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Antioxidant and cytotoxic properties of *Pavetta crassicaulis* Bremek. leaf crude extract and its isolated pure compound

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Pavetta crassicaulis Bremek.. (Family Rubiaceae), an important ethnomedicinal shrub/small tree, belonging to the family Rubiaceae, grows up to 4 meters tall. The plant is endemic to peninsular India. Plant parts have many medicinal properties used in the treatment of arthritis, boils and itches, hemorrhoidal pains, dropsy, epilepsy, urinary complaints, and anticephalagic. Fruits used as anthelmintic and flowers are eaten fried. The antioxidant experiments conducted were metal chelating activity, superoxide radicals, hydroxyl radical, DPPH radicals, ABTS radical scavenging assays. *In vitro* cytotoxicity was tested in DLA and EAC cancer cells by using Trypan blue dye exclusion technique and MTT assay. The results revealed that the leaf ethanolic crude extract of the *Pavetta crassicaulis* Bremek. has appreciable radical scavenging activity and its extracted pure compound, 2-Tert-Butyl-4,6-Bis(3,5-Di-Tert-Butyl-4-Hydroxybenzyl)Phenol, showed appreciable radical scavenging activity but not as good as the standards used. The cytotoxic experiment revealed that leaf ethanolic extract has considerable cytotoxicity activity and its pure compound 2-Tert-Butyl-4,6-Bis(3,5-Di-Tert-Butyl-4-Hydroxybenzyl)Phenol also showed noticeable activity which is not comparable to the standard Curcumin.

Keywords: Antioxidant, Cytotoxic, DLA, *Pavetta crassicaulis* Bremek, EAC, 2-Tert-Butyl-4,6-Bis(3,5-Di-Tert-Butyl-4-Hydroxybenzyl)Phenol.

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Introduction

Most of the free radicals divided into derivatives of oxygen like superoxide, hydrogen peroxide and derivatives of nitrogen like nitric oxide and peroxynitrite were an influence on biological systems[1].

Reactive oxygen species induce toxic effects like inactivation of enzymes and alteration of intracellular oxidation-reduction state[2]. It can influence the DNA modifications and chromosome aberrations leading to carcinogenesis[3,4].

The free radicals damage on the cell/tissues is neutralised by antioxidants such as á-tocopherol, carotenoids, glutathione, thiols, vitamin C etc., by scavenging and decreasing their formation. Most of the plants have several natural compounds exhibiting antioxidant and/or radical scavenger properties[5].There is clear evident proof that most of the phenolic compounds present in the plant possess antioxidant properties[6].

Many of the plant extracts are extensively used as medicinal compounds for the treatment of various ailments in different parts of the world, especially in under developing and developing countries[7-9].

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The Indian subcontinent is a vast repository of medicinal plants that are used in traditional medical treatments which also forms a rich source of knowledge[10]. India is one of the megadiversity centres on the planet having a diverse medicinal plant species, which is unexplored and most of them are endemic. India shares approximately 13 % of world's biodiversity, one among 17 mega diversity centres. Among the 34 hotspots in the world, India has four hotspots, viz., Eastern Himalaya, Indo-Burma, Western Ghats, and the Andaman and Nicobar Islands. The various indigenous systems use several plant species to treat different ailments[11]. In India, around 20,000 medicinal plant species have been recorded recently, but more than 500 traditional communities use about 800 plant species for curing different diseases[12].

Experimental medicinal plant and description

Pavetta crassicaulis Bremek. is an important ethnomedicinal shrub or small tree belonging to the family Rubiaceae. It grows up to four meters tall and is endemic to peninsular India. The leaves are often membranous with dark bacterial nodules, having small white tubular flowers, sometimes salviform or funnel-shaped with four spreading petal lobes. The flowers are

carried on terminal corymbs or cymes, the leaves are elliptical-oblong to elliptic-lanceolate, 6-15 cm long, and pointed at both ends. The flowers are white, rather fragrant, and borne in considerable number in hairy terminal panicle which is 6-10 cm long. The sepals are very small and toothed. The flowers tube is slender and about 1.5 cm long, with obtuse petals above half the length of the tube. The fruits are black when they dry, somewhat rounded and about 6 mm in diameter[13].

P. crassicaulis Bremek. is endemic to peninsular India and distributed in many states like, Mizoram, Gujarat, Uttara Pradesh, Chhattisgarh, Orissa Maharashtra and Karnataka[14-16].

