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CHEMICAL COMPOSITION, ANTIGENOTOXIC, ANTIOXIDANT AND ANTIPROLIFERATIVE ACTIVITIES OF N-BUTANOL FRACTION FROM *PTERIS VITTATA* L.

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Keywords:

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ABSTRACT: *Pteris vittata* L., a fern species in the Pteridoideae subfamily of Pteridaceae, is known to possess various medicinal properties. *P. vittata* is used as a folk medicine in the Eastern Ghats of Tamil Nadu, Western Ghats, and Vindhyan region of Madhya Pradesh, India. The present study was undertaken to evaluate antigenotoxic, antioxidant, and antiproliferative potential of *P. vittata* L. HPLC analysis was carried out for identification of polyphenols in n-butanol fraction of *P. vittata* L. (Pvitnol). The fraction significantly inhibited H_2O_2 and 4NQO induced genotoxicity in *E. coli* PQ 37 in SOS chromotest. Pvitnol fraction showed promising antioxidant potency in various *in-vitro* antioxidant assays *viz.* in DPPH, reducing power, superoxide anion scavenging, lipid peroxidation, site-specific hydroxyl scavenging, and non-site specific hydroxyl scavenging assays. Pvitnol was also effective in protecting pBR322 plasmid against damage caused by Fenton's reagent. The antioxidant activity may in part be accountable for its antigenotoxic activity obtained in SOS chromotest. An inhibition of 74.43% was obtained in MTT assay at a concentration of 200 µg/ml in MCF -7 cell line, which indicates its antiproliferative potential. Antiproliferative activity was further validated by confocal imaging and comet assay. Confocal studies showed nuclear condensation and fragmentation. Double strand breaks in DNA were introduced in the Pvitnol treated cells leading to apoptosis, as evidenced by confocal studies. HPLC analysis showed varying amounts of different polyphenols *viz.* ellagic acid, kaempferol, quercetin, and rutin, which may account for its bioactive potential.

INTRODUCTION: Cancer is one of the leading causes of morbidity and mortality worldwide, and over the next two decades, a number of new cases are expected to rise by about 70%. It has been revealed that a diet rich in plant-derived foods has a protective effect on human health. Identifying bioactive dietary constituents is an active area of research that can lead to new drug discovery.

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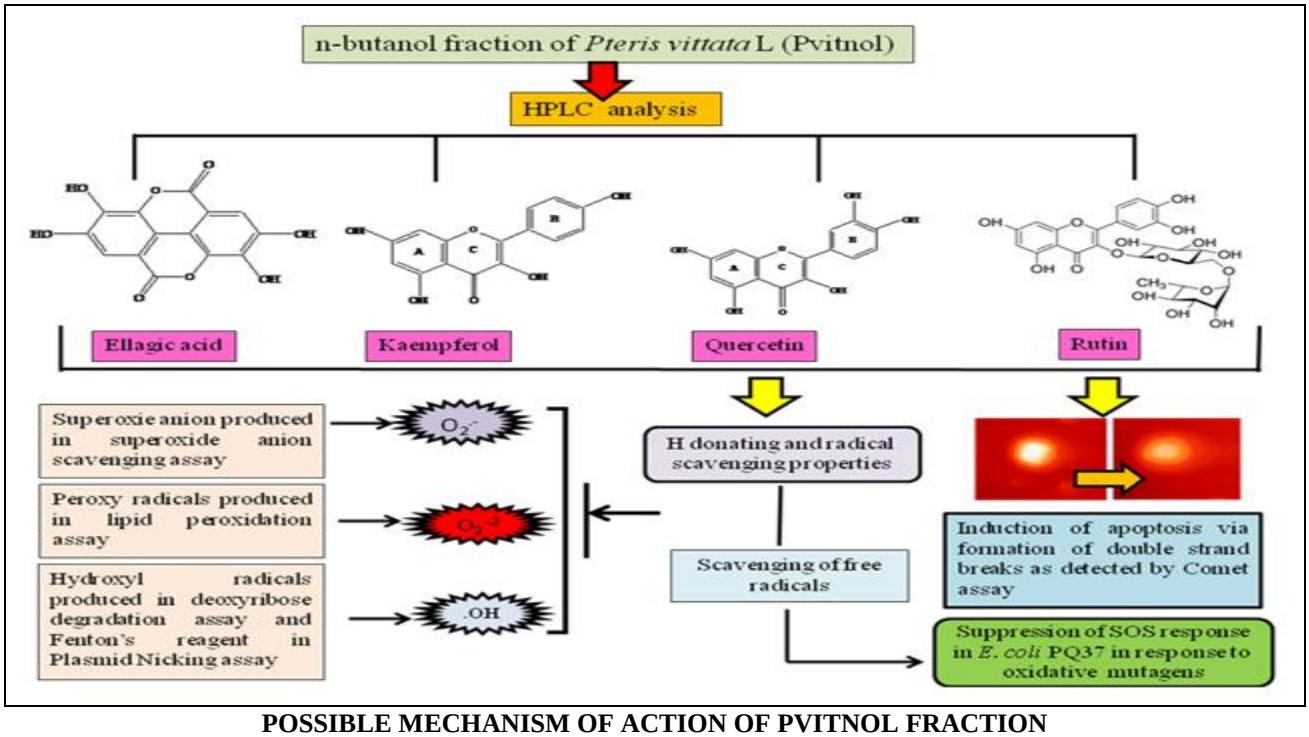
Polyphenols are being studied intensively for their role as potent inhibitors of cancer invasion and metastasis. Curcumin, resveratrol, gallic acid, chlorogenic acid, caffeic acid, carnosol, 6-gingerol, 6- shogaol, capsaicin, etc. and their related derivatives have been reported to reverse or retard tumorigenesis [1].

