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In-Vitro Antiproliferative Efficacy, Antioxidant Activity and LC-MS Based Metabolite Profiling of *Premna barbata* Stem Bark

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ABSTRACT

Premna barbata Wall. ex Schauer is used traditionally as folkloric medicines for the treatment of different pathological conditions. The first reported constituent from the bark of the plant was an iridoid glycoside premnosidic acid having antioxidant activity. Other species of this genus i.e., *Premna latifolia, Premna tomentosa* has shown to have antioxidant and cytotoxic activities. Despite the ethnomedicinal uses, no scientific evidence in support of antitumor activity on the stem bark of *Premna barbata* is reported so far. Hence, the current work aims to assess anticancer potentiality of different extracts of *P. barbata* on various cancer cell lines. Different extracts i.e., Petroleum ether extract (PBPE), Chloroform extract (PBCE), Ethyl acetate extract (PBEE) and Alcoholic extract (PBAE) were prepared and on each extract *in-vitro* antiproliferative activity was performed by using SRB assay. The most potent extract i.e., PBEE was then evaluated for antioxidant activity. Qualitative phytochemical investigation of different extracts indicates the presence of proteins, carbohydrates, steroids/triterpenoids, alkaloids, glycosides, flavonoids, and phenolic constituents. Ethyl acetate extract of *Premna barbata* gives potent cytotoxic activity in all cancer cell lines but more selectively to human colon cancer cell line (COLO-205) with GI^{50}-44.6 µg/ml. The phenolic and flavonoid content in ethyl acetate extract was 3.43±0.09 mg GE/g and 4.28±0.18mg QE/g respectively. Nineteen compounds were observed in positive (+) ESI mode chromatograms when LC-MS analysis was carried out. The LC-MS analysis by positive ionization mode, the predicted compounds such as Geniposidic acid (synonym: Premnosidic acid) and Rutin were detected. The cytotoxicity observed on cancerous cell lines in our study may be due to the presence of observed compounds. So, it can be concluded that *Premna barbat*a stembark has remarkable cytotoxic activity against different tumor cell lines but the effect is more on colon cell lines as compared to others.

Keywords: *Premna barbata*; Phytochemical; Cytotoxicity; antioxidant; LC-MS analysis

NOMENCLATURE

IARC	International Agency for Research on Cancer,
	P. barbata: *Premna barbata* Wall. ex Schauer
PBPE	Petroleum ether extract
PBCE	Chloroform extract
PBEE	Ethyl acetate extract
PBAE	Alcoholic extract
ADR	Adriamycin
MDA-MB-231	Breast cancer cell line
HOP-62	Lung cancer cell line
COLO-205	Colon cancer cell line
SiHa	Cervical cancer cell line
NCCS	National Centre for Cell Science
ATCC	American Type Culture Collection
DMEM	Dulbecco's Modified Eagle Medium
FBS	Fetal bovine serum
IC_{50}	Half maximal inhibitory concentration
DPPH	1,1-Diphenyl-2-picryl-hydrazyl
ABTS	2, 2′-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) diammonium salt
NO	Nitric oxide
BHT	Butylated hydroxytoluene
STD	Ascorbic acid
FCR	Folin-Ciocalteu's reagent
TPC	Total phenolic content
TFC	Total flavonoids content
GAE	Gallic acid equivalents
QE	Quercetin equivalents
ACN	Acetonitrile
ESI	Electrospray ionization
TIC	Total ion current

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1. INTRODUCTION

Cancer is a terrible disease which affects the health of the people globally. The risk factors of cancer can be intrinsic or extrinsic. Cancer-inducing factors may lead to genetic modification and interrupt the expression of

oncogenes and antioncogenes. According to IARC, 19.3 million new cases and about 10.0 million deaths in 2020 were reported all over the world.[1] One of the ways to treat this disease is chemotherapy and advancement in anticancer drugs helps in improving the patient care. A very successful advanced chemotherapeutic drug is used in treating many cancers but there are various adverse side effects of chemotherapy which includes gastrointestinal, myelosuppression, reproductive, nephron toxicities and hair follicle damage. moreover, these drugs also cause resistance to cancer therapy. The anticancer drug which is specifically cytotoxic towards the cancer cells only is considered to be ideal. Research findings suggest that the plant derived compounds and their synthetic derivatives are the substitutes for making better chemotherapeutic agents.[2] There is a need to discover new molecules from nature that can successfully destroy cancer cells and leave normal cells as such with very less toxicity. This is the reason why there is an increasing search for plants which possess potential anti-cancer activity. The genus *Premna* (Lamiaceae) constitutes more than 200 species, distributed around tropical and subtropical regions of the world. *P. barbata*; Lamiaceae is commonly called Aganyo in Kumaun region of Uttarakhand, and Bakhara in Ayurvedic system of medicine. This species is a popular medicinal plant and each of its parts has been utilized for the cure of numerous illnesses in Indian systems of medicine. Traditionally, many health problems like arthritic pain, dropsy, diarrhoea, fever, and herpes complex disease were treated by this plant. It has been reported to possess glycoside, iridoid glycoside, alkaloid, flavonoid, terpenoids, volatile oil, polysaccharide and fatty acids.[3,4,5] A new iridoid glycoside Premnosidic acid was first compound from the bark of the plant reported by Negi et al.[4] Earlier in some studies reports suggests that Premnosidic acid possesses the free radical scavenging and antioxidant activities.[6] Antibacterial activity has also been reported by the plant previously.[5]

