2010). Scoparinol, scoparic acid, scopadulcic acid, scopadulciol, and scopadulin have been isolated from this plant. This plant is used for diabetes, hypertension and other health conditions such as hemorrhoids, anemia, burns, and headaches (Edeoga et al. 2006).

Materials and Methods

Collection of plant material

Roots of *P. fascicularis*, *C. orchoides* and leaves of *S. dulcis* were collected from the Natore Owsodhi gram. Leaves of *B. Sapida* were obtained from Narshingdi where this plant is cultivated in commercial level.

Extraction of phytochemicals

For the extraction of phytochemicals, the leaf and root of the *selected plants* were dried at room temperature and was crushed into powder by electric blender (Pant et al. 2017). For methanolic extraction, powders were extracted in methanol apparatus at 55-85°C for 12-24 h. The extracts obtained were then dried using rotavapor drier at 55-85°C, and the solid extracts were preserved in refrigerator for further analysis. For aqueous extraction powders were dissolved in dH₂O and centrifuged at 8000 rpm for 10 min at room temperature and supernatant served as crude aqueous extract.

In vitro antioxidant assay

In order to examine the antioxidant properties of methanolic extracts of the test plants explants, DPPH free radical scavenging assay (Hatano et al. 1988) was employed. DPPH antioxidant assay is based on the ability of 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) a stable free radical, to decolorize in the presence of antioxidants. The percentage (%) inhibition activity was calculated from the following equation

$$\%I = \{(A_0 - A_1)/A_0\} \times 100$$

Where,

A₀ is the absorbance of the control, and

A₁ is the absorbance of the extract/standard.

Then % inhibitions were plotted against log concentration and from the graph IC₅₀ was calculated. Extracts (30 mg) of four plant samples were taken in four 1.5 ml centrifuge tube. Methanol (1 ml) was added to each of the tubes with the help of micropipette. Then the tubes were vortexed for 2-3 min so that plant extracts could mix with methanol. Subsequently, tubes were centrifuged at 10000 rpm for 5 minutes. Methanol (1.5 ml) solution and DPPH solution (1.5 ml) was then added in glass test tubes. Each test tube contained different concentrations of extract solutions (50 µg/ml, 100 µg/ml, 200 µg/ml, and 400 µg/ml). The negative control contained only methanol and DPPH solution, in contrast the positive control contained Ascorbic acid standard with methanol and DPPH was added. All the test tubes were incubated at room temperature for 30 min in dark chamber to complete the reaction. Finally, the absorbance of the solutions was measured at 517 nm wavelength using spectrophotometer.

Phytochemical properties

Phytochemical analysis was conducted using standard protocols (Obadoni and Ochuko 2002, Senguttuvan et al. 2014, Pant et al. 2017). Plants extracts were screened for glycosides, protein, carbohydrates, alkaloids, tannin, phlobatannin, flavonoid, steroids, saponins, phenolic compound, phytosterols, fats and for anthraquinones.

Fehling's test for glycosides

A small amount of grinded plant sample was taken and dissolved in water. Fehling's solution was added in test tube containing water dissolved plant crude extracts. Brick-red precipitation formation indicated the

presence of glycosides in the plant sample. In another test grinded plant sample was dissolved in methanol and few drops diluted H_2SO_4 was added. Then test tubes were boiled after adding NaOH solution for neutralization. Brick-red precipitation indicated the presence of glycosides.

General test for glycosides

Small amount of grinded plant sample was dissolved in water and a small portion of aqueous NaOH solution was added. A yellow color formation was considered the indicative of the presence of glycosides.

Biuret test for protein

First Biuret reagent was prepared according to Gornall et al. (1949). Few drops of Biuret reagent were added in four test tubes which containing different crude extracts. Upon completion of the reaction deep purple/lilac color formation confirms the presence of protein.

Benedict's test for simple carbohydrates

Different grinded plant sample (1 gm) was dissolved in water (10 ml) and filtered using Whatman 1 filter paper. From the stock solution, 5 ml were taken to other test tubes and added 5 ml Benedict reagent (Benedict 1909). Tubes were heated on to the boiling water bath for few minutes. A color change indicated the presence of simple carbohydrate.

Hager's reagent for alkaloid test

A few amount of water solution of different plant samples were neutralized by adding diluted H_2SO_4 in few drops. The solution was then treated with a small amount of Hager's reagent. The presence of alkaloid was confirmed by the formation of yellow crystals.

