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ORIGINAL ARTICLE

Evaluation of Free Radical Scavenging with *in vitro* Antiproliferative Properties of Different Extracts of *Pluchea lanceolata* (DC.) Oliv. and Hiern in Cancer Cell Lines

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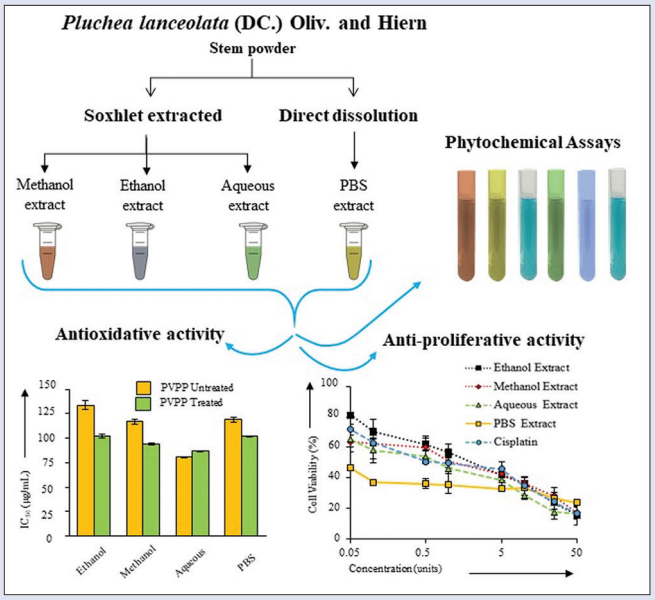
ABSTRACT

Background: Traditional medicinal plants have gained attention as a repository of pharmacologically active molecules. Extracts derived from *Pluchea lanceolata* (DC.) Oliv. and Hiern are reported to be antimalarial and can protect against chemical-induced neurotoxicity. There is limited research on solvents for extraction of metabolites from stem of *P. lanceolata* and their anti-cancer potential, which needs to be investigated. **Objectives:** The objective of the study was to investigate potency of stem powder, extracted in ethanol, methanol, aqueous, and phosphate buffer saline (PBS) solvents, for their phytochemical content, antioxidant potential, and *in vitro* antiproliferative nature in human cancer cell lines. **Materials and Methods:** The stem extracts of *P. lanceolata* were evaluated by phytochemical assays, antioxidant assays (2,2-diphenyl-1-picrylhydrazyl [DPPH] radical scavenging assay, hydrogen peroxide radical scavenging assay, nitric oxide radical scavenging assay, total antioxidant capacity, and assay of reducing power), and antiproliferative potential against cervical (ME-180 and HeLa) and hepatic (HepG2) carcinoma cell lines by MTT assay. **Results:** Quantification studies showed that the total phenolic content was in the range 7.44–38.91 mg GAE/g of stem extract, while the flavonoids were present in the range 29.05–109.62 mg QE/g of stem extract. Aqueous (DPPH antioxidant capacity assay$^{\pm PVPP}$, H_2O_2 free radical scavenging method^{-PVPP}, assay of reducing power^{+PVPP}, and total antioxidant capacity^{-PVPP}), methanol (H_2O_2 free radical scavenging method^{+PVPP}, NO radical scavenging assay^{+PVPP}, total antioxidant capacity^{-PVPP}), and ethanol (NO radical scavenging assay^{-PVPP}, assay of reducing power^{PVPP}) extracts had the highest antioxidant potential in respective assays. MTT findings demonstrated that the aqueous extract was more potent in ME-180 and HepG2 cell lines while the PBS extract caused maximal cytotoxicity in HeLa cells. HepG2 cells were more susceptible than ME-180 and HeLa cells for any of the extract or standard drug evaluated. **Conclusion:** Aqueous extract of *P. lanceolata* stem is the most promising extract for further cancer-cell toxicity.

Key words: Antioxidant, anti-proliferative, cancer cell lines, cisplatin, phytochemicals, polyvinylpolypyrrolidone

SUMMARY

- Methanol, ethanol, aqueous and phosphate buffer saline extracts of *Pluchea lanceolata* exerts antioxidant and anti-proliferative activity in a dose-dependent manner. Aqueous extract presented the most promising results on cancer cell lines and should be preferred extract for *in vitro, in vivo* and clinical studies.



