Phytochemical Analysis, *In Vitro* Antioxidant and Cytotoxic Activity of Extracts of *Paederia linearis* Hook. f. Root

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Abstract

Dried *roots* of *Paederia linearis* Hook. f were extracted with hexane, dichloromethane, ethyl acetate and methanol gave crude extracts of hexane, dichloromethane, ethyl acetate and methanol respectively. All crude extracts were analyzed the phytoconstituents, screened of *in vitro antioxidant* properties and evaluated the cytotoxic activity. Phytochemical analysis revealed the presence of various bioactive constituents, especially phenolics, flavonoids and tannin that were found to be major constituents in methanoic extract. The root extracts had moderate to good antioxidant activity when compared with the standards. The values of total phenolic, total flavonoid and tannin contents of methanolic extract were 51.27 $\pm$ 0.13 mg GAE /g, 36.41 $\pm$ 1.28 mg QE/g and 9.81 $\pm$ 1.85 %, respectively. All extracts were found to be non toxic toward *Vero cell* (% cell growth > 50%). Methanol extract exhibited selective cytotoxic activity against NCI-H187 cells with % cells growth inhibition value of 60.20%. With all these results, we could conclude that *Paederia linearis* Hook. f. root possessed antioxidant and selective anti-cancer activity and could be used as a new source of safe antioxidant. Moreover, the investigation on the anti-cancer constituents from this plant shoud be done in future.

Keywords: Phytochemical analysis, Antioxidant, Cytotoxic activity, *Paederia linearis* Hook. f.

Introduction

An antioxidant is a molecule capable of inhibiting the reactive free radicals. Free radicals are electrically charged molecules which have an unpaired electron that forces them to seek out and capture electrons from other molecules to neutralize themselves. Free radicals produced are capable of reacting with membrane lipids, proteins, enzymes, amino acids and other small substances resulting a various human diseases, such as cardiovascular disease, neural disorders, Alzheimer, Parkinson's disease, diabetes, ulcerative colitis, aging process, and cancer [1-5]. Therefore, antioxidants with potential free radical scavenging activities may have great in the protection of various diseases involving free radicals. Recently, the increasing of interest in natural antioxidants from plants has become a trend, because they are accepted to be safe [6-7]. It has been confirmed that the antioxidant properties of plant products is mainly due to effect of phenolic compounds, such as phenolic acid, flavonoids, tannins and phenolic diterpens [8-11]. Antioxidant properties of phenolic compounds arise from their high reactivity as ability to stabilize and delocalize the unpaired electron, and from their potency to chelate transition metal ions, and from excellent ability to act as hydrogen or electron donors [12]. Cancer is one of the most leading causes of death in world and a similar trend has emerged in the developing countries. Since almost all anti-cancer drugs currently being used in cancertherapy are highly toxic and produce severe damage to normal cells. Hence, the search of non-toxic natural products for cancer therapy represents an area of great interest in which plants had been the most important source [13,14]. Plants and herbs have been used as an important contributor to the quality of human life for thousands of years. Some of them are well known as medicinal herbs. The medicinal plant *Paederia linearis* Hook. f. is the member of the Rubiaceae family. It is widely grown in tropical areas and distributed throughout Thailand. *Paederia linearis* Hook. f. is a deep-rooted, tall slender, climbing plant with stinky lanceolate leaves. Its flowers are inflorescene with 0.5 – 1.0 cm long having white petal and purple corolla. The roots, seeds and leaves of this plant are of medicinal importance. In Thailand, it has been used as folkloric drug treating in various diseases. The roots are a little bitter, ophthalmic, laxative, diuretic, hemorrhoidal and are used as tonic. Root infusion or decoction was used for drink to relive fever and cough. Its leaves were used in, bronchitis, herpes labialis, ulcers, fever and curing snake bite poisons. The plant has been scientifically studied for chlolinesterase inhibitory activity [15,16]. To the best of our knowledge no other study on biological properties, including antioxidant activities was carried out so far on this plant. Therefore, this study aimed to determine for the first time, the phytochemical screening, evaluating of antioxidant and to preliminary evaluate the cytotoxic activity of the crude extracts of *Paederia linearis* Hook f. root. This study was also undertaken to determine total phenolic, total flavonoid, tannin content and reducing power ability of the methanolcrude extract of *Paederia linearis* Hook. f. root.

