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ANTI-OXIDANT AND ANTI-CANCEROUS PROPERTIES OF *PHANERA VAHLI* AGAINST PC-3- *IN VITRO*

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ABSTRACT:

Background: Prostate cancer is the fastest-growing cancer in men, leading to mortality. Many medicinal practices are recommended for various cancers. Ayurveda and homeopathy are also contributing a lot. Plants with antioxidant properties are now being harvested by different solvents to withdraw different chemical compounds to cure prostate cancer. **Objective:** The purpose of the current study was to examine the anti-cancer and antioxidant properties of aqueous extract of *Phanera vahli* (PV) flowers against PC-3 cancer cell lines. **Materials and Methods:** The total phenolic content of the extract was determined by using a folin-ciocalteu reagent. The DPPH, ABTS, and H_2O_2 assay were used to measure the free radical scavenging activity of the PV flower extract. The MTT assay was used to evaluate the cytotoxic efficacy of the aqueous extract of the plant flowers against PC-3 at various concentrations. **Results:** Based on the observations, the total phenolic content was present in the aqueous extract of PV flowers. **Conclusion:** According to the current study, PV flower extract significantly possesses antioxidant and anti-cancer properties against prostate cancer cell lines. This effect may be used in the future to the extract's ability for scavenging free radicals.

Keywords: *Phanera vahlii*, PC-3, Antioxidant, Phytochemical

INTRODUCTION

The cancer burden is growing globally, exerting physical, financial, and emotional stress on families and individuals. Many people in countries are preparing themselves for such types of diseases and are being overburdened financially due to it [1]. Prostate cancer is one of the most common cancers and every 25th male would be the victim of this cancer during his lifetime. [2,3]. It is the cancer of the prostate which is a walnut-shaped gland in males that helps in the nourishment and movement of sperm. Its cells migrate to bones and lymph nodes and there they cause metastatic cancer. Prostate cancer is the most frequent and second-highest cause of death for the male population in Western countries [2-5]. Its cancer is the fifth most common cancer globally and the second, most mortality-causing in males [6]. GLOBOCAN has reported 358,989 deaths worldwide and 1,276,106 cases of prostate cancer in 2018 and 19.3 million new cases of cancer in 2020, out of which 10.3% cases of only prostate cancer [7-10]. As per previous trends, approximately 2,88,300 cases will be affected by this cancer, and approx. 34,700 deaths will be reported this year and it is also predicted that 1.7 million cases and 5 lakh deaths will occur by 2030 [11]. Africa, Australia, the USA, and Asia are prone to this disease. Mutations in BRC1 and 2 genes, obesity, and multimember families are the risk factors of prostate cancer [12-13]. Prostatectomy, radiotherapy followed by androgen deprivation, and endocrine therapies like enzalutamide with docetaxel are the latest practices to treat this cancer [13]. Chemotherapy is the best for metastasized cancer but its efficacy is limited and it also leads to many side effects for patients. So herbal medication is required for such types of cancers [14].

Medicinal Plants

Plants such as *Ocimum gratissimum, Zingiber officinale, Allium sativum, Moringa oleifera, Asparagus recemosus, Mangifera indica, Punica grantum, Catharnthus roseus, Tinosporia cardifolia, Ocimum santum, Lantana camera* and many more possess anti cancerous properties [15]. Himalayan plants such as *Camellia sineis, Lantna camera, Tinospora cardifolia* and *Cedrus deodara* have shown antiproliferative activities in ethanolic extracts against LC-540 (Leydig Cell Testicular Tumour) cell lines of rats. The phytochemicals of plants are now used in preparations of herbal medicines and they could be used in cancer therapeutics [16]. The major phytochemicals of medicinal plants that play an important part in anti-cancerous activities are polyphenols and flavonoids. They have shown activities in arresting the cell cycle of cancerous cells, inducing apoptosis, suppressing cancer cell proliferation, and preventing oxidative stress [17].

Phanera vahlii (Wight & Arn.) Benth. -Taur

Family: Fabaceae

Synonyms: *Bauhinia vahlii, Bauhinia racemose*.