There is no scientific evidence which supports antitumor activity of plant so stembark of this plant were subjected to pharmacological screening. Other species of this genus i.e., *Premna latifolia* [7,8,] *Premna tomentosa* [9] have already been reported to have antioxidant and cytotoxic effects. Hence, the study aims to investigate the antiproliferative efficacy of different extracts of *P. barbata* on different types of cancer cell lines.

2. MATERIAL AND METHODS

2.1 Plant Material

The stembark was collected from Jones Estate village near Nainital district of Uttarakhand, India (1450 m altitude) in the month of November 2020 and authenticated from Botanical Survey of India, Northern regional center, Dehradun, Uttarakhand, India where a voucher specimen has been deposited. The voucher specimen number was Ref. BSI/NRC/tech./Herb (Ident.)/2020-21/572/28 December 2020. Plant was dried in shade (<40ºC), coarsely grinded and kept in tightly closed container.

2.2 Extraction of Plant Material

The dried plant (140 g) was coarsely powdered and then extraction was done with Petroleum ether (40-60ºC); using Soxhlet's apparatus. Rota-evaporator was used for concentrating the extract and concentrated extract was transferred to a china dish which was previously weighed and kept in a desiccator. The air dried defatted marc was further extracted with chloroform, ethyl acetate and ethanol. The yield of the extracts i.e. PBPE: Petroleum ether extract, PBCE: Chloroform extract, PBEE: Ethyl acetate extract and PBAE: Alcoholic extract were calculated and kept in a desiccator.

2.3 Phytochemical Screening[10,11]

The various extracts such as PBPE, PBCE, PBEE and PBAE were investigated for the qualitative estimation of chemical constituents as per standard methods.

2.4 *In vitro* antiproliferative activity

2.4.1 Cell Viability by Sulforhodamine B (SRB) Cytotoxicity Assay[12]

The cytotoxicity of the different solvent extract (PBPE, PBCE, PBEE, and PBAE) was assessed against four cancer cell lines i.e., breast (MDA-MB-231), lung (HOP-62), colon (COLO-205), cervical (SiHa) using SRB assay. The procurement of cell lines was done from NCCS, Pune and ATCC, USA. Cell lines were developed in RPMI 1640/DMEM medium consisting of 10% FBS and 2.0 mM L-glutamine. Cells were seeded in a 96 well microtiter plate for the current study in 100µL at a density of 5000 cells/well. The microtiter plates were incubated at 37°C with a humidified environment that contained 5% CO_2 after cell inoculation. After 24 h of incubation, Adriamycin (ADR) as positive control and various concentrations (10, 20, 40 and 80µg/mL) of test samples (PBPE, PBCE, PBEE, and PBAE) were taken. Then, the absorbance was recorded at test wavelength of (540 nm) on a MicroPlate reader. The execution of data was represented by GI^{50} value (GI^{50}= 50% growth inhibition).

2.5 *In-vitro* Antioxidant assay

2.5.1 DPPH Radical Scavenging Assay[13]

The antioxidant potential of potent extract (PBEE) was assessed by determining its ability to scavenge free radicals. For this assay, test solutions at the concentrations (20-80 µg/ml) were used and ascorbic acid (STD) was taken as reference standard.

2.5.2 ABTS Radical Scavenging Assay[14]

This assay was carried out according to the standard method.

2.5.3 NO Radical Scavenging Assay [15]

It was performed according to the standard procedure. The ascorbic acid was taken as standard and percentage inhibition was calculated.

2.6 Total Phenolic Content and Total Flavonoid Content[16]

2.6.1 Determination of Total Phenolic Content

FCR was used to calculate the amount of TPC in the extract. Linear equation obtained from the standard curve of gallic acid was used for the determination of TPC and was expressed in terms of gallic acid equivalent/g of test extract.

2.6.2. Determination of Total Flavonoid Content

The TFC was determined by aluminium chloride colorimetric assay. The content of total flavonoid was expressed in mg quercetin equivalent/g of test extract.

2.7 LC-MS analysis of potent extract of *P. barbata*

The LC-MS analysis of ethyl acetate extract (PBEE) of *P. barbata* was performed on a Waters, Synapt XS HDMS. The column was C-18 waters, Acquity BEH 2.1*100mm;1.7um; conditions were: (solvent) A- formic acid (0.1%) in water and B- formic acid (0.1%) in ACN; gradient: (i) 0 min 90/10, (ii) 2 min 90/10, (iii) 5 min 80/20, (iv) 10 min 70/30, (v)12 min 50/50, (vi)14 min 90/10; injection volume 4.0 µl; flow rate: 0.2 ml/min; ESI parameters: (+) ion modes; mass range 100–1200 m/z; Desolvation Gas: 900 Lts/Hr; Cone Gas: 50 Lts/Hr; Desolvation Temperature: 45ºC; Source Temperature: 12ºC; Collision energy 4ev; Capillary Voltage: 2.928keV; Cone Voltage: 50V; Gases used: N2 (6-7 bar), and Argon (5-6 bar).