Materials and Methods

Plant collection

The fresh root of *Paederia linearis* Hook f. were collected in February 2012 from Suwannaphum, Roi-Ed, Thailand. Their identities were checked with authentic plant and kept at the Natural Products Chemistry Research Unit, Phetchaburi Rajabhat University. Roots were cleaned and shade dried for 2 weeks at room temperature, ground into powder and stored in air tight container.

Chemicals

2,2-Diphenyl-1-picrylhydrazyl (DPPH), sodium carbonate (Na_2CO_3), ferric chloride ($FeCl_3$), aluminium chloride ($AlCl_3$), potassium ferriccyanide ($K_3Fe(CN)_6$), trichloroacetic acid (CCl_3COOH), potassium permanganate ($KMnO_4$), BHA (butylated hydroxyanisole), ascorbic acid (vitamin C), Folin-Ciocalteau reagent and gallic acid were perchased form Sigma-Aldrich Chemical Co.(Bangkok Science, Thailand). All other used chemical were of analytical grade.

Preparation of plant extract

Powder 2.5 kg of dried root of *Paederia linearis* Hook f. was soaked in 2.0 liters of hexane for 72 hours. The extract was filtered using Whatmann filter paper. This was repeated for 2 to 3 times and the similar extracts were pooled together. The combined extract was concentrated at 40°C to 50°C under reduced pressure using vacuum evaporator. The concentrated combined extract was used as hexane extract. The residual plants material was extracted successively by fractionation with increasing polarity of organic solvents (dichloromethane, ethyl acetate and methanol) in the same manner as followed for hexane.

Qualitative phytochemical screening

The obtained hexane, dichloromethane, ethyl acetate and methanol crude extracts were analyzed for presence of phytochemical constituents by standard method of qualitative phytochemical screening [17, 18]. The extracts were subjected for test for carbohydrate, amino acids, proteins, tannins, phenolics, alkaloids, antraquinones, saponins, flavonoids, terpenoids and steroids [17,18].

In vitro antioxidant methods

β-Carotene bleaching assay

Antioxidant activitis of all extract were determined according to modified version of β-carotene bleaching method [19]. A solution of β-carotene was prepared and 40 mg of linoleic acid, 400 mg of Tween 40 emulsifier, and 100 mL of distilled water were added to the β-carotene solution. Aliquots (4.8 mL) of the clearly emulsion was transferred into different test tubes containing 0.2 mL of ethanolic solution of different concentrations of the extract. A control, without antioxidant, consisting of 4.8 mL of the emulsion and 0.2 mL of ethanol. Readings of all samples were taken immediately (t = 0) and at 15 min intervals for 120 min on UV-Vis spectrophotometer at 470 nm. The synthetic antioxidant, butylated hydrotoluene (BHT) and vitamin C were used as positive control. All determination were performed in triplicate.

$$\% \text{ inhibition} = [(AA_{(120)} - AC_{(120)}) / (AC_{(0)} - AC_{(120)})] \times 100$$

Where $AA_{(120)}$ is the absorbance of the antioxidant at t = 120 min, $AC_{(120)}$ is the absorbance of the control at t = 120 minutes, $A_{C(0)}$ is the absorbance of the control at t = 0 min.

DPPH radical-scavenging assay

The radical scavenging activities of the extracts of *Paederia linearis* Hook. f. roots against 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical were determined by UV spectrophotometry. Radical scavenging activity was measured by a modified method from previously reported [20]. One mL of each extract with different concentration was placed in a test tube, and 3 mL of methanol was added followed by 0.5 mL of 1.0 mM DPPH in methanol. A blank solution was prepared containing the same amount of methanol and DPPH. Ascorbic acid was used as the antioxidant standard. The decrease in absorbance at 517 nm was determined by spectrophotometer after 1 h for all samples. All determinations were performed in triplicate. The % inhibition of DPPH free radical was calculated :

$$\% \text{ inhibition} = ((AC_{(o)} - AC_{(t)}) / AC_{(0)}) \times 100$$

Where $A_{C(o)}$ is the absorbance of the control at t = 0 min and $A_{C(t)}$ is the absorbance of the control at t = 1 h.