Common Name: Bauhinia climber, camel's foot

climber, Maloo Creeper,

Local Name: Taur

Phanera vahlii (Wight & Arn.) Benth-Taur plant is an evergreen climbing shrub that grows generally on the tops of trees up to 10-30m long with the help of tendrils. The stem is woody up to 20cm thick. Leaves may be to 40 cm, orbicular, bilobed, and chordate. White flowers up to 4 cm long are arranged in terminal corymbs inflorescence. Petals are spathulate and three stamens are present. Flat woody pods as fruit with 5 cm in length having rusty 6-12 seeds [18 -19]. It is found in soil having good moisture and fertility. Its seeds are edible and leaves are cooked as vegetables. It is used as food, fiber, and medicine by local inhabitants of the western Himalayas. Its leaves are used to, prepare platters or '*plates*' and '*dunus'*. White flowers arranged long in large terminal corymbs are used with salt and white pepper for headaches. They are also used as an aphrodisiac, tonic, and vermifuge, for stomachic, and for cordage for antihyperglycemic, antioxidant, and anti-inflammatory effects. Root extracts of *Bauhinia vahlii* were prepared to evaluate antimicrobial activities against gram-positive, and gram-negative bacteria and fungi strains [20-21].

Picture 1: *Phanera vahlii* flowers (Taken by myself at Mundkhar HMR HP)

PC-3 cancer cell line

There are some prostate cancer cell lines such as DU145, LNCaP, and PC-3. The PC-3 cancer cell line was obtained in 1979 from the prostate gland of a 62-year-old Caucasian male having metastatic cancer [22]. These cells do not respond to glucocorticoids and androgens but their growth is controlled by some epidermal factors [23]. PC3 cells have low acidic phosphatases and testosterone-5-alpha reductases and have more division potential as compared to LNCaP and DU145 [24-25]. They show adenocarcinoma similar to neoplastic cells of epithelial origin. These cell lines were investigated by long non-coding RNA (lncRNA) MALAT1. Metastasis Associated Lung Adenocarcinoma Transcript 1 (MALAT1) enhances prostate cancer so its inhibition in

expression may reduce or treat this cancer [26]. NOTCH3 gene protein increases stemness, docetaxel resistance, and lipid metabolism in PCa patients via MAPK and TUBB3 signaling pathways so it could be the biomarker and therapeutic target in PCa patients [27]. Androgen depletion therapy (ADT) is being used to regress PCa but leads to castration-resistant or recurrent prostate cancer (CRPC) due to the reactivation of the androgen receptor (AR) signaling axis. AR activity is retargeted with second-generation inhibitors such as enzalutamide (MDV3100) and it has validated AR as the chief growth regulator of PCa. So, AR-V (variant genes) rearrangement can act as a therapeutic target for PC-3 cells [28]. Cancer stem cells (CSCs) are the subpopulations of tumor cells that self-renew and differentiate. They are now being used as aspects of cancer therapeutic agents. Salinomycin is the antibiotic isolated from *Streptomyces albus* that is being used as an anti-potent agent against PC3 cells. It induces the arrest of G2 and M-phase, suppression of the beta-catenin pathway, and thus induces apoptosis in PC3 cells [29-30]. Ursolic acid which is a pentacyclic triterpenoid along with its derivatives has shown an autophagic response thus inhibiting the growth of PC3 cells by arresting their phase of the cell cycle. Akt/mTOR and Beclin-1 pathways are involved in Ursolic acid- -autophagy in PC3 cells [31].

MATERIALS AND METHODS

Plant material

Phanera vahlii flowers in good condition were collected from Hamirpur, Himachal Pradesh, India. Flowers were identified and authenticated by the Department of Agriculture, Abhilashi Group of Institutions, Mandi, HP.

The preparation of *Phanera vahlii* aqueous extract (PV-AE):

After being cut off from the plant, the PV flowers were shade-dried. The dried flowers were then powdered by using a mortar and pestle. The powder is stored between 4 and 8 °C until needed. Each of the examined PV's 125 g of powdered flowers was extracted using 2L distilled water through hot maceration until exhaustion. In each instance, the combined aqueous extract was evaporated at 30-40 °C under reduced pressure until it was scorched [32].

Cell line culture

The prostate cancer cell line (PC-3) was arranged by the National Centre for Cell Science (NCCS), located in Pune, India. The cells (10000 cells/well) were grown in DMEM medium supplemented with 10% FBS and 1% antibiotic solution for 24 hours in a 96-well plate. The cells were kept in a humidified incubator with 5% CO_2 at 37ºC in 95% air [33].

Chemicals

Folin-Ciocalteu reagent, Gallic Acid, DPPH, Na_2CO_3, Methanol, Ascorbic Acid, Ethylenediaminetetraacetic acid (EDTA), H_2O_2. The reagents and all chemicals used were of analytical grade.