Abbreviations used: DPPH: 2,2-diphenyl-1-picrylhydrazyl; PBS: Phosphate Buffer Saline; PVPP: Polyvinylpolypyrrolidone; GAE: Gallic Acid Equivalent; QE: Quercetin equivalent

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INTRODUCTION

Treatment of relapsed and metastasized cancers is a challenge. It routinely involves multiple cycles of surgery, chemo-and/or radio-therapy. The patients experience side effects such as colitis, hepatitis, and gastrointestinal toxicity which may become lethal.[1] At advanced stages, when chemotherapy and radiation become redundant, the nature of treatment shifts to palliative to improve the quality of remaining life.[2] Therefore, development of novel drugs for treatment with minimal side effects is urgent requirement for cancer patients. A survey of usage of

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clinically approved anticancer drugs between 1981 and 2006, in North America, Europe, and Japan has revealed that 47.1% (of 155) drugs were of either natural origin or derived from them or synthesized chemically by knowledge of their structure.[3] Considering the ongoing research on various plants to identify promising bioactive for different cancers, it is sensible to look at plants used in folk medicine.

Traditionally, *Pluchea* genus (Family: Asteraceae) has been used for treating various maladies in folk medicines including the Mayan civilization.[4,5] *Pluchea indica* (L.) Less has been reported to have multiple health beneficiary effects. Its tea preparation is commonly consumed in Southeast Asia and is reported to ameliorate hyperglycemia, dyslipidemia, and obesity.[6,7] Pretreatment with ethanol extract of its leaf (*P. indica* leaf ethanol extract [PLEE]) in streptozotocin-induced diabetic BALB/c mice prevented severity of liver damage and cytokine-induced β-cell apoptosis.[8,9] Further, nanoparticles prepared from leaf extract displayed higher cytotoxity and migration in buccal mucosa carcinoma cells (HO-1-N-1).[10] These may be attributed to the presence of high antioxidant levels.[11,12] PLEE and root extract prevent inflammation by reducing nuclear translocation of p65 subunit of NFkB[13,14] or 5-lipooxygenase pathway.[15] The hexane fraction of root extract of *P. indica* arrests glioblastoma cells by inducing autophagy.[16] Apoptosis by inducing p53-dependent pathway has been reported in glioma cell line GBM8401 and cervical carcinoma cell line HeLa using crude aqueous extracts of leaf and root extracts,[17] and in NPC-TW 01 and NPC-TW 04 using ethanolic extracts of roots.[18] Quinic acid esters are reported to inhibit metastasis associated proteins collagenase and metalloproteinase-2 and-9.[18] Beta-sitosterol and stigmasterol can neutralize viper venom-induced lethal, hemorrhagic, defibrinogenation, edema, and PLA (2) activity.[19]

Pluchea odorata (L.) Cass. is traditionally used medicinal plant of Central America, and its eudesmanes can arrest HL-60 growth and tumor invasiveness *in vitro*,[20] and reduce MCF-7 spheroid intravasation.[21] Fractions of dichloromethane extract of *P. odorata* were genotoxic in HL-60 and MCF-7.[22] Ethanol extracts of *P. carolinensis* have antileishmanial activity.[23]

Pluchea sagittalis (Lam.) Cabrera is used in folk medicine in South America. Ethanolic extracts of aerial parts act as antinociceptive and gastroprotective,[24] while the aqueous extract[25] and dichloromethane extract[26] possess anti-inflammatory properties in mice-model.[22] *P. lanceolata* (DC.) Oliv. and Hiern, is an important plant used in preparations of Indian Ayurvedic medicinal system. Decoctions prepared from it are recommended. The active compounds of *P. lanceolata* are pluchine, sorghumal, boehmerol acetate, moretenol, taraxasterol, neolupenol, pluchioside, sesquiterpene, pluchiol, stigmasterol, quercetrin, and isorhamnetin.[27] The methanolic extract of total plant parts of *P. lanceolata* suppresses the oxidative stress and gross chromosomal damage stimulated by cadmium chloride.[28] *P. lanceolata* extract attenuates oxidative stress caused by ferric nitrilotriacetate.[29] Hydroalcoholic extract of *P. lanceolata* rhizome can restore cognitive function in rats with ischemic hippocampal injury by normalization of glutathione peroxidase levels in brains.[30]

Based on the research cited above, a systematic evaluation and comparison of multiple extracts prepared directly from *P. lanceolata* stem on multiple cancer cells have not been carried out. Through this research, we hereby describe the cytotoxic properties of extracts of *P. lanceolata* in cancer cell lines of ME-180, HeLa, and HepG2.