2.8 Statistical Analysis

The data are characterized as means ± SEM (n=3). Statistical analysis was determined using the one-way ANOVA test (GraphPad Prism (version 6.0). Regression analysis was done by best fit method.

3. RESULTS AND DISCUSSION

Cancer has become a life threatening disease in developed as well as developing countries. The therapies currently in use include radiation, chemotherapy; possess many side effects as could be seen by high morbidity and mortality rates. This limits their use and demands new cancer management for this deadly disease. Therefore, attempts and researches are continuously made to explore naturally occurring anti-carcinogens that along with being potent can slow down or may even reverse the progression of cancer development. Due to the diverse phyto-metabolic contents which are having multiple biological activities; the herbal medicines are receiving great attention in cancer treatment. For centuries, medicinal plants have been used in treating many diseases. The literature survey can provide several plants that are used traditionally and can be studied further for exploring their biological potential. Several previous investigations have proven that many botanicals have curative potential against chronic diseases like diabetes, cancer, inflammation, stroke and ageing etc. Globally, around one-third of essential anti-tumor drugs are derived from plants and around 3000 plant species have been recognized to possess anticancer properties. As synthetic drugs have many adverse side effects there is an urgent demand for searching more safe new compounds that can kill cancer cells[1]. For this reason, the current work was aimed to analyse the possible therapeutic efficacy of *P. barbata* in various cancer cell lines. In the light of previous literature of genus *Premna*; some species such as *Premna latifolia* [7], *Premna tomentosa* [9,17], *Premna herbacea* [18], and *Premna serratifolia* [19] have been reported for cytotoxic activities against various cancer cell lines. The current study manifests the phytochemical investigation, antioxidant and antiproliferative efficacy of *Premna barbata* stem bark.

3.1 Extraction Yield

The yield in percent, colour and consistency of extracts are shown in Table 1. The percentage yield of the phytoconstituent is often different for all the parts of the plant and also between other plant species. The solvent systems used for extraction also cause variation in yield. The highest yield was observed in PBAE (4.85 % w/w), and the lowest was found in PBEE (0.98 % w/w). The percentage yields help in the assessment of solubility of a particular constituent of the plant in the given solvent; the percentage extractive values exceeded 30%, suggesting the notable quantity of polar compounds may be there in the polar extracts.[20]

Table 1. Percentage yield, colour and consistency of extracts of *P. barbata* stem bark

Extracts	Colour	Odor	Consistency	% Yield (w/w)
PBPE	Pale yellow	Characteristic	Semisolid	1.91
PBCE	Greenish brown	Characteristic	Semisolid	1.11
PBEE	Dark brown	Characteristic	Solid	0.98
PBAE	Brown	Characteristic	Semisolid	4.85

3.2 Phytochemical Analysis

The chemical screening of the plant extracts reflects the presence of many phytochemicals. (Table2) Tamta *et al* too divulged the existence of the similar constituents in crude extract of *P. barbata* [5]. Hence, our research was consistent with the above-mentioned results. Most of the phytoconstituents like glycosides (Iridoid glycoside), triterpenoids, flavonoids and phenolic compounds are present in PBEE.

3.3 *In vitro* Antiproliferative Activity

3.3.1 Effect of Different Solvent Extracts of P. Barbata by SRB Assay

SRB assay was used to check the cytotoxicity against various cancer cell lines (MDA-MB-231, HOP-62, COLO-205 and SiHa) of different histological origins. In this study, the different solvent extracts (PBPE, PBCE, PBEE and PBAE) of *P. barbata* was screened against