Oxidative stress has been implicated in many diseases, including cancer. Dietary antioxidants have emerged as effective agents for cancer prevention by reducing oxidative stress [2]. Chemopreventive agents acting through the antioxidant mechanism enhance the cellular capacity to combat oxidative stress and protect the

cells from the toxic effects of ROS, generated either endogenously or exogenously. These dietary supplements have an influence on enzymes involved in metabolism and affect the metabolism and disposition of carcinogen [3]. Mutations in somatic cells play a major role in cancer initiation and other stages of carcinogenesis. Mutational activation of oncogenes and loss of heterozygosity of tumor suppressor genes are the key contributors in cancer initiation and progression [4]. Advances in the understanding of cancer biology and cancer genetics has revealed that apoptosis and the genes that control it have a profound effect on the malignant phenotype. Oncogenic mutations disrupt apoptosis, leading to tumor initiation, progression, or metastasis [5]. Phytochemicals are known to induce apoptosis through several mechanisms such as release cytochrome c from mitochondria and activation of capsases [6, 7], increase in Bax [8, 9, 10], arrest of G_2/M phase, downregulation of expression of cyclin D1 in addition to upregulation of Cdk inhibitors [11, 12] and inhibition of NF-kB activation [13].

Attention is being focused on intracellular-signaling cascades as common molecular targets for various chemopreventive phytochemicals [14]. Anticarcinogenic properties of dietary phytochemicals may be attributed to detoxification mechanism and enhanced excretion of carcinogens, the suppression of inflammatory processes such as cyclooxygenase-2 expression, inhibition of mitosis, and the induction of apoptosis at various stages in the progression and promotion of cancer [15].

Out of 121 prescription drugs that are in use for the treatment of cancer, 90 of them are known to be derived from plant sources, and 74% of these drugs were discovered by pursuing studies on folklore sources and ethnomedicinally important plants [16, 17]. The present study was focused on *Pteris vittata* L., an ethnomedicinally important plant commonly known as the Chinese ladder brake fern. It is a fern species in the Pteridoideae subfamily of Pteridaceae. It is known as Yanai Vanaji in Kolli Hills, Eastern Ghats of Tamil Nadu, India, and Jasumba in Vindhyan region, Madhya Pradesh, India. It is known to possess wound healing properties, provides relief from body pain and swelling. In addition to having antiviral and antibacterial properties, it is used as a demulcent and hypotensive tonic [18, 19]. It is also used for flu prevention, treatment of dysentery, rheumatism, and other ailments. Keeping in the mind medicinal importance of this plant, the present study was designed to explore the suppression of the SOS response in *Escherichia coli* PQ 37, the antioxidant potential, and the antiproliferative activity of n-butanol extract of *P. vittata* L. on human MCF-7 breast cancer cells.



POSSIBLE MECHANISM OF ACTION OF PVITNOL FRACTION

MATERIALS AND METHODS:

Chemicals: 2,2-Diphenyl-1-picrylhydrazyl (DPPH), Ascorbic acid, Ethidium bromide, 3-(4,5-dimethyl-thiazol- 2- yl)- 2, 5- diphenyltetrazolium bromide (MTT), 4', 6-diamidino-2-phenylindole (DAPI) and Propidium iodide were obtained from HiMedia Pvt. Limited, Mumbai. O-Nitrophenyl-β- D-galacto-pyranoside (ONPG), p-nitrophenyl phosphate disodium (PNPP), 4-nitroqinoline 1-oxide (4NQO), sodium dodecyl sulfate (SDS), Dimethyl sulfoxide (DMSO), and Tris (hydroxymethyl) aminomethane were purchased from Sigma (St. Louis, MO, USA). Plasmid pBR322 was procured from Genei Pvt. Ltd., Bangalore.

Procurement of Bacterial Strain and Cell Line: *E. coli* PQ 37 strain was purchased from Pasteur Institute, France. MCF-7 breast cancer cells were obtained from the National Centre for Cell Science, Pune, and were routinely grown in DMEM supplemented with 10% fetal bovine serum (FBS) and antibiotic/antimycotic solution.

Plant Material: Plant was collected from the foothills of district Kangra (N 32° 07′ S 6.2″ E 076° 17′ 48.3″), Himachal Pradesh, India. The specimen was authenticated at the Botanical Survey of India (BSI), Northern Regional Center, Dehradun. A set of samples is deposited at the herbarium of BSI having accession No. 11455.

Preparation of Extract: Powdered fronds were extracted with 80% methanol. The supernatant was pooled and concentrated using a vacuum rotary evaporator (Buchi Rotavapor R 210) followed by lyophilization to obtain dry residue. The methanol extract was further fractionated using different solvents *viz.* hexane, diethyl ether, ethyl acetate, n-butanol, and water to obtain different fractions. The supernatant obtained was pooled and concentrated again followed by lyophilization to obtain the dry residues from the respective fractions. Preliminary assays showed that the n-butanol (Pvitnol) fraction was active, and therefore it was subjected to further detailed investigation.