MATERIALS AND METHODS

Preparation of extracts

Powder of *P. lanceolata* (stem) was collected from Maharashtra Arogya Mandal, Pune, India and stored in airtight containers. Extracts preparation in ethanol (MB106, HiMedia, Mumbai, India), methanol (AS058, HiMedia, Mumbai, India), or distilled water was as described previously.[28] In brief, *P. lanceolata* stem powder was extracted with solvent (ethanol, methanol or water) by conventional Soxhlet (4951, Goel Scientific, Vadodara, India) extraction at the temperature of 60°C. The extracts were dried by rotary evaporator (Aditya Scientific, Hyderabad, India) or the extract was concentrated using concentrator (5305000304, Eppendorf India Pvt. Ltd, Chennai, India). The extracted powder was stored in air-tight pack at room temperature. The phosphate buffer saline (PBS) extract was prepared by mixing dried plant powder in PBS or cell culture media.

Phytochemical screening

Qualitative occurrence of secondary metabolites-alkaloids, saponins, tannins, phenols, glycosides, terpenes, carotenoids, and quinones was detected as described previously.[31] Mayer's test for alkaloids was performed by treating equivalent volumes of extract with Mayer's reagent, and development of cream colored precipitate implied existence of alkaloid. Dragendorff's test for alkaloids was accomplished by treating equivalent volumes of extract with Dragendorff's reagent, and generation of red-colored precipitate suggested presence of alkaloid. Wagner's test for alkaloids was performed by treating equivalent volumes of extract with Wagner's reagent, and development of reddish-brown colored precipitate indicated existence of alkaloid. Hager's test for alkaloids was performed by treating equivalent volumes of extract with Hager's reagent, and development of yellow coloured precipitate suggested presence of alkaloid. Saponin was detected by dissolving equivalent quantity of extract in water followed by vigorous shaking. Formation of honeycomb-shaped persistent froth indicated the existence of saponins in the sample. Tannins were determined by mixing extract with 0.5% ferric chloride (GRM165-500G, HiMedia, Mumbai, India) and dark green/bluish green coloration of sample indicated the presence of tannins. Phenols were determined by adding equivalent volumes of extract to Folin Ciocalteu's reagent (RM10822, HiMedia, Mumbai, Maharashtra, India) and blue coloration of sample indicated the presence of phenols. Glycosides were identified by treating equivalent volumes of extract with glacial acetic acid (AS001, HiMedia, Mumbai, India) and some drops of 5% ferric chloride ($FeCl_3$) and concentrated sulfuric acid (H_2SO_4) (AS016-500ML, HiMedia, Mumbai, India). Reddish-brown coloration at the confluence and bluish green color in top layer solution indicated presence of glycosides. Terpenes were detected by mixing equivalent volumes of extract with chloroform and concentrated sulfuric acid. Reddish-brown coloration at junction of two solutions suggested the occurrence of terpenes. Steroids were detected by formation of orange color in solution consisting of equivalent volumes of extract with chloroform, glacial acetic acid and concentrated sulfuric acid. The presence of quinone was determined by the formation of green color upon addition of concentrated hydrogen chloride (RM5955-500ML, HiMedia, Mumbai, India) to the extract.

Removal of polyphenols from plant extracts using polyvinylpolypyrrolidone

The extracts were treated with equal amount of polyvinylpolypyrrolidone (PVPP) (PCT1003-100G, HiMedia, Mumbai, India) prepared in respective solvents and kept on shaking

incubator (CIS-24 PLUS, REMI, Mumbai, India) overnight.[32] The polyphenol-free supernatant was used for further experiments.

Quantification of phenols

The phenolic content was quantified as per the method given earlier.[32] Briefly, 1 ml of extract or standard gallic acid (GRM233, HiMedia, Mumbai, Maharashtra, India) solution with different concentrations was intermixed with 1N Folin-Ciocalteu reagent of 0.5 ml. The solution was kept for 5 min, 1 ml of 20% sodium carbonate (GRM1189, HiMedia, Mumbai, India) solution was added, incubated for 10 min at room temperature, followed by measuring absorbance at 730 nm using ultraviolet-visible (UV-Vis) spectrophotometer (UV-1800, Shimadzu Analytical (India)).