Reducing power ability assay

The reducing power of only the methanol crude extract was investigated by detection of the transformation of Fe^{3+} into Fe^{2+} under acidic medium. The Fe^{2+} can be monitored by measuring the formation of Perl's Prussian blue using spectrophotometer at 700 nm [21]. One mL of the sample (50-400 µg/mL in methanol), 2.5 mL of phosphate buffer pH 6.6 and 2.5 mL of 1% $K_3Fe(CN)_6$ were incubated at 50 °C for 20 min and 2.5 mL of 10% CCl_3COOH was added to the mixture and further centrifuged for 10 min at 3000 rpm. The absorbance was recorded at 700 nm using BHT and vitamin C as the standards. Increasing of absorbance of the reaction indicated the increased reducing power.

Quantification of total phenolic, total flavonoid and tannin contents

The assay for estimation of total phenolic and total flavonoid contents were measured using UV spectrophotometer (Shimadzu UV MINI 1240V) and performed in triplicate. Tannin contents was estimated using titration method.

Total phenolic contents

Total phenol contents in the methanolic extract was estimated using the modified Folin-Ciocalteu method [22]. An aliquot (0.5 mL) of the extract in methanol was mixed with 2.5 mL of Folin-Ciocalteu phenol reagent in a clean test tube and mixed well. Then 7.5 mL of Na_2CO_3 (0.2 g/mL) was added to the mixture. The tube was shaked and allowed to stand for 30 min at 40°C for color development. The absorbance was then measured at 765 nm using UV-Vis spectrophotometer. The total phenolic contents were determined from the calibration curve and presented as gallic acid equivalent (mg/g).

Total flavonoid contents

Flavonoid contents in only the methanol crude extracts of *Paederia linearis* Hook. f. root was determined by quercetin colometric method [23]. Briefly, 0.5 mL of the extract in methanol of plant was diluted with 1.5 mL distilled water and 0.5 mL of 10% (w/v) of $AlCl_3$ was added along with 0.10 mL of 1.0 M of CH_3COOK and 2.8 mL of distilled water. The mixture was then incubated at room temperature for 40 min. The absorbance of resulting reaction mixture was further measured at 415 nm using UV-Vis spectrophotometer. The quantification of flavonoids was done on the basis of standard curve of quercetin in methanol and the results were expressed in quercetin equivalent (mg/g).

Tannin contents

The analysis of tannin in methanolic extract of *Paederia linearis* Hook. f. root was performed according to The international Pharmacopoeia and AOAC method with some modification [24]. The methanolic extract solution was prepared by adding 250 mL of distilled water into 3.0 g of the extract. The 25 mL of solution was diluted into 1 L in conical flask, then 25 mL of indigo carmine solution and 750 mL distilled water were added. The aqueous solution of 0.1 M KMnO4 was used for titration until the blue color changed to green color. Few drops of KMnO4 were then added immediately into the green solution until the solution became golden yellow. Indigo carmine solution was prepared as following: 6.0 g indigo carmine was dissolved in 500 mL of distilled water under heating on hot plate. The solution was allowed to cool, 50 mL of conc. H_2SO_4 was added. The solution is diluted to 1 L and then filtered. The blank for this titration is a mixture of 25 mL indigo carmine solution and 750 mL of distilled water. The samples were analyzed in duplicates. The tannin contents in the methanolic extract of *Paederia linearis* Hook. f. root is calculated by using the formula:

$$\% \text{ Tannin} = \{(V\text{-}V_0) \times 0.004157 \times 250 \times 100\} / g \times 25$$

Where V and V_0 (mL) are the volume of 0.1 M aqueous solution of $KMnO_4$ for the titration of the sample and the blank, respectively, 0.004157 = tannins equivalent in 1 mL of 0.1 M aqueous solution of $KMnO_4$, g = mass (g) of the sample that taken for the measurement, 250 = volume (mL) of the volumetric flask and 100 is percentage (%).