Phytochemical Screening: HPLC analysis was performed using Shimadzu UHPLC (NEXERA) System, consisting of an LC-30 AD pump, and a diode-array detector (DAD). Conditions for analytical HPLC were: Nucleosil 100-C18 column (15 × 4.6 mm, 5 µm) with C18 guard column. Flow rate was 1 ml/min, and the injection volume was 5 µl. Detection was carried out at 280 nm. Identification of peaks was done on the basis of retention time and comparison with spectra of the standard chromatogram.

Antigenotoxic Activity:

SOS Chromotest: SOS chromotest was done according to the protocol given by Quillardet and Hofnung [20]. Culture of *E. coli* PQ37 was grown at 37 °C in Luria broth medium and diluted with the same to adjust OD_{600} to 0.4. Aliquots of 0.6 µl were dispensed in test tubes containing different concentrations of Pvitnol and 4NQO/H_2O_2. Positive control was prepared by exposing the bacteria to 4NQO and H_2O_2. After incubation of 2 h at 37 °C with constant shaking, 300 µl of samples were used for assay of β-galactosidase (β-gal) and alkaline phosphatase (Ap) activities each. The different concentrations of Pvitnol were also tested in the absence of mutagens for their genotoxic effect. The induction factor (IF) was calculated as the ratio of Rc/Ro where Rc is equal β-gal/Ap activity determined for the extract at concentration c, and Ro is equal to β-gal/Ap activity in the absence of extract. Antigenotoxicity was expressed as % inhibition of genotoxicity according to the formula:

$$\text{Inhibition (\%)} = 100 - (IF_1 - IF_o / IF_2 - IF_o) \times 100$$

Where, IF_1 is the induction factor in the presence of Pvitnol and mutagen (4NQO/ H_2O_2). IF_2 is the induction factor in the absence of the Pvitnol (only mutagen). IF_o is the induction factor of the blank.

Antioxidant Activities:

DPPH Radical Scavenging: [21] Three ml of 0.1 mM DPPH solution was mixed with 200 µl of fraction and absorbance was measured at 517 nm. Radical scavenging activity was calculated using the following formula-

$$\text{Inhibition (\%)} = (Ac - As/ Ac) \times 100$$

Where Ac is the absorbance of the control and As is the absorbance of the sample.

Superoxide Anion Scavenging: [22] One ml of a fraction of different concentrations was mixed with 156 µM NADH (1ml), 60 µM NBT (1ml), and 468 µM phenazine methosulphate (1ml) in phosphate buffer (pH = 8.3). The reaction was initiated with the addition of PMS. The reaction mixture was

incubated at 25 ºC for 10 min. The absorbance of the colored complex was measured at 560 nm. A decrease in absorbance of the reaction mixture indicated increased superoxide anion scavenging ability.

$$\text{Inhibition (\%)} = (Ac - As/Ac) \times 100$$

Where As is the absorbance of sample and Ac is absorbance of standard at maximum concentration.

Lipid Peroxidation: [23] The protective effect of the fraction was determined by mixing different concentrations (5-160 µg/ml) of it (1ml) with 0.15 M KCl and 0.5 ml of 10% egg yolk. Peroxidation was initiated by adding 100 µl of 10mM ferric chloride. After incubation at 37 ºC for 30 min., lipid peroxidation was monitored by the formation of thiobarbituric acid reactive substances (TBARS). TBARS were estimated by adding 2 ml of ice-cold HCl (0.25 N) containing 15% trichloroacetic acid (TCA), 0.5% thiobarbituric acid (TBA), and 0.5% butylated hydroxytoluene (BHT) to the reaction mixture, followed by heating at 100 ºC for 60 min. The samples were then cooled and centrifuged, and the absorbance of the supernatants was measured at 532 nm. Distilled water was used as a control, and rutin was used as a positive control. The protective effect of the fraction against lipid peroxidation was calculated as follows:

$$\text{Percentage inhibition} = (Ac\text{-}As/Ac) \times 100$$

Ac is the absorbance of the control, and As is the absorbance of the sample.

Hydroxyl Radical Scavenging Assay: [24, 25] This method comprised of non-site and site-specific scavenging of OH radicals depending upon the use of EDTA. For non-site specific hydroxyl radical scavenging assay, EDTA was added to the Haber-Weiss reaction mixture containing 2-Deoxyribose (10 mM), Fe (III) chloride (10 mM), EDTA (1mM), and H_2O_2 (10 mM) and ascorbic acid (1mM) without or with the fraction (5–160 µg/ml) in 50 mM phosphate buffer at pH 7.4. In site-specific hydroxyl radical scavenging assay, EDTA was replaced with the same amount of buffer. The reaction mixture was incubated for 1 h at 37 ºC. To 1 ml solution of the above mixture, 0.5% TBA in 25 mM NaOH and 10% TCA were added. The mixture was heated for 90 min. on a water bath at 80 ºC. The amount of pink chromogen (TBA-MDA adduct) produced was spectrophotometrically measured at 532 nm.

$$\text{Radical scavenging activity Inhibition (\%)} = (Ac - As/ Ac) \times 100$$

Where Ac is the absorbance of control and As is absorbance of sample of fraction.