Total Phenol Content Estimation:

Principle:

The Foiln-Ciocalteu phenol reagent is a mixture of phospho-tungstic acid and hetero-poly phosphomolybdic, where the molybdenum and tungsten are in 6+ states. When specific reducing agents are used to reduce the material, the molybdenum blue and tungsten blue, are produced.

Procedure:

The phenolic compounds were determined by the Folin-Ciocalteu reagent. The test sample dilutions were combined with 50µl of diluted folin ciocalteu reagent and 40 µl of aqueous Na2CO3 (1.0 M). According to the setup table, reaction mixtures were prepared, and allowed to stand for 15 minutes, and then absorbance was measured at 760 nm with the double-beam JASCO V-630 spectrophotometer. Gallic acid was prepared as a standard curve in a 50:50 v/v water mixture: methanol at concentrations ranging from 25 µg/mL to 300 µg/ml [34].

ABTS Radical Scavenging Activity

Principle

Either manganese dioxide or potassium persulfate oxidizes ABTS. ABTS cation radicle absorbs radiation of a wavelength of 743 nm and gives blue blue-green colour upon losing an electron by the Nitrogen atom of ABTS. When Trolox I is added which is a hydrogen-donating antioxidant, the colour is lost as the Nitrogen atom quenches the hydrogen, and absorbance is decreased at 743 nm [35-36].

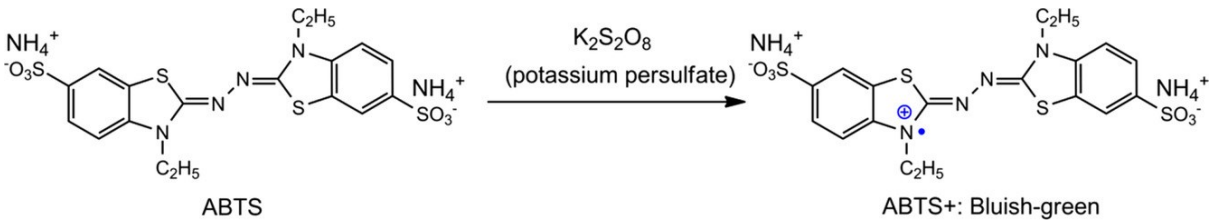


Fig.1: Oxidation of ABTS

Procedure

ABTS (SRL-Chem-Cat no.-28042) radicles were prepared by mixing APS (2.45 mM) and ABTS (7mM) solution, which was diluted 100X to prepare ABTS free radical reagent. Added 10µl of different stock of the standard (Ascorbic Acid - SD Fine- F13A/0413/1106/62, Concentration as per mentioned in excel sheet) and samples (As per mention in excel sheet) to the 200µl of ABTS free radical reagent in 96 well plate and incubated at RT for 10 min in dark. After incubation measure the absorbance of the decolorization at 750nm using a microplate reader (iMark, BioRad). Results were presented concerning negative control. IC-50 was calculated using Software Graph Pad Prism 6 [36-40].

DPPH Scavenging Assay:

Principle:

1,1-diphenyl-2-picrylhydrazyl (DPPH) is deep violet due to its picryl group. The colour of the molecule disappears when combined with a material that can donate a hydrogen atom, and

turning it into a pale yellow colour. The powder has a red colour and is stable [41].

(DPPH) + H-A → DPPH-H + A

(Purple) (Yellow)

Procedure:

0.1 ml of 0.1 mM DPPH solution was mixed with 5µl of a distinct stock of the test compound in a 96-well plate. The reaction was set up in triplicate, and blanks with 0.2 ml of DMSO/methanol. 5 µl of a compound at various concentrations were made in duplicate. The plate was rested for thirty minutes in the dark. After the incubation, the decolorization was measured at 495 nm. with the help of a microplate reader (iMark, BioRad). A reaction mixture having 20µl of deionized water was made control. In comparison to the control, the scavenging activity was expressed as "% inhibition". Utilizing Software Graph Pad Prism 6, IC-50 was computed [42-43].