Ferric chloride test for tannin

Water and methanolic extracts of four plant samples were stirred in dH_2O . Then 5% $FeCl_3$ solution was added in tubes. The presence of tannin was confirmed by the colored (blue, green, blue-black) precipitation.

Test for phlobatannin

5 ml of aqueous and methanolic solution of four plant extracts taken and added diluted HCl. The presence of phlobatannin was confirmed by red or reddish color precipitation formation.

HCl test for flavonoid

Aqueous and methanolic solutions of plants extracts were taken and concentrated HCl were added from the side of the test tubes. Presence of flavonoid is indicated by red color formation.

Salkowski's test for steroids

Aqueous solution (5 ml) of extracts were taken in test-tubes and chloroform (3 ml) and concentrated H_2SO_4 (2 ml) were added from the side of the test tubes. Red color production in the chloroform layer is the confirmation of presence steroids.

Frothing test for saponine

Solution (5 ml) of crude aqueous extracts of different plant samples was shaken strongly in a test tube. Produced of frothing and persistence of the froth upon warming for 2-3 minutes can be taken as a primary confirmation for saponine presence.

Test for phenolic compound

1gm of grinded plant sample was dissolved in 10 ml dH₂O. Small amount of neutral 5% ferric chloride solution was added to it in test tubes. Presence of phenolic compounds in the sample was detected based on the formation of a dark green color.

Test for phytosterols

0.5 gm grind sample was dissolved in acetic anhydride solution (2 ml). Few drops of concentrated H₂SO₄ was added very slowly by the side of the test tubes. A change in color detects the presence of phytosterols.

Ethanol emulsion test for fats

Solution (5 ml) of plant extracts was taken in different test tubes. Ethanol (2 ml) were added to each test tube and mixed by gentle shaking. Upon shaking, 2 ml dH₂O were added. Formation of emulsion was manually visualized to detect the presence of fat.

Test for anthraquinones

Powder (1 gm) of grinded plant sample was taken in different test tubes. Chloroform (5 ml) was added to each of the tubes and kept on boiling water bath for 5 min. After 5 minutes of boiling, remaining solutions were filtered and cooled at room temperature. Subsequently, 10% ammonia solution (5 ml of) was added to the filtrate. The tubes were shaken to visualize the formation of bright pink color as the indicator of the presence of Anthraquinones.

Toxicity assay

Brine Shrimp lethality assay was performed using the method of Meyer et al. (1982) with *Artemia salina* (nauplii) (24 hr post hatching). Ten organisms were used for each concentration. Different concentrations of solutions were prepared using different plant extracts and dose concentration was 5 µg/ml, 10 µg/ml, 15 µg/ml, 20 µg/ml and 25 µg/ml, respectively. Reading for lethality of the nauplii was obtained after 24 hours of given doses. Percentage (%) of inhibition was calculated by comparing four samples with the control samples which do not contain any plant extract.

Results and Discussion**Antioxidant potential**

In the present study, the antioxidant activity of the plant extracts of *B. sapida*, *P. fascicularis*, *C. orchioides* and *S. dulcis* were evaluated by DPPH free radical scavenging assay. The antioxidant activity was increased by increasing the concentration of the extract in a dose dependent manner. Different concentrations of the sample extracts viz. 50 µg/ml, 100 µg/ml, 200 µg/ml and 400 µg/ml were used in the experiment. *B. sapida* extracts showed the scavenging value of 12.90%, 30.74%, 44.59%, and 57.31% respectively in four different concentration with a IC₅₀ value of 302.16 µg/ml which means moderate free-radical scavenging activity are shown by this plant extracts. However, high antioxidant activity was observed in case of *P. fascicularis* extracts with IC₅₀ value of 121.87 µg/ml. The scavenging value of the extracts were 62.57%, 66.16%, 67.30%, 69.75 % respectively in four different concentrations mentioned above. 4.14%, 13.91%, 31.77% and 58.65% of scavenging value was obtained for *C. orchioides* extracts in 50 µg/ml, 100 µg/ml, and 200 µg/ml

and 400 $\mu\text{g/ml}$ concentrations, respectively. The calculated IC_{50} value of *C. orchoides* extract was 336.98 $\mu\text{g/ml}$. In case of *S. dulcis* extracts scavenging value of 35.18%, 63.67%, 65.97%, 69.02% respectively were obtained. *S. dulcis* root extracts showed a higher free-radical scavenging activity and the IC_{50} value was 173.36 $\mu\text{g/ml}$. Antioxidant potentiality of each of the extract was compared with the standard ascorbic acid. The results of DPPH radical scavenging assays of ascorbic acid (standard) and our four plant extracts are given in Table 1 and in the Figs 1 and 2.