Reducing Power Assay: Reducing power assay was carried out according to the method proposed by Oyaizu *et al*. [26] One milliliter of the fraction (25-400 µg/ml) was mixed with 2.5 ml of phosphate buffer (200 mM, pH 6.6) and 2.5 ml of 1% potassium ferricyanide. The mixture was incubated at 50 ºC for 20 min. A volume of 2.5 ml of 10% TCA (trichloroacetic acid) was then added to the mixture and centrifuged at 3000 rpm for 10 min. Then 2.5 ml of supernatant was mixed with an equal amount of distilled water and 0.5 ml of $FeCl_3$ (0.1%), and the absorbance was measured spectrophotometrically at 700 nm. An increase in absorbance of the reaction mixture was interpreted as an increase in reducing the activity of fraction, and the results were compared with rutin that was used as a positive control.

Percentage reduction ability was calculated as,

$$\text{Percentage reduction ability} = [1- (1\text{-}As/Ac) \times 100]$$

As is absorbance of sample and Ac is absorbance of standard (Rutin) at maximum concentration.

Plasmid Nicking Assay: Plasmid pBR322 DNA (0.5 µl) and Fenton's reagent (30 mM H_2O_2 + 50 µM ascorbic acid + 80 µM $FeCl_3$) were added to Pvitnol fraction of different concentrations and incubated at 37 °C for 30 min, and the final volume was made to 20 µl. Electrophoresis was done in TBE buffer (40 mM Tris base, 16 mM acetic acid, and 1 mM EDTA at pH 8) on 1% agarose gel [27] followed by measurement of band intensities using Gel Quant software.

Antiproliferative Activity:

Cell Viability and Cytotoxicity: Cells were washed with PBS (pH 7.4), trypsinized, and centrifuged. The cell pellet was suspended in medium and stained with trypan blue (0.4 % in PBS) to check viability. MCF-7 cells were seeded in 96 well plates at a density of 5×10^3 cells/well. After a time period of 24 h, cells were treated with various concentrations (12.5-200 µg/ml) of Pvitnol for 24 h. MTT was added followed by incubation

of 2 h. Media was removed from the wells, and 100 µl of DMSO was added per well to dissolve the purple-colored formazan crystals. Absorbance was measured at 570 nm.

Confocal Studies for Assessment of Nuclear Morphology: The morphology of the nucleus was examined using DAPI (4',6-Diamidino-2-Phenylindole, Dilactate) following the protocol of Bhushan et al. [28] with bit modifications. MCF-7 cells were cultured onto 18 mm coverslips in 6-well plates followed by Pvitnol treatment for 24 h. Cells were fixed with 4% paraformaldehyde and stained with 10 µg/ml DAPI in dark. Slides were scanned under a Nikon A1R Laser Scanning Confocal Microscope system (Nikon Corporation, Japan) using NIS Elements AR analysis software version 4.11.00 for observing nuclear morphology.

Neutral Single Cell Gel Electrophoresis Assay (Comet Assay): Comets that appear after lysis and electrophoresis in neutral solution are evidence of double-strand breaks in DNA, whereas comets produced under alkaline conditions are evidence of single-strand breaks or alkali labile lesions [29]. It can be postulated that DSBs may be the executors of apoptosis [30, 31]. Concentrations of Pvitnol used in this assay were 50, 100, 200, and 400 µg/ml. Control cells without extract were cultured in parallel using the same conditions. Following treatment for 24 h, cells were mixed with 100 µl of low melting agarose (LMPA) in PBS buffer at pH 7.4 and spread evenly on slides precoated with normal melting point agarose (NMPA). Another layer of LMPA was spread on the cell suspension layer. The slides were kept undisturbed at 4 ºC in the dark for 30 min to solidify and then immersed in pre-chilled lysis buffer (2.5 M NaCl, 100 mM EDTA, 10 mM Tris–HCl, pH 8.3, 1% Triton X-100 and 10% DMSO) for 45 min. Slides were placed in electrophoresis buffer (300 mM sodium acetate, 100 mM Tris–HCl, pH 8.3) for 45 min, and electrophoresis was carried out at 16V and 300 mA for 30 min. Staining was performed with 20 µg/ml ethidium bromide, and slides were analyzed under a fluorescent microscope (Nikon, Germany) equipped with excitation filters of 450-490 nm. All the experiments were performed in triplicates for the accuracy of results. Visual image analysis of DNA damage was carried out in accordance with previously reported protocols [32, 33].

A total of at least 50 non-overlapping comet images per gel were visually assigned a score on an arbitrary scale of 0 (round and intact without discernible tail) to 4 (almost all DNA migrated towards the tail without apparent head) based on the perceived comet tail length migration and relative proportion of DNA in the comet tail.

An overall score was calculated for each gel by applying the following formula: (percentage of cells in class 0 × 0) + (percentage of cells in class 1 × 1) + (percentage of cells in class 2 × 2) + (percentage of cells in class 3 × 3) + (percentage of cells in class 4 × 4). Consequently, the total score was in the range of 0 to 4. A mean DNA damage score for each slide was obtained by dividing the total damage score gained by the total number of comets analyzed.