Calculations

DPPH Scavenging activity = ((Abs $_{Control}$- Abs $_{Sample}$)/Abs$_{Control}$) ×100

Hydroxyl Free Radical Scavenging Assay:

Principle:

A kit of H_2O_2 assay for measuring hydrogen peroxide includes cell culture, supernate, urine, serum, plasma, and other biological fluids. This kit's objective is to quantify low-level H_2O_2 concentrations in biological matrices. A color reagent reacts with xylenol orange dye in an acidic solution with sorbitol and ammonium iron sulphate to produce a mixture of purple colour which is directly proportional to the amount of H_2O_2 in the sample.

Procedure:

Following were added in the order to the 96-well plate: 10µl of plant extract (concentration as specified in the excel sheet), 24µl of phosphate buffer (50 mM, pH 7.4), 10µl of ascorbic acid (-SD Fine- F13A/0413/1106/62) and 24.14 mg of deoxyribose (SRL-84384), 88µl FeCl3 (Fischer Scientific-Cat no.-23585) (10mg/ml), 28 µl H2O2 (Neurochem Laboratories-HP6520) (6%), water up to 33 ml, and 10µl of plant extract (Concentration as per mentioned in excel sheet). Gallic acid (SRL-Cat no.-5995-86-8) with a concentration as indicated in the Excel sheet was standard. After incubation, 10% TCA (Fischer Scientific-Cat no. 28444) and 50µl of 1% TBA (HiMedia-Cat no. RM1594) were added to each well. A chromogen in pink was created. Then the absorbance was measured at a wavelength of 540 nm. [42-43].

Calculation:

Scavenging activity was calculated by the following formula...

$$\frac{A(control)-A(sample)}{A(control)} x\,100$$

A (Sample): Absorbance of the extracts/standard.

A (control): Absorbance of the control and

In vitro Cytotoxicity Evaluation of aqueous extract of PV flowers on PC-3 cells

Principle:

The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide, which is known as the

MTT reagent, is a mono-tetrazolium salt made up of three aromatic rings. Two of which are phenyl moieties and one of which is thiazolyl, encircling a positively charged quaternary tetrazole core ring with four nitrogen atoms. Upon reduction of MTT, the core tetrazole ring is disrupted and formazan, a violet-blue water-insoluble dye, is formed. The MTT reagent is reduced to formazan by metabolically active cells and can cross both the cell membrane and the inner membrane of the mitochondria in viable cells, most likely because of its positive charge and lipophilic structure [44].

Procedure:

In cell culture MTT Solution (a final concentration of 250µg/ml) was added and incubated for 2 h. After incubation, the culture supernatant was removed and the cell layer matrix was dissolved in 100 µl Dimethyl Sulfoxide (DMSO) and then read in an Elisa plate reader (iMark, Biorad, USA) at 540 nm and 660 nm. IC-50 was calculated by using the software Graph Pad Prism -6. Images were captured under an inverted microscope (Olympus ek2) by using a Camera (AmScope digital camera 10 MP Aptima CMOS) [44- 46].

RESULTS

Total Phenol Content Estimation

Table 1: Antioxidant property of PV using DPPH assay compared to Ascorbic acid.

Sample Code	Phenolic Content (µg/mg sample)
PV	407.33

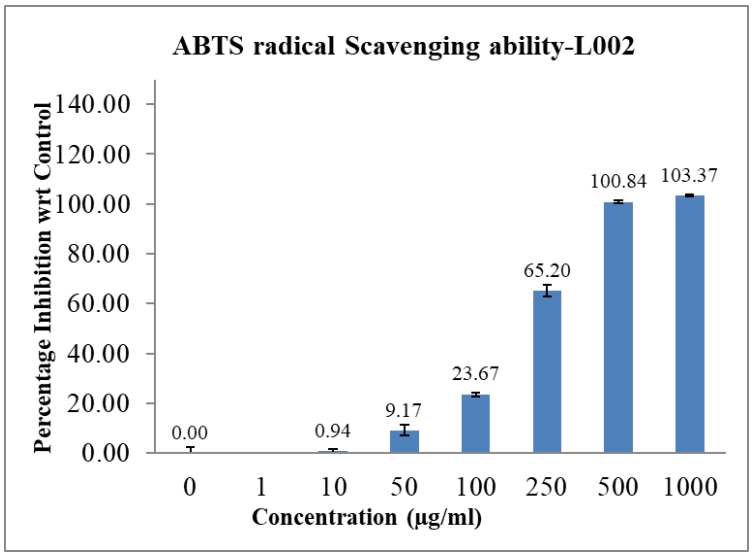
Folin ciocalteu reagent was used to determine total phenolic content. Results obtained from the experimental work that the Aqueous extract of *Phanera vahli* (PV) flowers possesses a content of 407.33µg/mg.