Cytotoxicity bioassay

The cytotoxicities of all plant extracts were determined employing the colorimetric method as described by Brien et al. [25]. Briefly, cells at a logarithmic growth phase were harvested and diluted to 2.2×10^4 cells/mL for KB and 3.3×10^4 cells/mL for MCF-7 and NCI-H187. Solution (5 µL) was diluted with 5% DMSO. Portions 45 µL of cell suspension were distributed into 384-well plates, and incubated at 37 °C in 5% CO_2 incubator. After the incubation, each well were stained with 12 µL of 62.5 µg/mL resazurin, and the plates was then incubated at 37°C for 4 h. Fluorescence signal was measured using SpectraMax M5 multi-detection microplate reader (Molecular Devices, USA) at the excitation and emission wavelengths of 530 nm and 590 nm. The activity was expressed as % inhibition of cell growth which was calculated by the equation:

$$\% \text{ inhibition} = [1\text{-}(FUT / FUC)] \times 100$$

Where FU_T and FUC were the mean fluorescent unit from treated and untreated conditions, respectively. The positive substance, doxorubicin, exhibited cytotoxic activity against epidermoid carcinoma of the mouth (KB), small cell lung cancer (NCI-H187), and breast adenocarcinoma (MCF7) cells with IC50 values of 0.495, 0.108, and 8.87µg/mL, respectively. Water and 0.5% DMSO were used as negative control. Cytotoxicities of all extracts against Vero cells (African green monkey kidney cell line, ATCC CCL-81) were also evaluated using Green Fluorescent Protein (GFP)-based assay which was described by Hunt et al [26]. Elipticine (IC50 = 0.540 mg/mL) and 5% DMSO are used as a positive and negative control, respectively. The activity was calculated and expressed as % cell growth.

Statistical analysis

Experimental results were means ± SD of three parallel measurements and analyzed by SPPSS (Version 17.0). The IC_{50} values were calculated from linear regression analysis and all linear regression in this paper was processed and analyzed by computer program: Microsoft Excel 2007.

Results and discussion

Extraction and phytochemical screening

The crude extracts of *Paederia linearis* Hook. f. root were prepared and investigated for their physical characteristics and percentage of yields (% yields). The results of the color and consistency for the various test extracts of *Paederia linearis* Hook f. were depicted in Table 1. Brown wasthe color observed with the hexane and dichloromethane extracts while ethyl acetate and methanol were observed as reddish brown. The consistency of the various extracts was found to be oil and sticky in nature. The % yields of extraction was found to be maximum (22.76%) with methanol solvent while minimum (1.01%) with hexane. Preliminary phytochemical screening of the different fractions of this plant revealed that the maximum number of constituents such as, alkaloids, carbohydrates, proteins, antraquinones and amino acids were present in methanolic extract. Steroids and terpenoids were found to be present in hexane and dichloromethane extracts whereas were absent in methanol extract. The presence of saponins was also detected as the component in ethyl acetate and methanol extracts. Flavonoids and tannins were seem to be major constituent in methanolic extract and the results were given in Table 2. The presence of tannins, alkaloids and saponins can support the traditionally use of *Paederia linearis* Hook f. root in treating boils, shingles and snake bite healing. Furthermore, the presence of phenolic compounds in the plant extracts indicates that some extracts may have ability as an antioxidant and anti-cancer agents.

Table 1 Physical characteristics and % yield of *Paederia linearis H.* f. root extracts.

Solvent	Color of extract	Consistency	Sense of touch	Amount of extract (g)	% yield
Hexane	Brown	Oil	Sticky	25.28	1.01
Dichloromethane	Brown	Semisolid	Sticky	58.69	2.35
Ethyl acetate	Reddish brown	Semisolid	Sticky	149.81	5.99
Methanol	Reddish brown	Semisolid	Sticky	568.92	22.76

Antioxidation activities

Inhibition of β-carotene bleaching

This anti-oxidation assay is one of the rapid method to screen anti-oxidants. The method is mainly based on the opinion that linoleic acid,

an active unsaturated fatty acid, gets oxidized by reactive oxygen species produced from oxygenated water. The products formed will initiate the β-carotene oxidation. As a result, β-carotene will be oxidized and broken down in part; subsequently, the system looses its chromophore and characteristic orange color, which can be monitored spectrophotometrically. Thus, the anti-oxidation activity of the extracts were measured by the bleaching of β-carotene. The addition of the extracts, BHT and vitamin C at

Table 2 Phytochemical constituents of *Paederia linearis Hook* f. root extracts.