Quantification of flavonoids

Flavonoid content was determined as given previously.[33] Equivalent volumes of extract or standard solution of Quercetin (551600-M, Sigma – Aldrich, MO, USA) with the concentration range of 100–500 µg/ml were intermixed with distilled water and 5% sodium nitrite (GRM3959, HiMedia, Mumbai, India) solution, incubated for 5 min, and 150 µl of 10% aluminium chloride (206911, Sigma-Aldrich, MO, USA) solution was added. After incubation of 5 min, 500 µl of 1M sodium hydroxide (GRM1183-500G, HiMedia, Mumbai, India) and 275 µl of distilled water were added, mixed, and read at 510 nm using UV-Vis spectrophotometer.

2,2-diphenyl-1-picrylhydrazyl free radical scavenging capacity

Antioxidant activity by 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay was measured with spectrophotometric method as described previously.[34] In brief, 1 ml DPPH (RM2798, HiMedia, Mumbai, India) prepared in ethanol was added to 0.5 ml extract solution or ascorbic acid (TC094-100G, HiMedia, Mumbai, India) of concentrations from 0 µg to 100 µg, and volume was trumped up to 2 ml using methanol and incubated for 30 min. The absorbance of prepared solution was taken at 517 nm against blank. The antioxidant activity was presented as IC_{50} value (µg/ml) based on percentage of inhibition of DPPH as calculated below.

$$\text{Percent scavenging activity} = \frac{\left(\left(A_{\text{control}} - A_{\text{sample}}\right) \times 100\right)}{A_{\text{control}}} \quad (1)$$

Where, $A_{control}$ is absorbance reading of control solution and A_{sample} is absorbance reading of sample solution measured at given wavelength.

Hydrogen peroxide free radical scavenging assay

Antioxidant effect of hydrogen peroxide (H_2O_2) was evaluated as described previously.[35] In brief, 1 ml extract solution or ascorbic acid of concentration ranging from 0 µg to 100 µg was treated with 0.6 ml, 40 mM hydrogen peroxide (H325-500, Fisher Scientific, NH, USA) prepared in PBS (pH 7.4). Absorbance was read at 230 nm against blank. Antioxidant capacity was represented as IC_{50} value (µg/ml) based on percentage of inhibition of hydrogen peroxide as calculated by Equation 1.

Nitric oxide radical scavenging assay

The scavenging effect was evaluated as described previously.[36] Briefly, 10 mM sodium nitroprusside (RM987-100G, HiMedia, Mumbai, India) in PBS (pH 7.4) with plant extract or ascorbic acid of concentrations of 0 µg/mL to 100 µg/mL was incubated at 25°C for 5 h. Next, 0.5 ml of supernatant was aspirated, and 0.5 ml 1 mM Griess reagent (G7921, Thermo Fisher, Massachusetts, USA) prepared in distilled water was added. The absorbance of the solution was read at 546 nm. Antioxidant capacity was represented as half maximal inhibitory concentration, i.e., IC_{50} value (µg/ml).

Total antioxidant capacity

The total antioxidant capacity was established spectrophotometrically.[37] Briefly, 1 ml plant extract or ascorbic acid of concentration from 0 to 100 µg and 1 ml of reagent containing 0.6M sulfuric acid, 28 mM sodium phosphate (MB047-250G, Thermo Fisher, Massachusetts, USA), and 4 mM ammonium molybdate (A7302-100G, Sigma-Aldrich, MO, USA) was prepared. The solution was heated at 95°C for 90 min, cooled to room temperature, and measured at 695 nm against blank. Antioxidant capacity was presented as IC_{50} value (µg/ml).

Assay of reducing power

The assay of reducing power was determined spectrophotometrically.[38] Briefly, equivalent volumes (2.5 ml) of extract solution or ascorbic acid of concentrations 0–100 µg/ml, 0.2M PBS (pH 6.6), and 1% potassium ferricyanide (GRM627-500G, Himedia Labs, Mumbai) was mixed, heated at 50°C for 20 min, cooled, 2.5 ml of trichloroacetic acid (GRM6274-500G, HiMedia, Mumbai, Maharashtra, India) (w/v) was added, and centrifuged at 3000 rpm for 10 min. The upper layer of the solution was taken to which equivalent volume of methanol and one-fifth of its volume of 0.1% ferric chloride solutions were added and read at 700 nm. Antioxidant capacity was represented as IC_{50} value (µg/ml).