ABTS Radical Scavenging Assay

Table 2: Antioxidant property of PV using ABTS assay compared to Ascorbic acid.

Sample code	IC50 value (µg/ml)
Ascorbic Acid	2.197 ± 0.054
PV	171.4 ± 0.035

Antioxidant property (ABTS) was observed dose-dependent manner in PV Aqueous extract (IC_{50}= 171.4 ± 0.035) as compared to the standard ascorbic Acid (IC_{50} = 2.197 ± 0.054 µg/ml). 171.4 of PV was found equivalent to 2.197 µg of the standard Ascorbic acid.

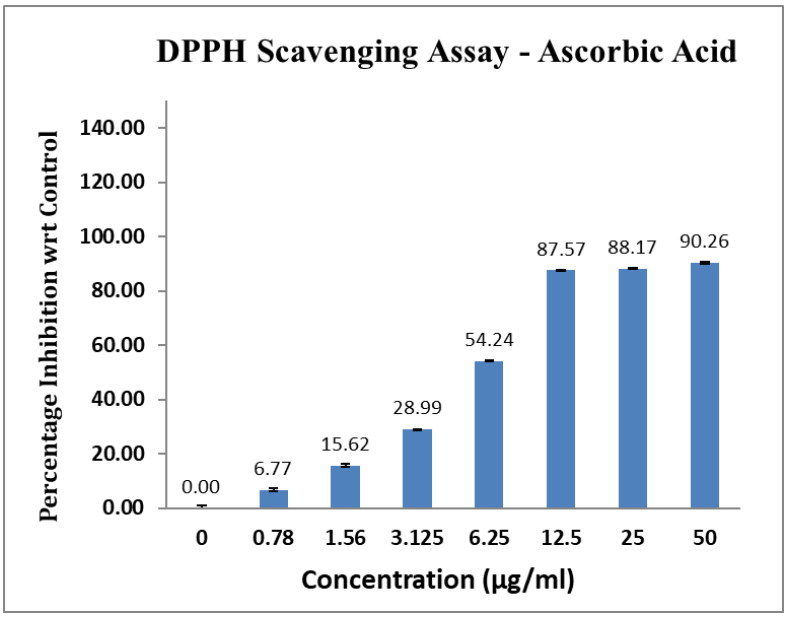


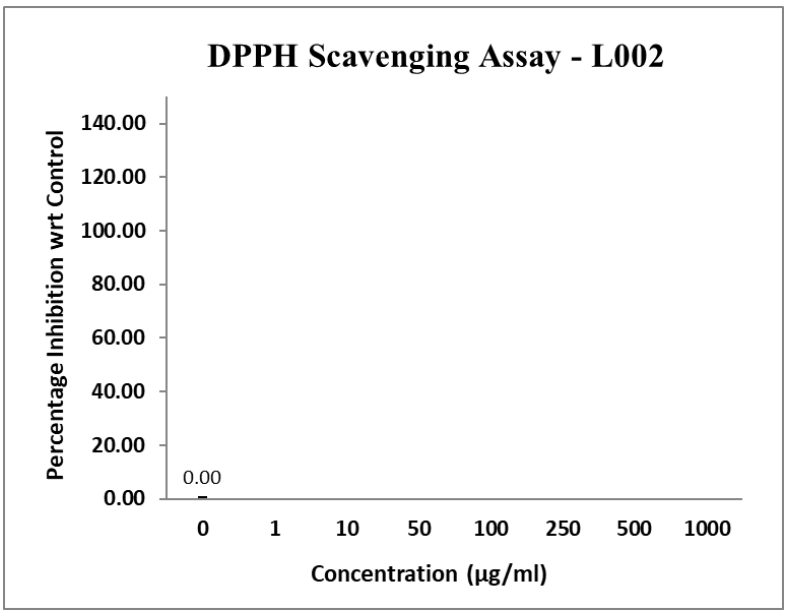
Graph 1: Antioxidant property of PV using ABTS assay

DPPH Scavenging Assay: DPPH scavenging activity was not observed in PV as compared to standard ascorbic acid (IC_{50}= 5.201 ± 0.038 µg/ml).

Table 3: Antioxidant property of PV using DPPH assay compared to Ascorbic acid.