Constituents	Test	Extracts			
		Hex	DCM	EtOAc	MeOH
Carbohydrate					
General test	Molisch's test			–	– –
Free reducing sugars	Fehling's test	-	-		
Combined reducing sugars	Fehling's test	-	-	–	– –
Amino acids and proteins	Biuret test	-	-	-	
Tannins and phenolics	Ferric chloride test				– – –
	Gelatin test	-	-	-	– – –
	Iodine test	-	-	-	
	Nitric acid test	-	-	-	–
Alkaloids	Dragendorff's reagent	-	-		
	Mayer's reagent			–	–
	Wagner's reagent	-	-		
Anthraquinones	Bornträger's test				–
Saponins	Frothing	-	-		
Flavonoids	Shinoda's test	-	-	-	– – –
	Sodium hydroxide test	-	-	-	
Terpenoids	Liebermann-Buchard's test	–	+	–	-
Steroids	Liebermann-Buchard's test	-	+	–	-

– – – = very strong response; – – = strong response; + = moderate response; – = negative response

various concentrations prevented the bleaching of β-carotene to different degrees. The presence of different antioxidants can hinder the extent of β-carotene bleaching by neutralizing the linoleate-free radical and other free radicals formed in the system [27]. Figure 1 shows the antioxidant activity of the extracts in the comparison with those of BHT, and vitamin C. The antioxidant power decreased in order BHT > methanol extract > ascorbic acid > ethyl acetate extract > dichloromethane extract > hexane extract. The increased bleaching of β-carotene inhibition of all tested sample were seem to be correlated with concentrations. In comparison, the methanol extract exhibited relatively significant antioxidant effect, while standard vitamin C showed lower antioxidant activity than that of methanol extract probably due to ascorbic acid remaining in the aqueous phase of the emulsion are quite diluted in lipid phase thus less effective to protect the linoleic acid. The 50% inhibition (IC_{50}) were accomplished with 33.71 ± 0.98 mg/mL of BHT, 48.25 ± 0.07 mg/mL of vitamin C and 36.20 ± 0.16 mg/mL of methanol extract.

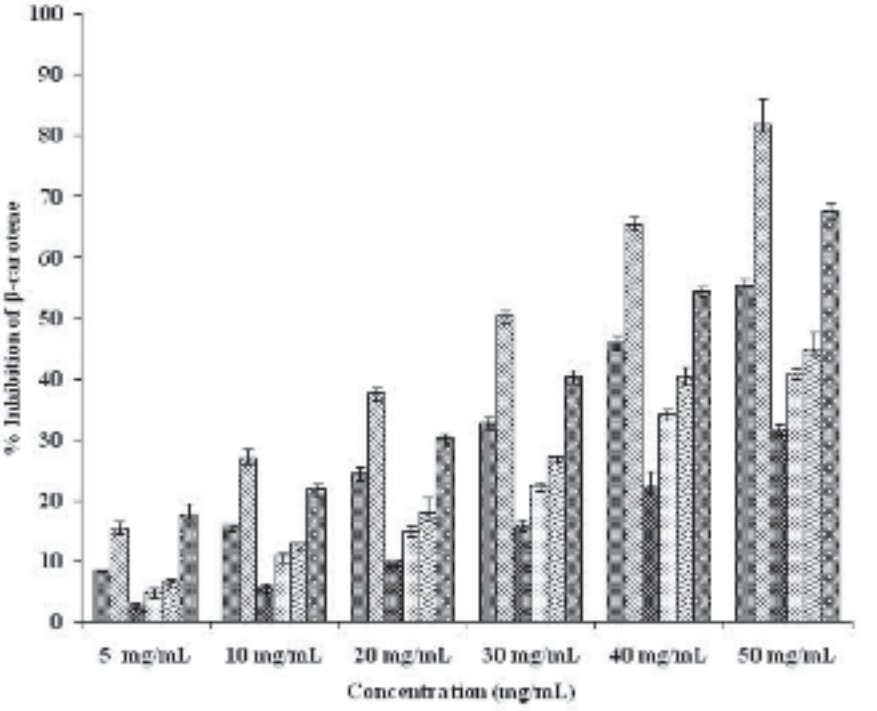

Figure 1 Effect of vitamin C, BHT and all extracts of *Pseuduvaria trimera* (Craib) Airy Shaw leaves on the inhibition of β-carotene bleaching. Values are presented as mean ± SD, (n=3).
ascorbic acid; BHT; hexane extract; dichloromethane extract; ethyl acetate extract; methanol extract.