Cell culture and *in vitro* cell viability

Authenticated cell lines (ME-180, HeLa and HepG2) were bought from National Centre for Cell Science, India. The cells were grown in RPMI, MEM and DMEM media (HiMedia, Mumbai, India), respectively, and supplemented with 10% FBS (16000044, Thermo Fisher, Massachusetts, USA) and 1% antibiotic solution (A002A, HiMedia, Mumbai, India). The cells were passaged using trypsin-EDTA (TCL007, HiMedia, Mumbai, Maharashtra, India) upon 80%–90% confluence. Cells were observed daily for morphology and the presence of any contaminant. For antiproliferative assay, cells were seeded in a 96 well plate with density of 5×10^4 cells/well and incubated overnight at 37°C in 5% CO_2 incubator. The cells were treated with extract having concentration range of 0–50 mg/ml or 0–50 µM Cisplatin and left overnight in the incubator. Next day, 5 mg/ml of MTT (219459291, MP Biomedicals, California, USA) was added per well and incubated for 2 h at 37°C. Formazan crystals hence formed, were solubilized by adding 100 µL DMSO (TC185, HiMedia, Mumbai, India) and incubating for 10 min at room temperature. The absorbance was taken at 570 nm and reference was set at 630 nm.

Statistical analysis

All experiments were performed in triplicate. All the values were expressed as mean ± standard error of mean. The data were analyzed by Student–Newman–Keuls test using SigmaPlot version 14 (Systat software Inc., US) and IC_{50} values were calculated using OriginPro, version 2021 (OriginLab Corporation, Northampton, MA, USA).

RESULTS AND DISCUSSION

Phytochemical analysis

All evaluated extracts showed the presence of secondary metabolites [Table 1]. Alkaloids (only by Dragendorff's assay), terpenes, phenols, and flavonoids were present in all the extracts. Steroids, quinones, and carotenoids were absent in all the extracts evaluated.

Table 1: Phytochemical screening of *Pluchea lanceolata* extracts

Assay	Extract			
	Aqueous extract	Ethanol extract	Methanol extract	PBS extract
Mayer's test for alkaloids	–	–	–	–
Dragendorff's test for alkaloids	+	+	+	+
Wagner's test for alkaloids	–	–	–	–
Hager's test for alkaloids	–	–	–	–
Saponins	+	–	–	–
Tannins	+	–	+	–
Phenols	+	+	+	+
Glycosides	–	+	–	–
Flavonoids	+	+	+	+
Terpenes	+	+	+	+
Steroids	–	–	–	–
Quinones	–	–	–	–
Carotenoids	–	–	–	–

For all the experiments (n=3). PBS: Phosphate buffer saline. +: Present; -: Absent

Table 2: Quantification of total phenolic and flavonoid content of *Pluchea lanceolata* extracts

Extracts in solvent	PVPP	Total phenolic content (mg GAE/g of plant extract)	Total flavonoid content (mg QE/g of plant extract)
Ethanol	–	38.91±0.13[a,b,c,d]	82.96±0.77[a,b,c,d]
	+	7.44±0.67	38.77±2.09
Methanol	–	20.37±0.13[a,e,f]	109.62±0.37[a,e,f]
	+	7.60±0.40	31±1.6
Aqueous	–	28.77±0.03[a,g]	51.40±0.36[a,g]
	+	7.71±0.40	29.88±2.5
PBS	–	18.70±0.21[a]	34.62±1.26[a]
	+	8.46±0.58	29.05±1.27

[a-g]Column wise values with different superscripts indicate significant difference (P<0.001). [a]Between: PVPP and + PVPP for same solvent, [b-g]For: PVPP for different solvents, [b]Between ethanol and PBS, [c]Between ethanol and methanol, [d]Between ethanol and aqueous, [e]Between methanol and aqueous, [f]Between methanol and PBS, [g]Between aqueous and PBS. Expressed as mean±SEM (n=3). SEM: Standard error of mean; GAE: Gallic acid equivalent; QE: Quercetin equivalent; PBS: Phosphate buffer saline; PVPP: Polyvinylpolypyrrolidone. +: Present; -: Absent

Aqueous extract had exclusive presence of saponins, while ethanol extract had the presence of glycosides.

Results for quantification of phenolic content were computed using standard gallic acid calibration curve. Whole ethanol extract had the maximum phenolic quantity (38.91 ± 0.13 mg GAE/g of stem extract) followed by aqueous extract (28.77 ± 0.03 mg GAE/g of stem extract) [Table 2]. In contrast, PVPP-induced depletion shows maximal phenolic (8.46 ± 0.58 mg GAE/g of stem extract) presence in PBS extract, while other extracts had similar levels (~7.44–7.71 mg GAE/g of stem extract) of phenolic content. Total flavonoid content was computed from linear calibration curve of quercetin. Methanol and ethanol (109.62 ± 0.37 and 82.96 ± 0.77 mg QE/g of stem extract, respectively) extracts had maximum content of flavonoids in the absence and presence of PVPP, respectively, while PBS extract had the least of flavonoids in PVPP untreated (34.62 ± 1.26 mg QE/g of stem extract) and treated (29.05 ± 1.27 mg QE/g of stem extract) systems. Here, our phytochemical analyses on *P. lanceolata* suggest its potential for biological evaluation.