The plant has medicinal properties used in the treatment of arthritis, boils and itches, hemorrhoidal pains, visceral problems, dropsy[17], epilepsy, hemorrhoids[18], skin diseases[19], anticephalagic, fat burner, aphrodisiac[20], urinary complaints and fruits used as anthelmintic and flowers are eaten fried[21].

The preliminary qualitative phytochemical analysis of *P. crassicaulis* Bremek. methanolic crude extract revealed the presence of alkaloids, steroids and terpenoid with moderate antimicrobial activity[22-23].

Despite many works on *P. crassicaulis* Bremek, antioxidant and cytotoxicity properties have not been studied in detail. Therefore, the study aimed to provide basic data on the antioxidant and cytotoxic study of *P. crassicaulis* Bremek plant parts.

Materials and Methods

Plant collection and authentication

The leaf of *P. crassicaulis* Bremek. was collected from Shringeri taluk, Karnataka in April 2014.(13.4198° N, 75.2567° E) The plant was identified by Prof. K G Bhat, Udupi and a voucher specimen was conserved under the reference number KU/AB/RN/AS/002.

Extraction

The samples were dried in the shade for 20 to 25 days, mechanically powdered and subjected to Soxhlet extraction using ethanol and aqueous extracts[24]. The crude extracts were collected in air-tight plastic containers and stored in cool condition.

GC-MS analysis

Plant extracts were subjected to gas chromatography-mass spectrometry (GC-MS); obtained spectra were analysed. GC Model: Thermo Trace GC Ultra, MS Model: Thermo DSQ II, Ionization: Electron Impact Ionisation (EI), Chemical Ionisation (CI), Mass Range: 1 - 1074 m/z.

Chemicals used

2,2-Diphenyl-2-picrylhydrazyl(DPPH), 2,2-azino-bis(3-ethylbenzothiazoline)-6-sulfonic acid (ABTS), ascorbic acid, butylated hydroxyl anisole (BHA), Ferrozine, gallic acid, ferrous chloride, Folin– Ciocalteu reagent, nitro blue tetrazolium sodium salt (NBT), Nicotinamide adenine dinucleotide phosphate reduced (NADH), phosphate buffered saline (PBS) and trichloroacetic acid (TCA), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, Trypan blue. All other chemicals and solvents used were of analytical grade.

Qualitative phytochemical screening for phenols and flavonoids

Total phenolic content

Total phenolic content was determined by the Folin-Ciocalteau method[25]. A dilute concentration of extract (0.5 mL) was mixed with 0.5 mL of 1:1 diluted Folin-Ciocalteu reagent and 4 mL of sodium carbonate (I M). The mixtures were allowed to stand for 15 min, and the absorbance was measured against the blank at 765 nm colourimetrically. A standard curve was plotted using different concentrations of Gallic acid (standard, 0-1000 µg/mL of ethanol). The total phenolic content of solvent extracts was estimated as µg Gallic acid equivalents (GAE)/mg of extract. All samples were analysed in triplicate.

Determination of total flavonoid content

Total flavonoid content was determined[26] using quercetin as standard. Briefly, 5 mL of extracts (200 µg) in Millipore water was mixed with 300 µL of sodium nitrite (5 %) and 300 µL of aluminum chloride (10 %), this reaction mixture was incubated at room temperature for 6 min followed by the addition of 2 mL of sodium hydroxide (1 M). Later, the volume in each test tube was made up to 10 mL by adding 2.4 mL of Millipore water. Absorbance was measured against the blank at 510 nm. Total flavonoid content of the extract was expressed in terms of equivalent to Quercetin (EQ, µg/mg of dry mass).

In vitro antioxidant activity

Total antioxidant capacity

Total antioxidant capacity[27] of extracts were analysed using 300 µL of extracts at different concentrations (0-160 µg) was combined with 3 mL of reagent mixture (4 mM ammonium molybdate, 0.6 M sulfuric acid and 28 mM of sodium phosphate). The tubes were capped and kept for incubation at 95 °C for 90 min, after cooling to room temperature, the absorbance of the content was measured at 695 nm against the blank. The total antioxidant capacity of each extract is expressed as equivalents of ascorbic acid.