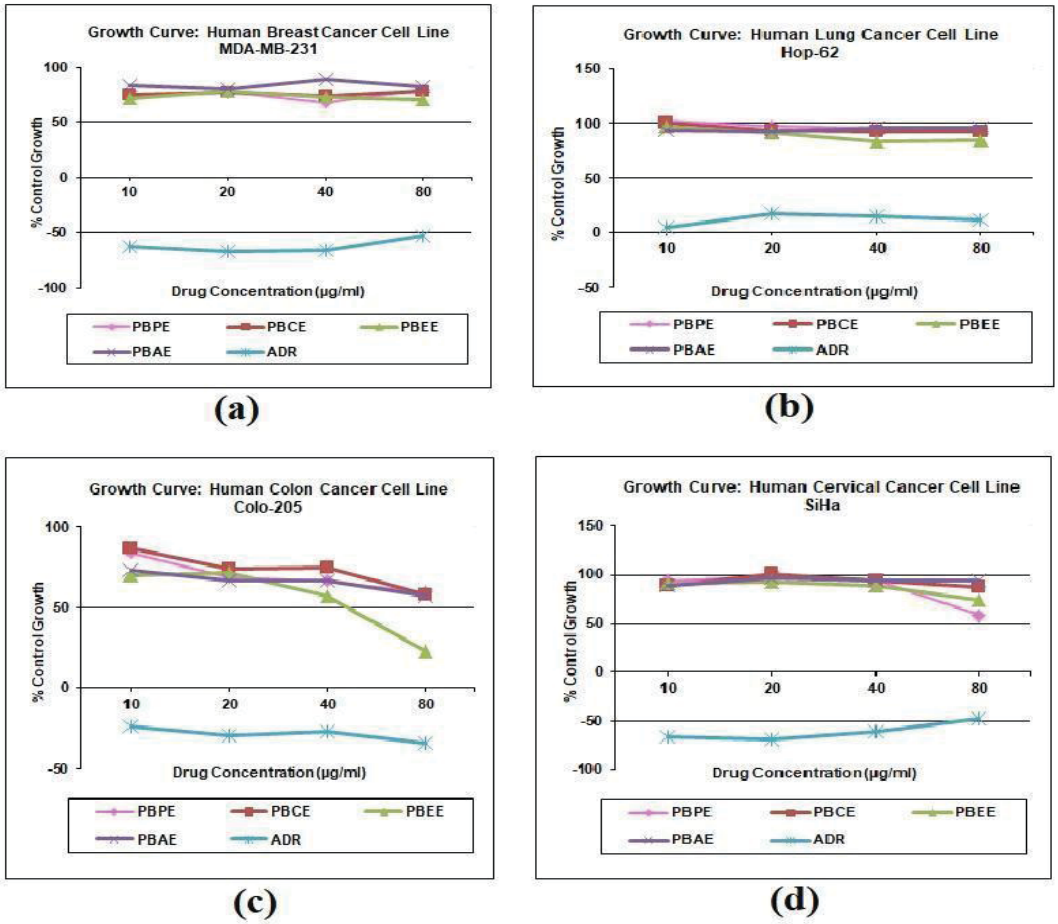

Figure 1. Effect of different solvent extracts on human cancer cell lines; (a) MDA-MB-231; (b) HOP-62; (c) COLO-205; (d) SiHa, The cells were treated with various concentrations and cytotoxicity was measured by SRB assay. Values were expressed as mean ± SEM.

Table 2. Preliminary phytochemical screening of *P. barbata* stembark

Class of constituents	Tests	PBPE	PBCE	PBEE	PBAE
Amino acids	Ninhydrin	-	-	-	-
Proteins	Biuret	-	-	-	+
	Million's	-	-	-	+
Carbohydrates	Molisch's	-	-	+	+
	Fehling's	-	-	+	+
Steroids	Salkowski Reaction	+	-	-	-
Triterpenoids		-	-	+	-
Alkaloids	Mayer's	-	+	-	-
	Dragendorff's	-	+	-	-
	Hager's	-	+	-	-
Glycosides	Sodium hydroxide reagent	-	-	+	+
	Keller kiliani	-	-	-	-
	Borntrager's	-	-	-	-
	Trim-Hill reagent	-	-	+	-
	Foam	-	-	-	-
Flavonoids	Shinoda's	-	-	+	-
Phenolic compounds	$FeCl_3$	-	-	+	+
	Lead acetate solution	-	-	+	+

(+) present, (−) absent

Table 3. *In-vitro* antitumor activity of different solvent extracts of *P. barbata*

Plant	Cancer Cell lines	Sample name	Anti-proliferative activity GI^{50} (µg/ml)
P. barbata (Stembark)	MDA-MB-231	PBPE	>80
		PBCE	>80
		PBEE	>80
		PBAE	>80
		ADR	<10
	HOP-62	PBPE	>80
		PBCE	>80
		PBEE	>80
		PBAE	>80
		ADR	<10
	Colo-205	PBPE	>80
		PBCE	>80
		PBEE	44.6
		PBAE	>80
		ADR	<10
	SiHa	PBPE	>80
		PBCE	>80
		PBEE	>80
		PBAE	>80
		ADR	<10

All values were expressed in mean ± SEM (n=3); GI_{50}: 50% growth inhibition, that drug concentration at which there is 50% decrement in the net protein increase.

four cancer cell lines i.e. breast cancer (MDA-MB-231), lung cancer (HOP-62), colon cancer (COLO-205), and cervical cancer (SiHa), and the results are compiled in Table 3. The ethyl acetate extract (PBEE) shows more cytotoxicity on colon cancer cell line (Colo-205) with GI_{50} (50% of maximal concentration that inhibited cell proliferation), (44.6µg/ml) when compared to the other cell lines shown in Figure 1, and Table 3. Perhaps, this is because of the presence of complex phytoconstituents and many of them solubilize in ethyl acetate. The PBEE contains bioactive phytoconstituents like glycosides (Iridoid glycoside), triterpenoids, flavonoids, and phenolic compounds which could be responsible for various biological activities of *P. barbata*. Previously, these classes of constituents have already reported antioxidant and anticancer potential.[21,22,23,24] PBEE showed significant cytotoxic activity when compared with other extracts. Hence, this extract was further subjected to check antioxidant activity.