Antioxidant activity

The presence of phenols and flavonoids in all extracts, led to the estimation of antioxidant activity in each of the extract for both pre-and post-PVPP systems.

DPPH free radical scavenging activity

All extracts had significant dose-dependent DPPH free radical scavenging activity (methanol extract, P < 0.050, other extracts P < 0.001). The aqueous extract of *P. lanceolata* had the most effective DPPH antioxidant capacity (IC_{50} values of 80.60 ± 0.53 µg/mL and 86.66 ± 0.73 µg/mL for pre-and post-PVPP treatment, respectively) [Table 3 and Figure 1a]. Least DPPH activity was observed in ethanol and PBS extracts. Interestingly, ascorbic acid had nearly equivalent or less DPPH activity as compared extracts tested.

Hydrogen peroxide activity

All the extracts had significant dose-dependent hydrogen peroxide-free radical scavenging activity (P < 0.001). Aqueous and methanol extracts had most effective hydrogen peroxide scavenging activity in PVPP untreated and treated systems (IC_{50} of 50.36 ± 2.91 and 80.94 ± 0.48 µg/mL, respectively) [Table 3 and Figure 1b]. Minimal hydrogen peroxide activity was observed in methanol and ethanol (IC_{50}-75.78 ± 1.30 and 100.43 ± 2.01 µg/mL, respectively) in PVPP untreated and treated systems.

Nitric oxide scavenging activity

All the extracts except aqueous extract demonstrated a significant dose-dependent nitric oxide scavenging activity (P < 0.001). Ethanol (IC_{50}= 84.41 ± 2.33 µg/mL) and methanol extracts (IC_{50}= 80.97 ± 0.78 µg/mL) of *P. lanceolata* showed significant free radical scavenging activity in PVPP untreated and treated systems [Table 3 and Figure 1c].

Assay of reducing power

All the extracts of *P. lanceolata* showed concentration-dependent increase in the reducing power (P < 0.001). Ethanol (IC_{50}-81.06 ± 1.09 µg/mL, PVPP untreated) and aqueous (IC_{50}= 67.52 ± 1.731 µg/mL, PVPP treated) extracts of *P. lanceolata* showed significant free radical scavenging activities [Table 3 and Figure 1d]. Ascorbic acid had low reducing power than the extracts.

Total antioxidant capacity

All the extracts had significant dose-dependent Total antioxidant capacity (methanol P < 0.001, others P < 0.05). The methanol (86.49 ± 0.27 µg/mL, PVPP untreated) and aqueous extracts (74.24 ± 0.65 µg/mL, PVPP treated) had significant total antioxidant activity compared to others [Table 3 and Figure 1e].

Table 3: IC_{50} (µg/mL) values of antioxidant assay of *Pluchea lanceolata* extracts

	DPPH free radical scavenging activity		Hydrogen peroxide assay		Nitric oxide scavenging activity		Assay of reducing power		Total antioxidant capacity	
	–	+	–	+	–	+	–	+	–	+
PVPP										
Ethanol	133.81±4.36***	102.16±1.83	63.03±1.20***	100.43±2.01	84.41±2.33**	95.35±1.11	81.06±1.09*	78.31±0.25	92.97±0.70***	78.41±1.44
Methanol	117.18±2.59***	93.83±0.97	75.78±1.30**	80.94±0.48	95.36±0.49***	80.97±0.78	86.49±1.34***	94.03±0.91	86.49±0.27***	90.40±0.53
Aqueous	80.60±0.53*	86.66±0.73	50.36±2.91***	98.63±1.75	96.00±1.34***	82.06±0.82	91.90±0.34*	67.52±1.73	111.76±1.49***	74.24±0.65
PBS	118.91±2.83***	101.68±0.71	71.15±1.44***	81.00±0.97	101.47±3.41	97.86±1.43	90.50±0.88***	97.21±0.87	100.58±0.54**	92.93±2.20
Ascorbic acid	159.25±4.10***	100.43±0.94	64.50±0.51***	99.15±1.48	100.10±0.60***	82.89±0.40	90.69±1.77***	101.50±1.36	104.26±0.62*	100.31±1.40