Inhibition of DPPH free radicals

The roots extracts of *Paederia linearis* Hook. f. showed effective DPPH radical scavenging activity as shown in Figures 2. The scavenging effect increased with increasing concentration of the extracts. The IC_{50} values of hexane, dichloromethane, ethyl acetate and methanol extracts were 625.02 ± 0.21, 551.26 ± 0.45, 596.73 ± 0.98 and 37.52 ± 0.13 µg/mL, respectively. The methanol extract showed significant highly antioxidant activity as compared to all other extracts. However, scavenging activity of vitamin C, a known antioxidant, used as a reference standard for the DPPH free radicals scavenging assay; it significantly inhibits DPPH free radicals with the IC_{50} value of 7.36 ± 1.31 µg/mL, was relativelymore pronounced than that of other extracts (Figure 2).

The comparison of antioxidant activities of all crude extracts showed that the methanol fraction was grater thane other extracts from above tested methods. Thus, this fraction was selected to use for determination of reducing power ability and estimation of total phenolics, total flavonoids, and tannin contents.

Reducing power ability

The measurement of the reducing ability, detection of Fe3+-- Fe2+ transformation in the presence of methanol extract was investigated. As shown in Figure 3, the reducing power of methanolic fraction and the standard agents increased with an increased in their concentration. At concentration 400 µg/mL, methanol extract (1.29 ± 0.05) showed greater absorbance than vitamin C (0.68 ± 0.02) while weaker than BHT (2.93 ± 0.6). BHT showed the excellent reducing power compared with methanol extract and vitamin C in all tested concentrations. Methanol extract exhibited a higher reducing power than ascorbic acid, suggesting that the methanol extract had stronger electron-donating capacity.The reducing power of methanol extract of *Paederia linearis* Hook. f. root was found to be increase as a linear relationship (R^2 = 0.9303; y = 0.3022x – 0.3664) with a increasing its concentration. The antioxidant activity has been reported to be concomitant with development of reducing power [28]. Hence, reducing capacity of the methanolic extract served as a indicator of its moderate antioxidant activity.

Total phenolics, total flavonoids and tannin contents

It has been recognized that phenolic acids and flavonoids showed antioxidant activity and related effects on human nutrition. The strong response of the presence of phenolic, flavonoid and tannin constituents from the preliminary phytochemical analysis including having of higher antioxidant activity when compared with the crude extracts of hexane, dichloromethane and ethyl acetate prompted us to analyze the quantitative of total phenolics, total flavonoids and tannins. The phenolic contents was measured in term of gallic acid equivalent (the standard curve equation; y = 0.010x + 0.052, R^2 = 0.997). The content of phenolic compounds in methanolic extract was 51.27 ± 0.13 mg gallic acid equivalent/g. The flavonoid amount of the methanolic extract was determined from the calibration curve established by quercetin at 415 nm. The regression line was y = 0.006x - 0.007, R^2 = 0.991. The value was found to be 36.41 ±1.28 mg quercetin equivalent /g plant extract. In addition, the percentage of tannin contents of methanol extract was also determined

using titrimetric method. The value was found to be 9.81 ± 1.85 %. These results suggested that the greater levels of antioxidant activity of methanolic fraction compared to hexane, dichloromethane and ethyl acetate extracts were due to the presence of highly phenolic contents including flavonoids and tannins.