RESULTS AND DISCUSSION:

Phytochemical Screening: Results of HPLC analysis revealed that Pvitnol harbors ellagic acid (301.00 ppm), kaempferol (44.065 ppm), quercetin (18.502 ppm), and rutin (15.614 ppm) in varying amounts. The major constituent in Pvitnol was ellagic acid at a concentration of 301.001 ppm followed by kaempferol (44.065 ppm), quercetin (18.502 ppm), and rutin (15.614 ppm) detected at a wavelength of 280 nm and retention time of 15.559, 17.184, 16.45 and 15.031 min, respectively **Fig. 1, Table 1**. The compounds were identified by comparison with a chromatogram of reference compounds obtained under the same conditions.

Antigenotoxic Activity: Pvitanol was found to be non-genotoxic as it was unable to induce DNA damage, as evident from the results (IF of 0.96 at highest tested dose). In antigenotoxicity studies, Pvitnol effectively reduced induced IF by H_2O_2 and 4NQO, suggesting that it has the potential to protect DNA from genotoxic effects of tested mutagens. It significantly modulated the IF induced by H_2O_2 and 4NQO with inhibition of 77.03 % and 77.86 %, respectively, at the highest tested concentration **Fig. 2**. *E. coli* PQ 37 harbors *uvrA* mutation, which abolishes excision nucleotide repair. This process is active on lesions and affects DNA conformation, thereby blocking transcription. The presence of various polyphenols in the fraction as revealed by HPLC analysis may be responsible for inhibiting ROS that break DNA strands. It has

been demonstrated by many researchers that DNA damage protective activity may be attributed to antioxidant activity [34, 35].

One of the major constituents in Pvitnol was ellagic acid (301.001 ppm) which is known to exhibit antigenotoxic potential against direct and indirect mutagens. Many researchers have reported antimutagenic effects of ellagic acid, kaempferol, rutin, *etc*. in various assays. Kaur *et al*., [36] isolated ellagic acids from *Terminalia arjuna* and studied its antimutagenic activity in TA98 and TA100 strains of *Salmonella typhimurium* and concluded that it showed moderate activity against NPD but was effective against 2-AF [36].

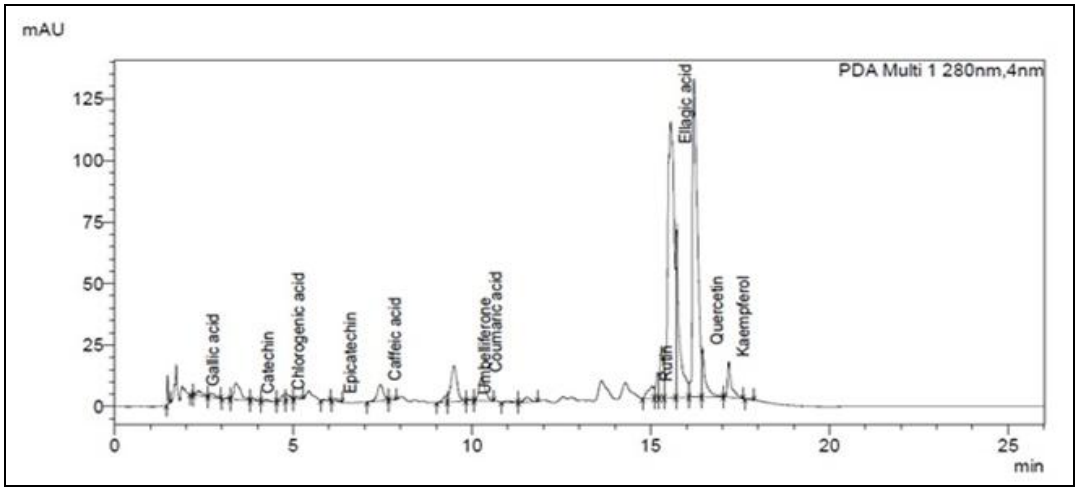


FIG. 1: HPLC CHROMATOGRAM SHOWING RETENTION TIME OF POLYPHENOLS DETECTED IN PVITNOL FRACTION OF *PTERIS VITTATA* L.

TABLE 1: QUANTITATIVE ANALYSIS OF MAJOR PHYTOCONSTITUENTS IDENTIFIED BY HPLC IN PVITNOL FRACTION OF *PTERIS VITTATA* L.

Name	Ret. Time	Area	Height	Conc. (ppm)	Area/Height
Gallic acid	2.347	18298	1693	1.283	10.805
Catechin	3.877	8529	1108	3.574	7.697
Chlorogenic acid	4.736	21695	2602	2.838	8.337
Epicatechin	6.181	7143	1106	1.876	6.460
Caffeic acid	7.431	75857	6533	5.451	11.611
Umbelliferone	9.939	6065	680	1.296	8.923
Coumaric acid	10.264	115301	9660	3.930	11.936
Rutin	15.031	60602	4964	15.614	12.207
Ellagic acid	15.559	1368623	112289	301.001	12.188
Quercetin	16.450	134621	19391	18.502	6.942
Kaempferol	17.184	103419	14544	44.065	7.111