3.4 *In-vitro* Antioxidant Assay

3.4.1 DPPH Radical Scavenging Assay

The PBEE and STD (ascorbic acid) manifest activity with an IC_{50} of 45.40±0.17 and 25.16±0.19 µg/mL respectively shown in Table 4 & Figure 2.

3.4.2 ABTS Radical Scavenging Assay

In this method, the efficacy of extract was analysed by the reduction of blue coloured ABTS+ radical by the antioxidant which results in decolourization. The IC^{50} value of PBEE was found to be 43.61±0.53µg/mL shown in Table 4 & Figure 2. BHT (standard) demonstrated significantly higher antioxidant capacity with IC^{50} value of 24.12±0.20µg/mL.

3.4.3 NO Radical Scavenging Assay

PBEE showed nitric oxide inhibitory assay with an IC_{50} (82.68±0.74µg/mL) shown in Table 4 & Figure 2. The IC_{50} value of standard STD (ascorbic acid) was 43.02±0.63µg/mL.

Table 4. *In-vitro* Free radical scavenging activity of PBEE

Sample	DPPH	ABTS	NO
PBEE	45.40±0.17µg/Ml	43.61±0.53 µg/Ml	82.68 ± 0.74 µg/mL

Free radical assays were used to estimate the antioxidant capacity of PBEE from *P. barbata*. The generation mechanism of free radicals will change with the type of chemicals used. For this, PBEE was examined for its ABTS, DPPH, and NO scavenging activities which exhibited potent results with IC_{50} values of (45.40±0.17, 43.61±0.53, and 82.68±0.74 µg/mL) respectively. (Table 4) The DPPH assay is the most acceptable assay used for assessing the free radical scavenging potency of the samples. Antioxidants from plant materials are capable of visually distinguishable quenching of the steady purple to the yellow coloured DPPH radical.[13] ABTS is a blue green compound, and the reduction in its colour is used to calculate the antioxidant potential of compounds. The active compounds have the power to give electrons/protons to the $ABTS^{\cdot+}$ radical form. This is a spectrophotometric technique which is uncomplicated and facile for screening and routine analyses. $ABTS^{\cdot+}$ is more reactive than $DPPH^{\cdot}$.[14] Nitric oxide is a free radical, regulating several bodies functions such as neuronal messenger, vasodilatation and antitumor activities.[15] The ethyl acetate extract (PBEE) significantly inhibited NO with the IC_{50} being 82.68±0.74µg/mL in contrast with standard having IC_{50} value 43.02±0.63µg/mL. The results indicated that PBEE contains the active compounds of the plant that are capable of inhibiting free radicals and thus useful as antioxidants. Antioxidants show their effects by detoxification of the reactive oxygen species. Thus ameliorates immunity and decreases the chance of cancer and degenerative diseases.[25]