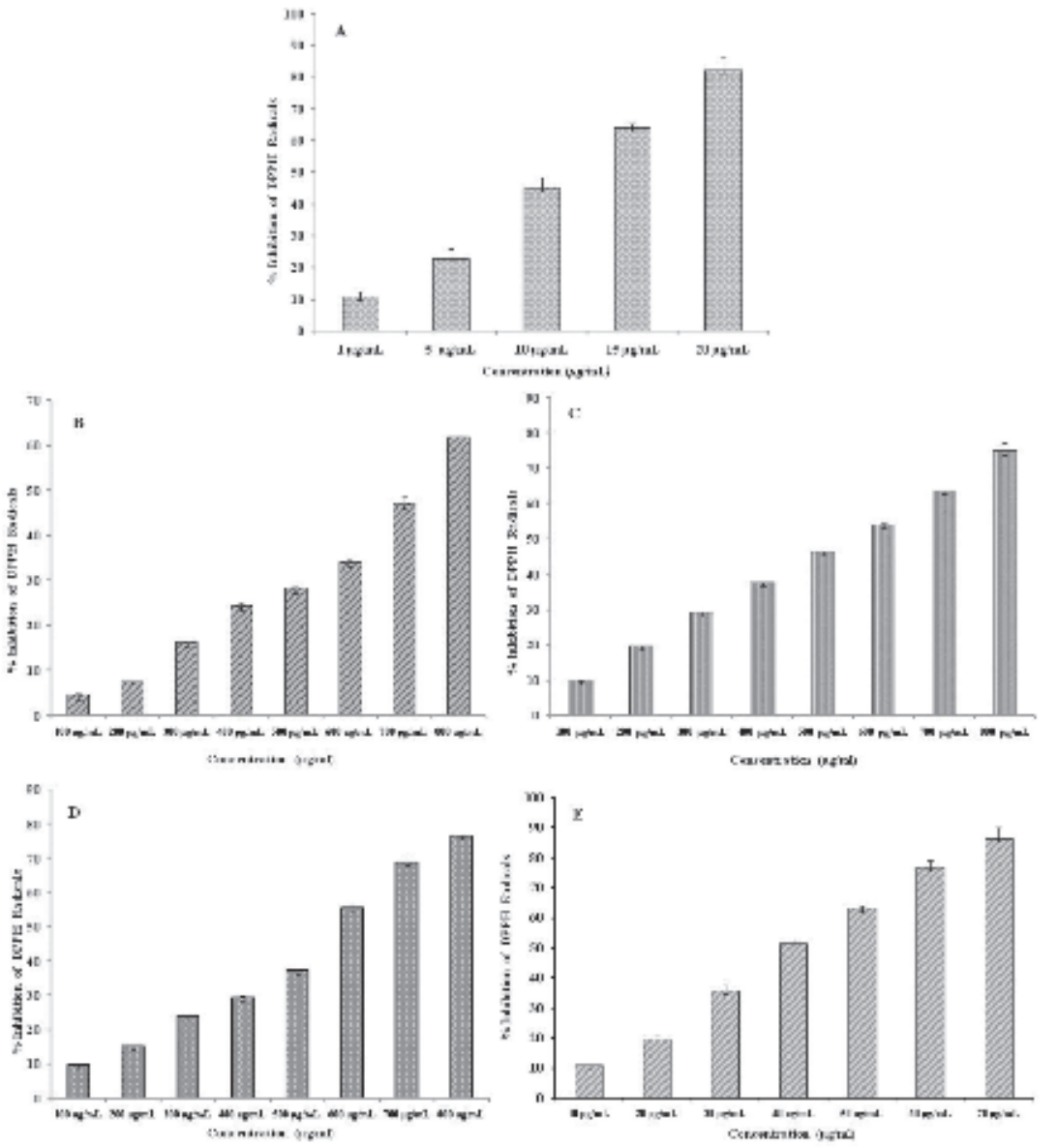

Figure 2. DPPH free radicals scavenging activity of standard vitamin C and crude extracts; A) vitamin C, B) hexane, C) dichloromethane, D) ethyl acetate and E) methanol. Each value is presented as mean ± S.D (n=3).