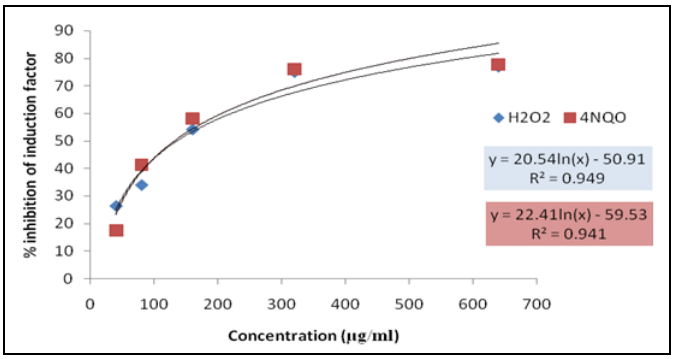


FIG. 2: EFFECT OF PVITNOL FRACTION FROM *PTERIS VITTATA* L. ON THE INDUCTION FACTOR OF H_2O_2 (0.1 mm) AND 4NQO (20 µg/ml) IN SOS CHROMOTEST

Zahin *et al*., [37] investigated the antimutagenic potential ellagic acid in Ames *Salmonella* tester strains against NaN_3 and Methyl methanesulfonate, in the absence of S9, as well as against promutagens (Benzo[a]pyrene and 2-aminofluorene) with Aroclor induced rat liver S9. A dose-dependent anti-mutagenic trend against both Benzo[a] pyrene and 2-aminofluorene was observed. His^+ revertants were reduced by 78.6% to 88.9%, respectively. Kaempferol from *Lilium candidum* was reported to inhibit DNA damage induced by the radiomimetic zeocin in *Hordeum vulgare* and human lymphocytes *in-vitro*. Kaur *et al*., [38] evaluated the antigenotoxic potential of *Cassia fistula* fruits and found that the extracts suppressed the mutagenicity of 2-aminofluorene (2-AF) in TA98 tester strain of

Salmonella typhimurium. This activity was attributed to the presence of rutin and catechins, and gallocatechin. Barcelos and coauthors, [39] reported the antigenotoxic effects of quercetin and rutin against Aflatoxin B1, methyl methane-sulfonate or doxorubicin-induced DNA damage in human hepatoma HepG2 cells using the comet assay.

Antioxidant Activity: An IC_{50} of 49.21, 90.08, 55.44, 6.73, 49.68, 44.44 µg/ml was seen in DPPH, reducing power, superoxide anion scavenging, inhibition of lipid peroxidation, site-specific hydroxyl scavenging, and non-site specific hydroxyl scavenging assays, respectively **Table 2** and **3** which signifies that Pvitnol fraction has hydrogen donating as well as radical scavenging ability. Bioactive compounds, as revealed by HPLC analysis, may be responsible for antioxidant activity. In superoxide radical scavenging, lipid peroxidation, and hydroxyl radical scavenging assays, the extract was more potent than the positive control, rutin. The role of phenolic compounds as scavengers of free radicals has been emphasized by many researchers [40, 41].

In a study carried out by Han *et al*., [42] high DPPH radical scavenging and lipid peroxidation inhibition activities of ellagic acid have been reported. Kaempferol is considered to be a strong antioxidant compared to rutin since it showed a strong DPPH radical scavenging activity (IC_{50} value of 4.349 µg/ml) compared to rutin [43].

TABLE 2: DPPH, REDUCING POWER AND SUPEROXIDE RADICAL ANION SCAVENGING CAPACITY OF PVITNOL FRACTION OF *P. VITTATA* L.

Conc. (µg/ml)	DPPH	Reducing power ability	Superoxide anion scavenging capacity
		Inhibition % ± S.E.	
25	23.77 ± 1.496[a]	24.29 ± 3.7[a]	19.25 ± 0.067[a]
50	54.43 ± 1.089[b]	49.29 ± 1.02[b]	42.9 ± 0.06[b]
100	79.25± 1.445[c]	73.32 ± 1.73[c]	71.64± 0.063[c]
200	86.72 ± 0.782[d]	80.98 ± 1.65[d]	85.85± 0.113[d]
400	90.4 ± 0.591[e]	89.69 ± 2.09[e]	92.92 ± 0.29[e]
F ratio	1650.077*	420.563*	70135.39*
HSD	1.4153	6.0358	0.5424
Reg. Eqn.	y = 23.884 ln(x) - 43.075	y = 29.277 ln(x) - 81.768	y = 27.446 ln(x) - 82.915
R^2 value	R^2 = 0.8803	0.9539	0.9508
IC_{50} (µg/ml)	49.205	90.082	55.441
IC_{50} (standard)	41.006	81.278	158.71

* represents significance at $p \leq 0.05$, data shown are Mean ± S.E. of experiments performed in triplicates, superscript letters indicate significantly different values. All values were compared among each other as well as with standard. Ascorbic acid was used as standard in DPPH assay whereas rutin was used in reducing power and Superoxide anion scavenging capacity assay

TABLE 3: PROTECTIVE EFFECTS OF PVITNOL FRACTION AGAINST LIPID PEROXIDATION AND HYDROXYL RADICAL SCAVENGING ABILITY IN SITE-NON SITE SPECIFIC DEOXYRIBOSE DEGRADATION ASSAY