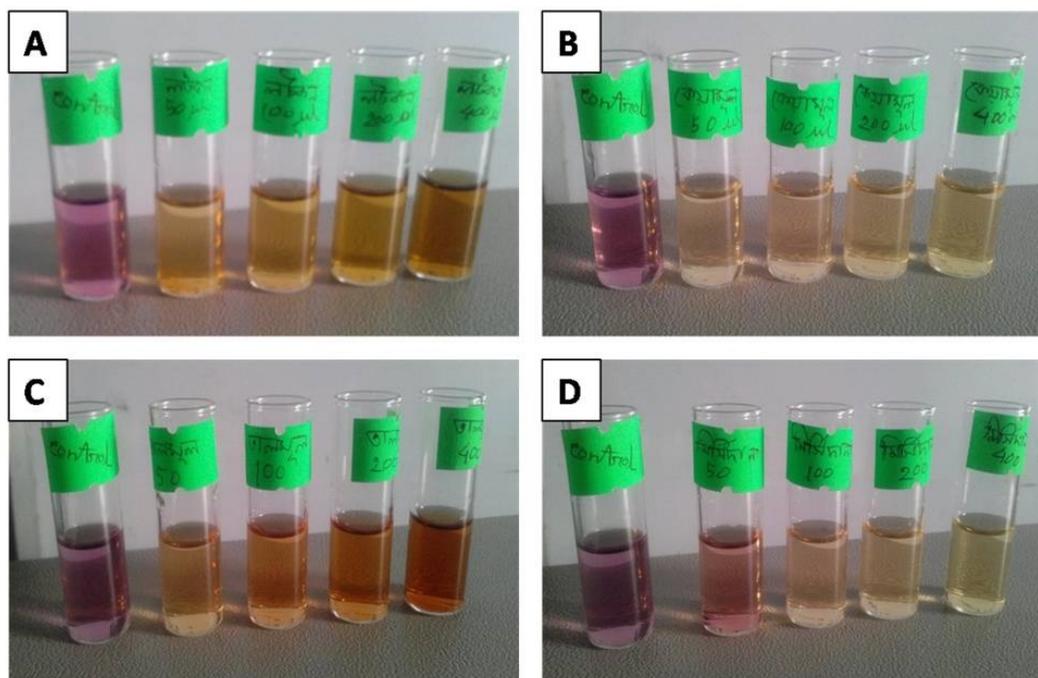


Fig.1. Decolorization of DPPH solution by (A) *B. sapida* extracts, (B) *P. fascicularis* extracts, (C) *C.orchoides* extracts and (D) *C. orchoides* extracts.

Table 1. Percentage (%) of scavenging by DPPH free radical scavenging assay of four (4) different plant sample extract along with the standard ascorbic acid.

Concentrations ($\mu\text{g/ml}$)	% of scavenging				
	Ascorbic acid	<i>B. sapida</i> (leaf)	<i>P. fascicularis</i> (root)	<i>C. orchoides</i> (root)	<i>S. dulcis</i> (leaf)
0	0	0	0	0	0
50	68.59	12.90	62.57	4.14	35.18
100	72.64	30.74	66.16	13.91	63.67
200	77.45	44.59	67.30	31.77	65.97
400	78.23	57.31	69.75	58.65	69.02