Sample code	IC_{50} value (µg/ml)
Ascorbic Acid	5.201 ± 0.038
PV(L002)	Not active





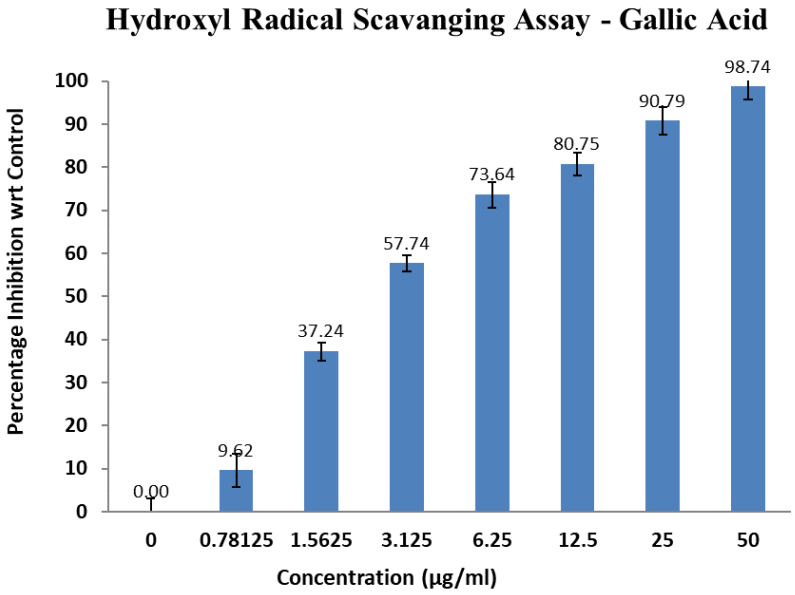
Graph 2&3: Comparative account of Antioxidant properties of PV and Ascorbic acid using DPPH

Hydroxyl Radical Scavenging Assay

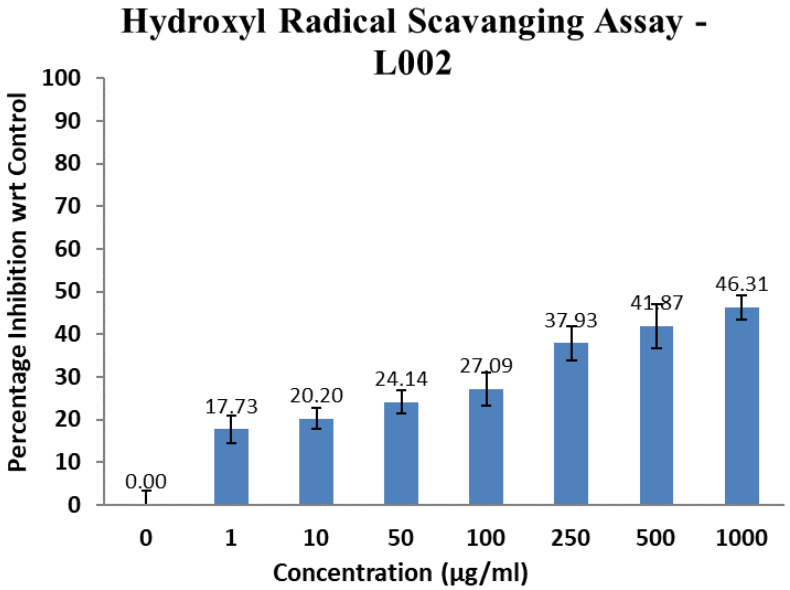
Based on the results obtained from the study, Hydroxyl Free Radical Scavenging Activity was not observed in permissible limits in PV (IC_{50}= Above Dose Limit) in comparison to standard Gallic acid (IC_{50} = 2.758 ± 0.043 µg/ml).

Table 4: Antioxidant property of PV using H_2O_2 assay compared to Gallic acid.

Sample Code	IC_{50} Value (µg/ml)
Gallic Acid	2.758 ± 0.043
PV(L002)	Above Dose Limit



Graph 4: Comparative account of Antioxidant property of PV using H_2O_2 assay compared to Gallic acid