+: Present; -: Absent. Results were expressed as mean±SD (n=3) and analysed using Student's t-test between PVPP untreated and treated groups for test of significance wherein *, ** and *** represent statistical significance of P<0.05, P<0.01 and P<0.001 respectively. SD: Standard deviation; PVPP: Polyvinylpolypyrrolidone; PBS: Phosphate buffer saline; DPPH: 2,2-diphenyl-1-picrylhydrazyl

Based on the evaluation of extracts by different antioxidant assays, we found that methanol and aqueous extracts had the maximal antioxidant activities. Antioxidant assays on entire extracts showed the presence of metabolites other than phenols which impart free radical scavenging activity. In comparison to routinely used standard (ascorbic acid), most of the extract possessed higher antioxidant capacity. In addition, the PVPP-induced phenol quenching resulted in improved scavenging activity in few extracts. This attributes to certain bioactive that may have been sequestered from reaching higher antioxidant potential. Considering purification process may lead to misjudging the most suitable extract with antiproliferation capacity, and to confirm the suitability of these extracts over other extracts, we evaluated all the extracts in entirety for potential cytotoxic activity in cancer cells.

Pluchea lanceolata extracts have anti-proliferative potential

Cervical cancer cell lines ME-180, HeLa, and hepatic cancer cell line, HepG2 were treated with stem extracts (ethanol, methanol, aqueous, and PBS) for 24 h. The MTT results showed that the aqueous extract was more potent than ethanol, methanol, and PBS extract for ME-180 (1.18 ± 0.12 mg/ml) and HepG2 (0.004 ± 0.0039 mg/ml) cell lines, while PBS extract was the most cytotoxic in HeLa (3.43 ± 0.12 µg/ml) than other extracts [Figure 2 and Table 4]. Except for the results of HeLa treated with PBS extract, HepG2 was the most sensitive cell line to the *P. lanceolata* extracts to undergo apoptosis.

Though our antioxidant assays suggested methanol and aqueous extracts had maximal antioxidant potential, *in vitro* cytotoxicity showed PBS extract is most potent against HeLa, while aqueous was effective against ME-180 and HepG2. We can conclude that, of all the extracts evaluated, aqueous extract is the most suitable for further evaluation. However, it is noteworthy that HeLa had maximal cytotoxicity by PBS extract. The underlying reason may be differential presence of cytotoxic/bioactive agent in ethanol and aqueous extracts, which is common in plant based research.[39]

CONCLUSION

Pluchea genus is used in traditional medical practices for the management of various diseases. Our study correlated antioxidant potential of extracts of stem of *P. lanceolata* with cancer cell toxicity. Broadly, aqueous extract is most suitable candidate for future research. This work orients the direction to which solvent-based extracts of *P. lanceolata* ought to be used for isolation of bioactive molecules from the plant. However, further investigation corroborating the antitumor effects in *in vivo* systems is warranted. Further, isolation of bioactive responsible to cancer cell apoptosis may be determined as performed for *P. odorata* and *P. indica*.

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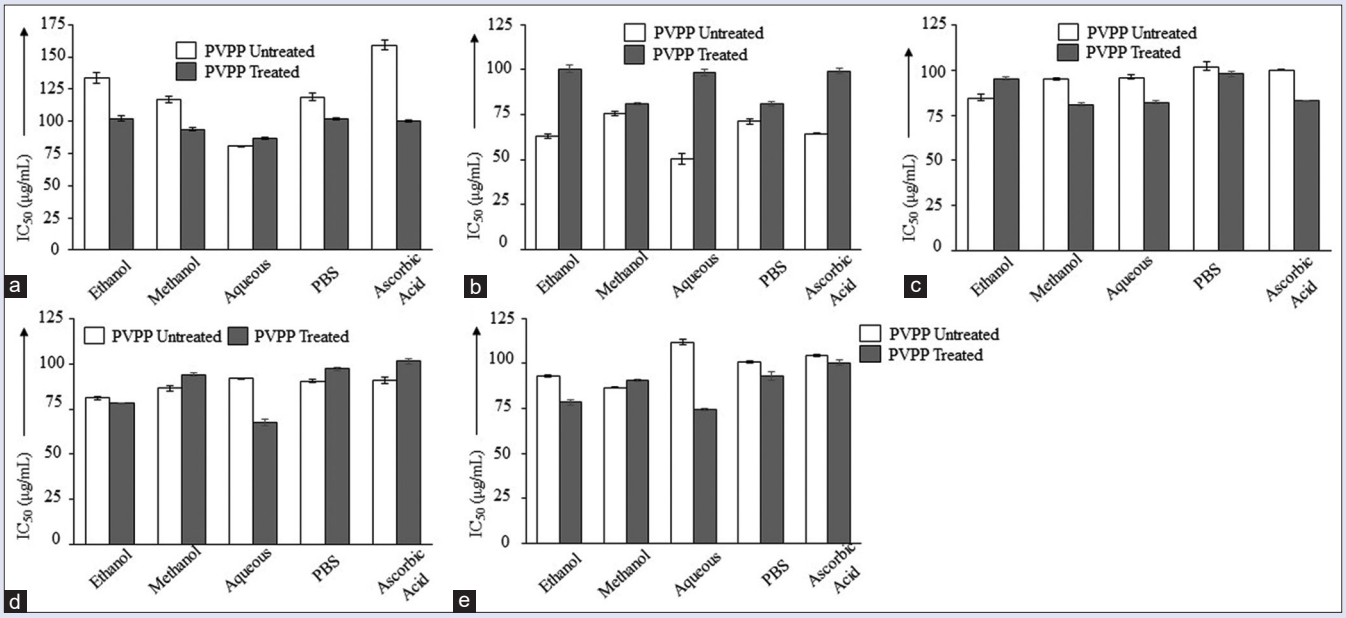