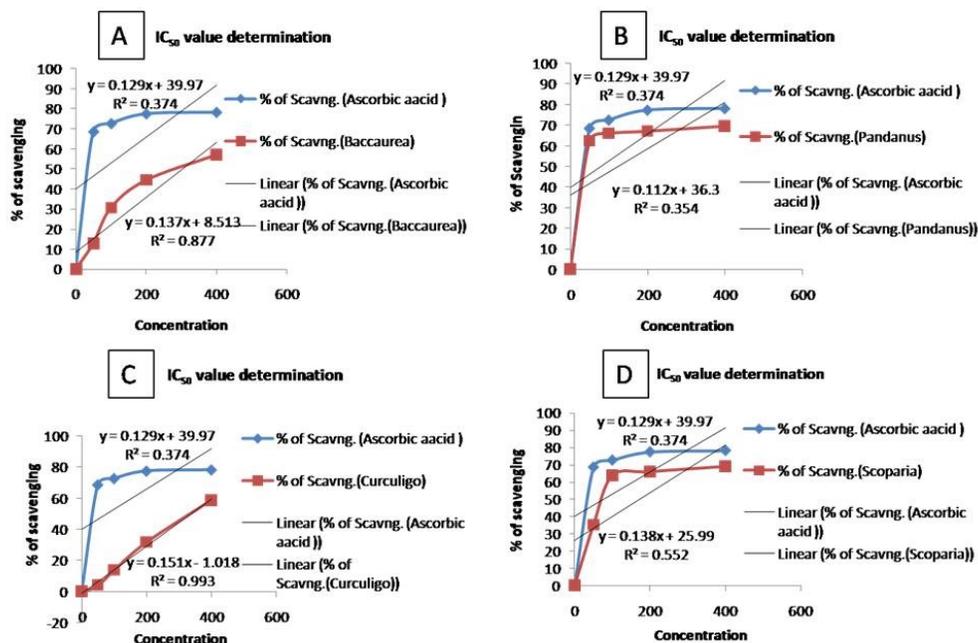


Fig. 2. DPPH free radical scavenging activity of the plant extracts at different concentrations along with ascorbic acid standard ($\mu\text{g/ml}$) (A). *B. sapida* leaf extract, (B). *P. fascicularis* root extract, (C). *C. orchoides* root extract, and (D). *S. dulciss*.

Phytochemical properties of four plant extracts

14 phytochemical properties of medicinally important four plant extracts were investigated in the present study and the results of the study are summarized in the Table 2.

Toxicity test of four plant extracts

Brine-shrimp lethality bioassay was conducted at five different concentrations (5 $\mu\text{g/ml}$, 10 $\mu\text{g/ml}$, 15 $\mu\text{g/ml}$, 20 $\mu\text{g/ml}$ and 25 $\mu\text{g/ml}$). Methanolic extracts of four plant extracts were used. *Baccaurea* leaves, *Scoparia* leaves, *Curculigo* roots extracts did not show any cytotoxic activity in any of those five different concentrations. Only *Pandans fascicularis* extracts showed cytotoxicity in a dose dependent manner (Table 3). From the percentage (%) lethality of brine-shrimp, probit analyses were carried out by probit analysis software (Fig. 3). From the dose value, lethal concentration 50 or LC_{50} value were calculated. LC_{50} value of *P. fascicularis* root of methanolic extract was found 25.64 $\mu\text{g/ml}$ with 95% confidence limits. The results suggest that the *P. fascicularis* root extract is highly toxic to cells at least against brine-shrimp larvae.

Aqueous and methanolic extracts of the four plant extracts were used for the qualitative analysis of the four plant extracts. *Baccaurea* leaves showed positive results for the test of presence for glycosides, carbohydrate, steroids, phenolic compound and phytosterols but negative the presence of protein, alkaloid, tannin, phlobatannin, flavonoid, saponine, fats and anthraquinones for both the aqueous and methanolic extracts.

Table 2. Qualitative analysis of the phytochemical screening of aqueous and methanolic extracts of four medicinally important plant samples from Bangladesh. Here, "+" refers to presence and "-" refers to absence.

Compound name	<i>B. sapida</i> (leaf)		<i>P. fascicularis</i> (root)		<i>C. orchoides</i> (root)		<i>S. dulcis</i> (leaf)	
	Aqueous extract	Methanolic extract	Aqueous extract	Methanolic extract	Aqueous extract	Methanolic extract	Aqueous extract	Methanolic extract
Fehling's test for glycosides	+	+	-	-	+	-	+	-
General test for glycosides	+	+	-	-	+	-	-	-
Biuret test for protein	-	-	+	-	-	-	+	-
Benedict's test for simple carbohydrate	+	+	+	+	+	+	+	+
Hager reagent for alkaloid test	-	-	-	+	-	+	-	+
Ferric chloride test for tannin	-	-	+	-	+	+	-	-
Test for phlobatannin	-	-	-	-	-	+	-	-
HCl test for flavonoid	-	-	+	-	-	-	-	-
Salkowski's test for steroids	+	+	+	-	-	-	-	-
Frothing test for saponine	-	-	-	-	-	-	+	-
Test for phenolic compound	+	+	+	+	+	+	-	-
Test for phytosterols	+	+	-	-	-	-	+	-
Ethanol emulsion test for fats	-	-	-	-	-	-	+	-
Test for Anthraquinones	-	-	-	-	-	-	-	-

In case of *Pandanus fascicularis* root extracts, for both aqueous and methanolic extracts negative result was found for the test of presence for glycosides, phlobatannin whereas the test of presence for carbohydrate was positive. In case of test of presence for protein, tannin and flavonoid, the aqueous extracts showed positive but methanolic extracts showed opposite. Negative results were found for the aqueous extracts of the test of presence for saponine, phytosterols, fats and anthraquinones.