Concentration (µg/ml)	Lipid peroxidation Inhibition % ± S.E.	Deoxyribose site-specific inhibition % ± S.E.	Deoxyribose non site specific inhibition ± S.E.
5	45.4 ± 1.11[a]	8.63 ± 0.69[a]	1.21 ± 0.76[a]
10	56.9 ± 0.972[b]	27.42 ± 0.56[b]	17.03 ± 0.96[b]
20	60.82 ± 0.715[c]	34.56 ± 0.71[c]	28.45 ± 1.48[c]
40	65.56 ± 0.26[d]	39.57 ± 2.88[c]	55.37 ± 0.59[d]
80	73 ± 0.459[e]	54.84 ± 0.42[d]	63.8 ± 0.38[e]
160	77.56 ± 1.414[f]	75.94 ± 0.55[e]	74.6 ± 1.07[f]
F ratio	256.414*	327.11*	2306.279*
HSD	3.445	6.055	2.854
Reg. Eqn.	y = 8.8755ln(x) + 33.559	y = 17.469ln(x) - 18.227	y = 22.01ln(x) - 33.51
R^2 value	0.972	0.9561	0.978
IC_{50}	6.375	49.678	44.441
IC_{50} (standard)	7.793	137.043	136.286

* represents significance at $p \leq 0.05$, data shown are Mean ± S.E. of experiments performed in triplicates, superscript letters indicate significantly different values. Values were compared with each other as well as with standard based on HSD values. Rutin was used as standard

Kaempferol and some of its glycosides are known to have a wide array of pharmacological activities, such as antioxidant, anti-inflammatory, anti-microbial, anticancer, cardioprotective, neuro-

protective and antidiabetic (reviewed in Calderón Montano *et al*. [44] Kaur and Coworkers [45] reported the antioxidant, antigenotoxic and antiproliferative potential of methanolic extract from *Pteris vittata* L. in several *in-vitro* assays.

It was found that kaempferol was active at a concentration range of 1-50 µg/ml in DPPH assay and 1-100 µg/ml in deoxyribose degradation assay. Festa *et al*., [46] have reported significant antioxidant activity of ellagic acid against H_2O_2 and Bleomycin-induced DNA damage in mammalian cells *in-vitro*. High radical scavenging activity can be attributed to the hydrogen donating ability of phenolic compounds in Pvitnol. Pvitnol has potential to scavenge hydroxyl radicals as verified by results of DNA nicking and deoxyribose degradation assays. Pvitanol effectively protected pBR322 DNA against damage induced by Fenton's reagent. DNA was maintained in superociled form (Form I), possibly by hydrogen abstraction mechanism. Form II (single-stranded nicked DNA) and Form III (double-stranded nicked and linear DNA) DNA was minimized **Fig. 3**.

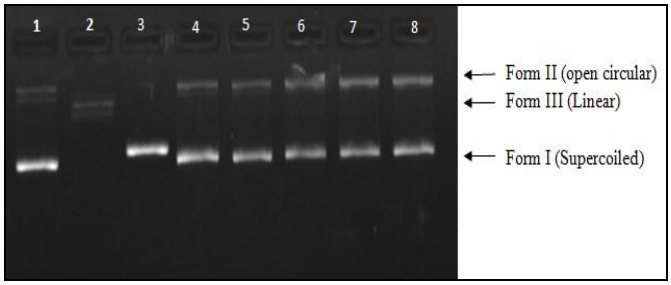


FIG. 3: DNA NICKING ASSAY FOR DIFFERENT CONCENTRATIONS OF PVITNOL FRACTION OF *PTERIS VITTATA* L. LANE 1: DNA DAMAGE CONTROL; LANE 2: FENTON'S REAGENT; LANE 3: POSITIVE CONTROL (RUTIN); LANE 4-8: FENTON'S REAGENT + DIFFERENT CONCENTRATIONS OF PVITNOL (25, 50, 100, 200, 400 MG/ML RESPECTIVELY)

Cancer cells show a cellular redox imbalance compared with normal cells and have been related to oncogenic stimulation. DNA mutation is a critical step in carcinogenesis, and elevated levels of oxidative DNA lesions (8-OH-G) have been noted in various tumors. DNA damage has been predominantly linked with the initiation process [47]. Ehtyl acetate fraction from *Cassia fistula* showed protection against thioacetamide-induced oxidative stress in male wistar rats [48]. The protective activities of Pvitnol on DNA may be attributed to the presence of flavonoids and phenolic compounds like ellagic acid, kaempferol, and rutin, which can prevent the production of ROS by forming complexes with cations like Cu and Fe that are involved in hydroxyl radical formation [49]. Some phenolic compounds and extracts have been reported to have cytoprotection capability [50, 51]. There is compelling evidence from these reports that phenolic compounds can protect DNA damage caused by oxidants through antioxidant activities.

Antiproliferative Activity: Pvitnol exhibited the potency to inhibit the growth of human MCF-7 breast cancer cells. An inhibition of 74.43% was observed at a concentration of 200 µg/ml of Pvitnol in MTT assay ($y = 21.21 \ln(x) - 37.16$, $r^2 = 0.987$). Several medicinal plants are known to have apoptosis-inducing ability [52]. The anti-proliferative potential of plant extracts toward human breast cancer MCF-7 cells has been reported earlier. It was observed that on treatment with Pvitnol fraction at a concentration of 150 µg/ml, nuclei of MCF-7 cells showed condensation and fragmentation, whereas the control cells showed prominent nuclei without any indication of apoptosis **Fig. 4**.