Figure 1: *Pluchea lanceolata* extracts evaluated for *in vitro* antioxidant activities. IC_{50} values were calculated for percent scavenging activity at different concentrations. (a) 2,2-diphenyl-1-picrylhydrazyl free radical scavenging assay, (b) hydrogen peroxide free radical scavenging assay, (c) nitric oxide assay, (d) assay of reducing power and (e) total antioxidant assay. All the values are expressed as mean ± standard deviation (n = 3)

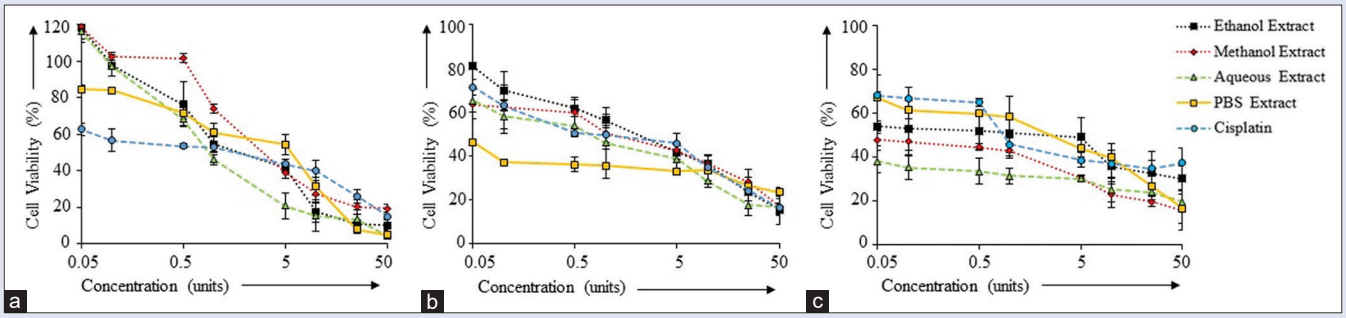

Figure 2: Cell viability assay on cervical cancer cell lines (a) ME-180, (b) HeLa, and liver carcinoma cell line (c) HepG2 cells using *Pluchea lanceolata* extracts (mg/ml) of ethanol (black), methanol (red), aqueous (green), phosphate buffer saline (orange), and standard chemotherapy drug cisplatin (µM) (blue)

Table 4: IC_{50} values of *Pluchea lanceolata* extracts and cisplatin

Cells	Extract				
	Ethanol extract (mg/ml)	Methanol extract (mg/ml)	Aqueous extract (mg/ml)	PBS	Cisplatin (µM)
ME-180	2.16±0.53	4.21±0.62	1.18±0.12	2.43±0.63 mg/ml	0.72±0.35
HeLa	1.65±0.31	0.93±0.16	0.60±0.49	3.435±0.12 µg/ml	0.87±0.12
HepG2	0.4±0.34	0.08±0.03	0.004±0.0039	1.24±0.25 mg/ml	1.6±0.13

For all the experiments (n=3). PBS: Phosphate buffer saline

Conflicts of interest

There are no conflicts of interest.

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