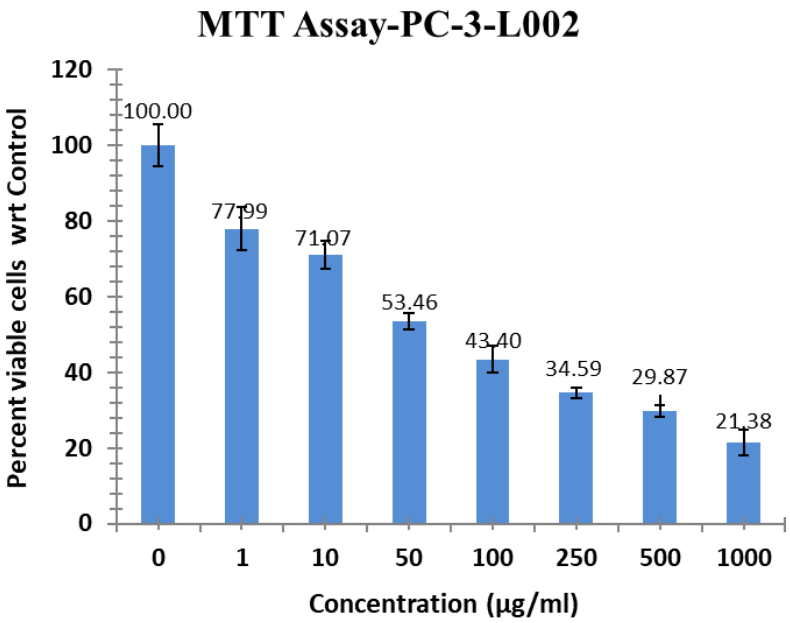
Graph 5: Comparative account of Antioxidant property of PV using H_2O_2 assay compared to Gallic acid

In -Vitro cytotoxicity evaluation of _Phanera vahlii_ flower aqueous extract on PC-3 cell lines

Based on the results obtained from the MTT assay, it was observed that when the PC-3 cell line was exposed to different concentrations of the sample, cytotoxic activity was observed in PV (IC_{50} = 83.74 ± 0.15454 µg/ml). The IC_{50} is the concentration of an inhibitor/sample/ formulation at which the viable cells are reduced by half.

Table 5: Cytotoxic activity of PV extract against PC-3

Sample code	IC_{50} value (µg/ml)
PV	83.74 ± 0.15454



Graph 6: PC-3 cell line exposed to different concentrations of PV

DISCUSSION

So far cancer is understood, it is the deformation of cellular mechanisms in different ways. DNA is damaged by rupturing nuclear envelopes causing the death of normal cells. Its uncontrolled nature of growth explains extra lipid metabolism and starvation of normal cells [47]. There are so many treatment models for cancer. But Chemotherapy is effective with side effects. Many countries are now switching over to medicinal plants that have some secondary metabolites [48]. Phenols, carotenoids, and terpenes are effective antioxidants used to get rid of free radicals which are the causes of cancer. It has no side effects. More than 3500 plant species are now being treated to cure such dreadful diseases [49-51]. *Phanera vahlii* is also such a plant that is being used for many other ailments but least for its cancerous properties. It is an evergreen climbing shrub.

used as food, fiber, and medicine by local inhabitants of the western Himalayas. Its leaves are used to, prepare platters or '*plates*' and '*dunus'*. Root extracts of *Bauhinia vahlii* were prepared to evaluate antimicrobial activities against gram-positive, and gram-negative bacteria and fungi strains [52-54]. Bark possesses anti-inflammatory and anti-diabetic activities in the stem. The extract showed the presence of flavonoids, phytosterols, alkaloids, glycosides, and phenolic compounds. The ethanolic and chloroform extracts showed a significant reduction in blood glucose levels in rats [55]. Methanol extract of plants possesses the highest level of phenols, tannins, and flavonoids [56]. The plant is traditionally used as medicine for its antioxidant, anti-inflammatory, and antidiabetic activities. It maintained the ecological balance used in the food, medicine, and handicraft industry [57-58]. However, in the current study, the extract is not active in DPPH and Hydroxyl radical scavenging assay but the presence of its phenol content to 407.33µg/mg and value in ABTS scavenging assay IC_{50}= 171.4 ± 0.035, making plant extract effective in treating cancer cells. This study confirms the IC_{50} value for cytotoxicity by MTT assay (IC_{50} = 83.74 ± 0.15454 µg/ml).

CONCLUSION

The result of the current study suggests that the aqueous extract of PV flowers has antioxidant and anti-cancerous efficacy against PC3 cells, which might be due to its free radical scavenging activity (ABTS) and due to its phenol contents. Thus, it is concluded that the study of this extract can be an alternative treatment for prostate cancer.

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CONFLICTS OF INTEREST

All authors declared no conflict of interest.

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