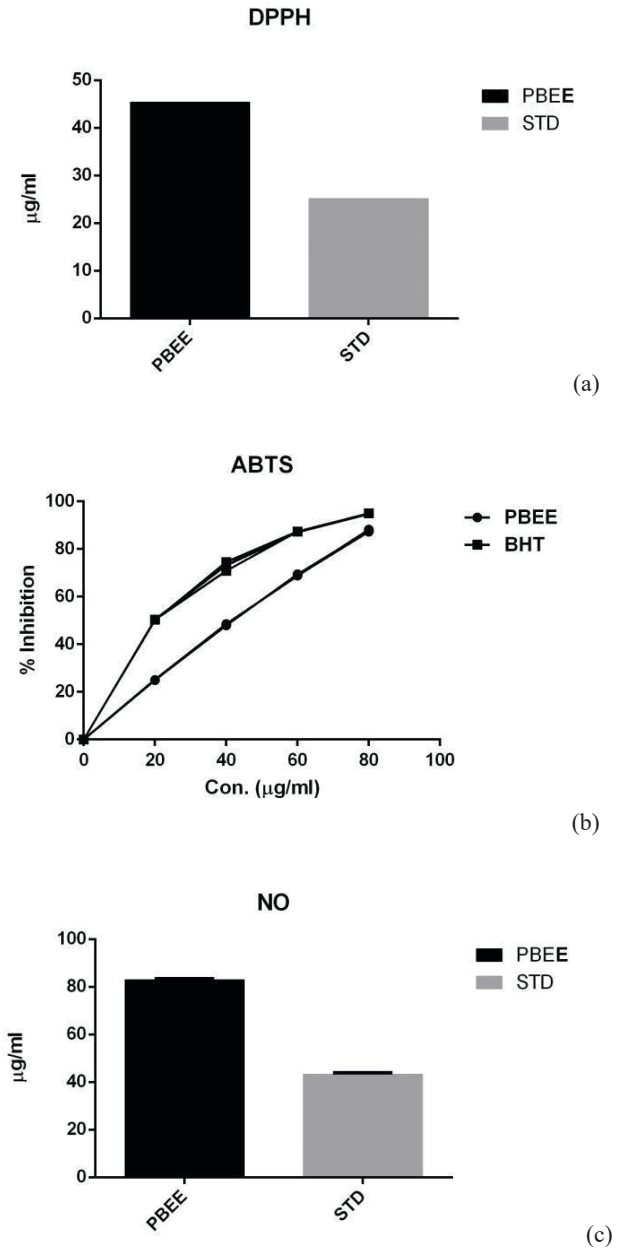

Figure 2. ***In-vitro*** **Free radical scavenging activity; (a): DPPH assay; (b): ABTS assay; (c): NO assay, Data are presented as mean± SEM (n=3). The values with P <0.01 when compared against STD was considered as statistically significant. Standard (STD) Ascorbic acid for DPPH, NO radical scavenging assay and BHT for ABTS radical scavenging assay**

3.5 Total Phenolic and Flavonoid Content

The results were obtained from a gallic acid linear regression (y = 0.0269x - 0.0124, r²- 0.999) and expressed in GAE/g shown in Table 5 & figure 3. The phenolic content possessed in PBEE was found to be 3.43±0.09 mg GAE/g.

The results were obtained from the quercetin linear regression (y = 0.0125x + 0.0026, r² -0.993). We observed that PBEE possesses 4.28±0.18mg QE/g quercetin equivalents of flavonoids (Table 5 & Figure 3).

Table 5. Total phenolic and flavonoid contents of antioxidant potent extract (PBEE).

Sample	TPC (mg GAE/g)	TFC (mg QE/g)
PBEE	3.43 ± 0.09	4.28 ± 0.18

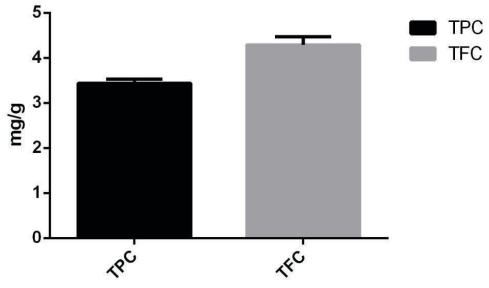

Figure 3. Total phenolic and flavonoid content of PBEE. The values are given as means of triplicate analyses (n=3).

Phenolic compounds are the chief constituents which are present in the plant with redox potential. The endogenous components of the plant can be dissolved in different solvents by using specific extraction procedures and these components can be polar or nonpolar. Flavonoids and condensed tannins are specialised metabolites with antioxidant efficacy and their potency based on the number and position of free hydroxyl groups.[16] Total phenolic and flavonoid contents of the PBEE extract was 3.43±0.09 gallic acid equivalents/g, and 4.28±0.18 quercetin equivalents/g respectively. (Table 5)

This is the first-time report on the free radical scavenging activity of *P. barbata* and in-depth phytochemical screening for identification of the active polyphenolic compounds should be carried out. Flavonoids reduce reactive oxygen formation, promote chelation of trace elements and thus prevent production of free-radicals. They also combat reactive molecules, up-regulate and protect antioxidant defence.[23] In the same manner, phenolic substances also confer oxidative stress tolerance in plants.[24] The free radical scavenging efficacy of PBEE may be proposed due to the high flavonoids and phenolic contents. Both components have many evidenced mechanisms against cancerous cells which include the activation of apoptosis, cell-cycle specific arrest, down regulation of matrix metalloproteinases-2, metalloproteinases-9 actions, inhibition of cell proliferation and decreased levels of B-cell lymphoma-2.[26]

3.6 LC-MS Analysis of Ethyl Acetate Extract (PBEE) of *P. barbata*

The analysis of phytochemical constituents in PBEE of stem bark was performed by LC-MS. Nineteen compounds were observed in positive (+) ESI mode chromatograms shown in Figure 4a. The LC-MS analysis by positive ionization mode, the predicted compounds such as Geniposidic acid