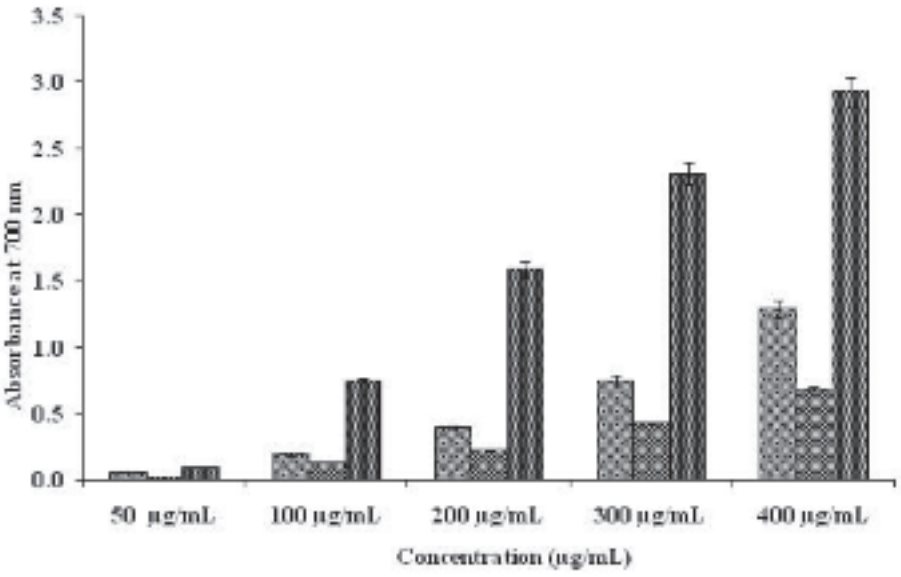

Figure 3. Reducing power of methanol extract, vitamin C and BHT on Fe^{3+}--Fe^{2+} transformation. Values are presented as mean ± SD, (n=3), methanol extract; ascorbic acid; BHT.

Cytotoxic effects

The hexane, dichloromethane, ethyl acetate, and methanol crude extact were evaluated in vitro for their cytotoxicity against KB, NCI-H187, and MCF7 cell lines and the results were summarized in Table 3. The results showed that all the extracts exhibited % inhibition values less than 50% against KB and MCF-7 cells and were consequently considered to be inactive. The hexane, dichloromethane, and ethyl acetate extracts were inactive to inhibit cell growth of small cell lung cancer NCI-H187. Interestingly, the methanolic extract exhibited moderate cytotoxic activity against NCI-H187 cell line with % inhibition value of 60.20%. This results indicated that methanolic extract contained the selective cytotoxic constituents. The having cytotoxicity of methanolic extract was due to the presence of basic potential antioxidant molecules including tannins, alkaloids, saponins, and flavonoids which were proved to be phytochemical constituents of *Paederia linearis* Hook. f. root. However, the components responsible for the cytotoxic activity of the plant are not clear. Therefore, further work is necessary to isolate and characterize those constituents.

The cytotoxicities of all extracts against *Vero cells* of American green monkey kidney cells were also evaluated using GFP-base method. The results showed that the extracts were non-cytotoxic with % cell growth more than 50% (hexane (86.86%), dichloromethane (88.86%), ethyl acetate (106.36%) and methanol (166.27%)).

Table 3 The results of cytotoxic activities against KB, NCI-H187 and MCF7 of *Paederia linearis Hook* f. root extracts.

Extracts	Activity		
	KB	NCI-H187	MCF7
Hexane	Inactive	Inactive	Inactive
Dichloromethane	Inactive	Inactive	Inactive
Ethyl acetate	Inactive	Inactive	Inactive
Methanol	Inactive	Active*	Inactive

Inactive at % inhibition < 50 , Active at % inhibition > 50, * % Inhibition = 60.2

The non-toxicities of all extracts suggested that *Paederia linearis* Hook. f. root is safe for using as folkloric drug.

Conclusion

The results obtained in the study represented that the root extracts of *Paederia linearis* Hook f. contain various types of phytochemical compounds. Among the extracts, methanolic extract showed remarkable antioxidant activity related to the containing of phenolics, flavonoids and tannins. These results supported the use of *Paederia linearis* Hook f. root as the folkloric drugs. Methanol extract also showed selective inhibition of NCI-H187 cancer cells with non-cytotoxic property to Vero cells. The root of *Paederia linearis* Hook f. may be the good source of an effective and safe natural antioxidants and anti-cancer agents. However, further work is necessary to isolate, characterize its bioactive components and also evaluate for their various biological activities.

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