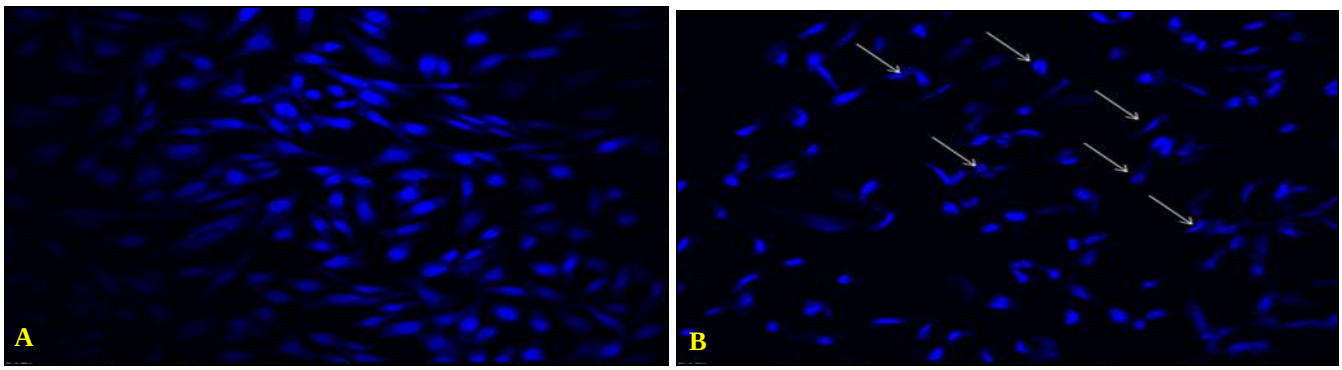


FIG. 4: NUCLEAR MORPHOLOGICAL ALTERATIONS OBSERVED IN MCF-7 CELLS AFTER TREATMENT WITH TEST FRACTION FOR 24 h. (A) CONTROL (B) PVITNOL FRACTION (150 µg/ml). CELLS WERE STAINED WITH DAPI. ARROWS DESIGNATE CELLS EXHIBITING NUCLEAR CONDENSATION, FRAGMENTATION, AND FORMATION OF APOPTOTIC BODIES

Phenolic compounds are responsible for many cellular responses like cytotoxicity, cell cycle arrest, and apoptotic activities by activating a cascade of molecular events. Kaur *et al.*, [53] investigated antiproliferative activities of n-hexane fraction of *Cassia fistula* in human cervical cancer (HeLa) cells and concluded that it effectively reduced the viability of cells by inducing G0/G1-phase arrest of the cell cycle. It also increased p53 and Bad and decreased Bcl-2 gene expression, and promoted caspase-3 activity. Ellagic acid is known to induce apoptosis in HOS cells through upregulation of Bax and activation of caspase-3 [42]. Reduction of cancer cell proliferation and induction of apoptosis in human osteosarcoma MG-63 cells by epiafzelechin has been reported by Kaur *et al.* [54] Bhosle *et al.*, [55] have also reported modulatory effects of ellagic acid in radiation-induced oxidative stress and cytotoxicity in tumor cells.

DNA single- and double-strand breaks are induced due to oxidative stress by oxygen and various radical species and have been implicated in several human diseases, including cancer. Antioxidants from natural sources have shown the ability to protect DNA against such injury. The neutral SCGE comet assay is an appropriate tool for the detection of DSBs and possible cell death mechanism (apoptosis or necrosis) [56]. SCGE comet assay profile confirmed that the majority of DNA migrated towards the tail of the comet instead of the head, which is a strong indication that Pvitnol can cause double-strand breaks (DSB) **Fig. 5**. These DNA breaks could have lead to the execution of apoptosis.

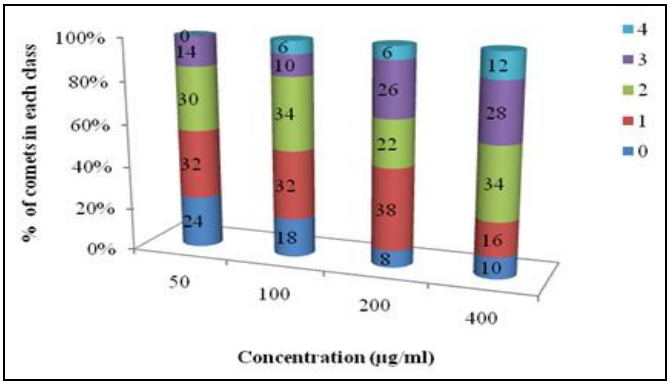


FIG. 5: DETECTION OF DSBS IN MCF-7 CELLS TREATED WITH VARIOUS CONCENTRATIONS OF PVITNOL FRACTION EVALUATED USING COMET ASSAY. *P≤0.05

CONCLUSION: Results of the present investigation strongly indicate that Pvitnol fraction of *Pteris vittata* L. harbors various polyphenolic constituents, which may be major contributors to its antigenotoxic, antioxidant and antiproliferative properties. The fraction could be used as a potential source of antioxidants linked with health benefits and an alternate adjunctive. It is conceivable that *P. vittata* could be exploited as one of the potential sources for plant-based pharmaceutical products. Further research is underway to explore its chemotherapeutic potential and mechanism of action *in-vivo* against oxidative mutagens.

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