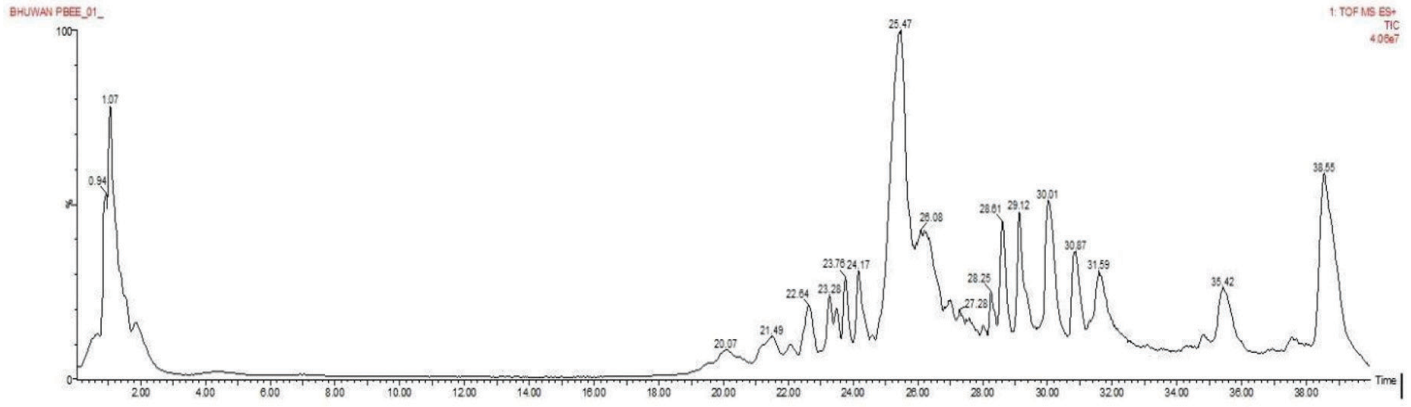

Figure 4. (a) TIC chromatogram of PBEE, in positive (+ESI) ionization mode

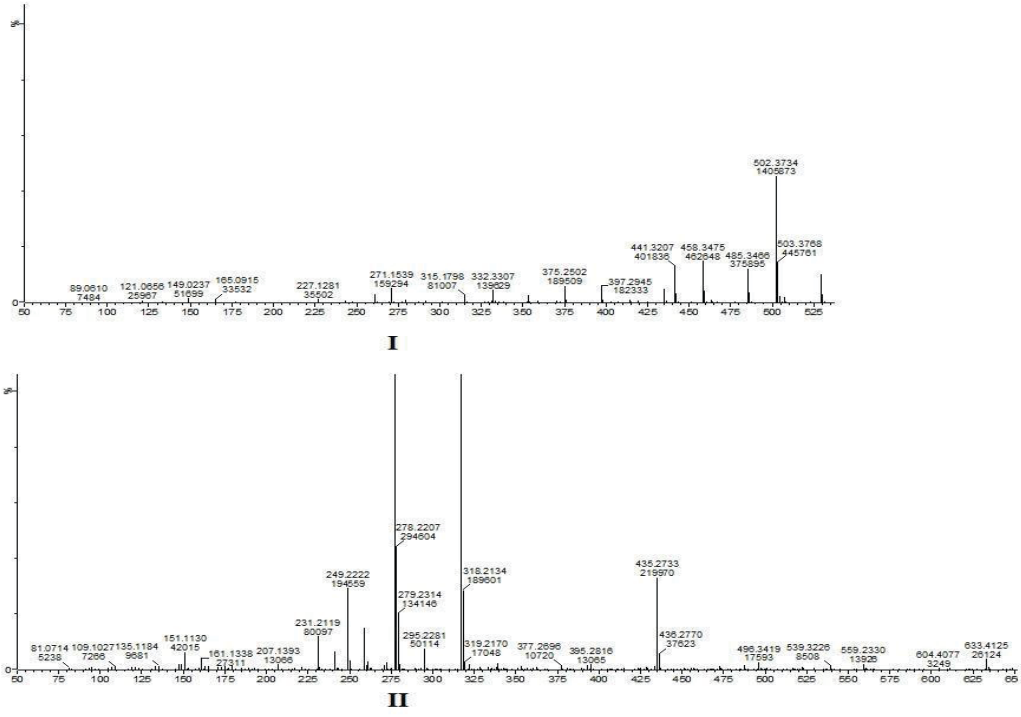

Figure 4. (b) Mass spectra of predicted molecule I: Geniposidic acid (m/z: 375.25 [M+H]+, m/z: 397[M+Na]+) and II: Rutin (m/z: 633.41 [M+Na]+, m/z 611.27 [M+H]+)in ethyl acetate extract (PBEE) of *P. barbata*

(synonym: Premnosidic acid) and Rutin were detected.[27] The mass spectrum (MS) of individual compounds is shown in Figure 4b. Therefore, the molecular weight of Geniposidic acid (m/z: 375.25 $[M+H]^+$, 397.10 $[M+Na]^+$) and rutin (m/z: 633.14 $[M+Na]^+$, m/z 611.16 $[M+H]^+$ fully matched fragmentations were revealed by Pubchem. The Top peaks of predicted compounds Geniposidic acid and Rutin are shown in figure 4c. Along with this, our results also confirmed with the literature reported by Kim *et al.*[28] and Yingyuen *et al.*.[29]

The complete information related to compounds identified by LC-MS. Based on the LC-MS interpretation, the predicted compound Geniposidic acid (synonym; Premnosidic acid) was detected and this compound belongs to the class of iridoid glycosides. Earlier, Premnosidic acid was reported in this plant by Negi *et al*.[4] Although, it has been proved that iridoid compounds have antioxidant and antitumor activity.[21] Along with another compound rutin, a flavonoid is of great therapeutic significance and it showed cytotoxic effects.[30] Plant extracts possessing antioxidant principles showed cytotoxicity towards many tumor cell lines and in experimental animals.[25] So, the cytotoxicity observed on cancerous cell lines in our study may be due to presence of this compound and further research is on-going in our laboratory to isolate the compound(s) responsible for the cytotoxic action on tumor cells. The present study reported for the first-time that *P. barbata* stembark has antiproliferative and antioxidant effects by *in-vitro* studies.