Curculigo roots showed positive results for both the extracts for the test of presence of carbohydrate, tannin and phenolic compounds but negative for the protein, flavonoid, saponine and fat. The aqueous extracts of the *Curculigo* roots contains glycosides but not the alkaloid, phlobatannin, steroids, phytosterols and anthraquinones. Only the methanolic extracts of the *Curculigo* roots contain the alkaloid and phlobatannin.

The aqueous extracts of *Scoparia* leaves showed positive results for the test of presence for glycosides, protein, carbohydrate, saponine, phytosterols and fats but negative results for the general test for glycosides, alkaloid, steroids and anthraquinones. On the other hand, the methanolic extracts of the roots indicated negative results for the presence of glycosides, protein, tannin, phlobatannin, flavonoid, saponine, phenol and fats but positive for carbohydrates and alkaloids.

Table 3. Brine-shrimp cytotoxicity of the methanolic extract of *P. fascicularis* root.

Dose µg/ml	Log dose	Number	Kill	%kill	Corr%	Emp probit	Expt probit	Work probit	Weight	Final probit
5	0.6989628	10	1	10	10	3.72	3.343838	3.89	2.08	3.328856
10	0.9999897	10	0	0	0	0	4.042734	3.34	4.390001	4.037427
15	1.176079	10	4	40	40	4.75	4.451563	4.78	5.580001	4.451915
20	1.301017	10	4	40	40	4.75	4.741631	4.74	6.16	4.745999
25	1.397926	10	5	50	50	5	4.966625	4.99	6.34	4.974107

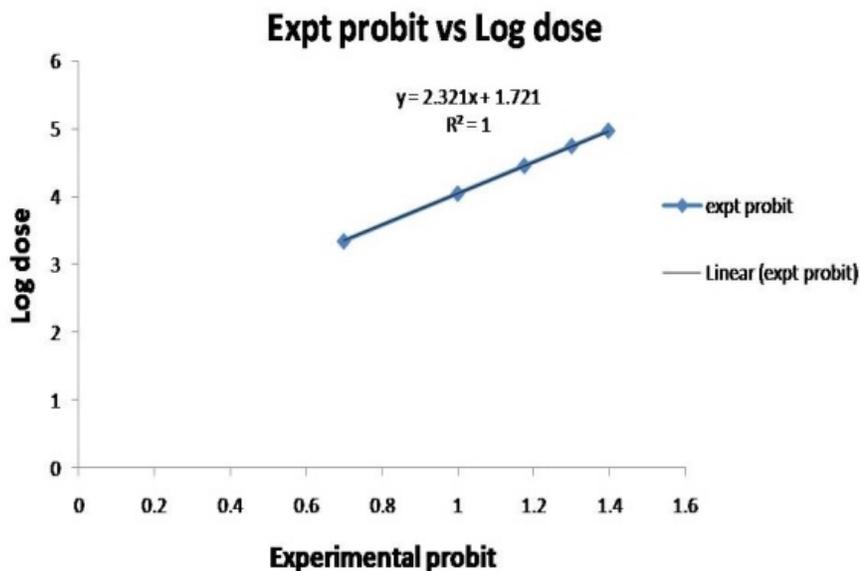


Fig. 3. Plot of log doses versus probit for calculation of LC_{50} of methanolic extract of *P. fascicularis* root. Finney's statistical method of probit analysis was used for the analysis of the data.

Conclusion

The results of the study demonstrate that each of the four medicinally important plant extract has antioxidant activity where *P. fascicularis* and *S. dulciss* plant extract has highest antioxidant activity close to the standard ascorbic acid (positive control). So these plants are the significant source of natural antioxidant that can be used in cosmetic, food supplement, drug designing etc. From the phytochemical analysis, we found that each of these plant species possesses pharmacologically active biomolecules which may be the attribute of their folk medicinal use. From the result of preliminary cytotoxicity testing (Brine-shrimp lethality assay), only *P. fascicularis* extracts found to be the cytotoxic LD50 value of *P. fascicularis* root extract was found 25.64 µg/ml. Further biochemical investigations are in progress for purification and characterization of bioactive molecule present in the above described four medicinal plants.

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