4. CONCLUSION

Premna barbata Wall. ex Schauer stembark has exhibited cytotoxic activity against numerous cancer cell lines but it has shown more activity on colon cancer cell

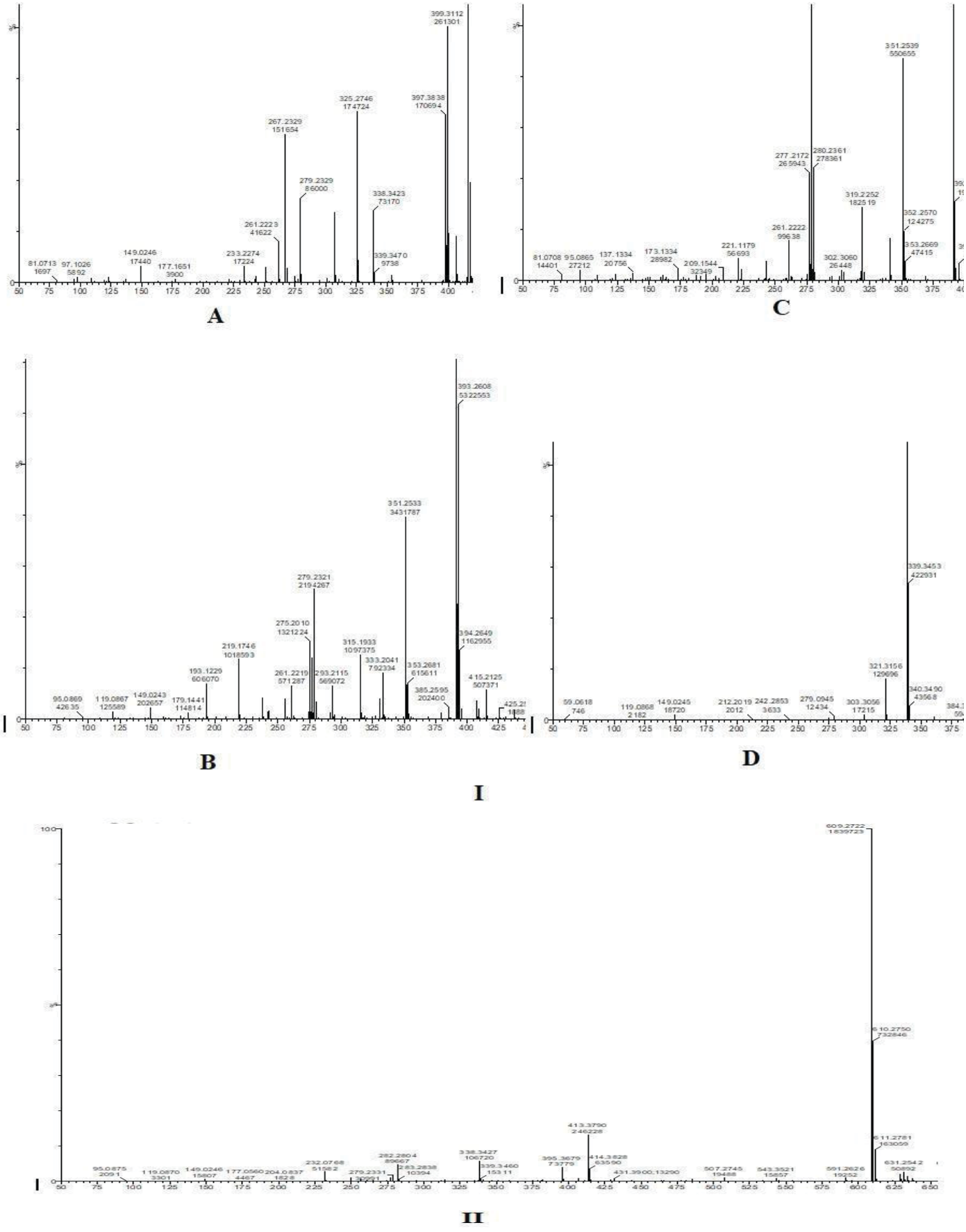

Figure 4. (c) Top peaks of predicted compounds I: (Geniposidic acid) A: m/z: 397.3838, B: m/z: 393.2603, C: m/z: 392.2495, and D: m/z: 212.2019. II: (Rutin) m/z: 609.27, 610.27, and 611.27

lines. As the plant contains various compounds, it may be concluded that the bioactivity of ethyl acetate extract was due to the synergistic effects of these compounds in the extract. These findings suggest that *P. barbata* stem bark is a good source of active compounds against cancer.

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