Total reductive capability

Total reduction capability[28] of extracts was analysed using different concentration of extracts (0-300 µg) in 1 mL of water was mixed with 2.5 mL of phosphate buffer (0.2 M, pH 6.5) and 2.5 mL of potassium ferricyanide (1 %). The mixtures were incubated at 50 °C for 20 min, and 2.5 mL of 10 % trichloro acetic acid were added. Then the mixture was centrifuged for 10 min at 3000 rpm. 2.5 mL of water and 0.5 mL of ferric chloride (0.1 %) was added to 2.5 mL of supernatant. The absorbance was measured at 700 nm against blank. A higher absorbance of the reaction mixture indicated greater reducing power. Total reducing capacity of each extract is expressed as equivalents of quercetin.

Scavenging of superoxide radicals

Superoxide radical scavenging activity was determined by the NBT reduction method[29]. The reaction mixture contained 6 µM EDTA, 0.0015 % NaCN, 2 µM riboflavin, 50 µM NBT, various concentrations of extract, and phosphate buffer (67 mM, pH 7.8) in a final volume of 3 mL. The tubes were uniformly illuminated with an incandescent lamp for 15 min, and the optical density was measured at 560 nm before and after illumination. The percentage inhibition of superoxide radical generation was evaluated by comparing the absorbance values of control and experimental tubes.

Scavenging of hydroxyl radical

Hydroxyl radicals generated from Fe^{2+}/ascorbate/H system degrades deoxyribose producing thiobarbituric acid reacting substance (TBARS)[30]. The efficacy of the extracts to inhibit TBARS formation was assessed. The reaction mixture contained 2.8 mM deoxyribose, 0.1 mM $FeCl_3$, 0.1 mM EDTA, 1 mM H_2O_2, 0.1 mM ascorbic acid, 20 mM KH_2PO_4–KOH (pH 7.4), and various concentrations of extracts in a final volume of 1 mL. The reaction mixture was incubated for 1 h at 37 °C. The TBARS formed was measured[31] and the percentage inhibition was calculated from the optical measurements of control and experimental tubes.

Scavenging of DPPH radicals

Stable radical, 2, 2-diphenyl-1-picryl hydrazyl (DPPH) in methanol was used as a substrate to evaluate antioxidant activity. The method is based on the reduction of DPPH radical in the presence of hydrogen donating antioxidants leading to the formation of a non-radical form DPPH-H by the reaction. DPPH in its radical form has an absorption peak at 515 nm which disappeared upon reduction by antioxidant compounds. Absorbance was measured 20 min after the reaction was started.

Radical scavenging activity was calculated using the following formula

$$Percentage\ inhibition = \frac{Absorbance\ of\ control - Absorbance\ of\ test}{Absorbance\ of\ control} \times 100 \quad \ldots (1)$$

IC_{50} value was calculated using the following formula

$$IC_{50} = \frac{Sum\ of\ extract\ concentration}{Sum\ of\ percentage\ of\ inhibition\ at\ diff\ conc.} \times 50 \quad \ldots (2)$$

Scavenging of ABTS radicals

ABTS (2, 2-azobis-3-ethylbenthiozoline-6-sulfonic acid) radical scavenging activity[32] of the extract was determined based on the principle that the oxidation of ABTS to its cation radicals by ferryl myoglobin is formed in the reaction of H_2O_2 and metmyoglobin. Briefly, the stock solutions of 500 µM ABTS diammonium salt, 400 µM myoglobin (MbIII), 740 µM potassium ferricyanide, and 450 µM H_2O_2 were prepared in PBS (pH 7.4). Metmyoglobin was prepared by mixing equal volumes of myoglobin and potassium ferricyanide solutions. The reaction mixture (2 mL) contained ABTS (150 µM), MbIII (2.25 µM), and varying concentrations of extracts in PBS. The reaction was initiated by adding 75 µM H_2O_2 and oxidation reaction was monitored at 734 nm.

Metal chelating activity

The chelation of ferrous ions[33] experiment was done with 3 mL of extracts at different concentrations taken in different test tubes followed by the addition of 50 µL of ferrous chloride (2 mM). The reaction was initiated by the addition of 20 µL ferrozine (5 mM), and then the mixture was shaken vigorously and allowed to stand for 10 min at room temperature. After equilibrium, absorbance of the solution was measured at 562 nm against the blank. EDTA was used as standard for comparison. Percentage of inhibition and IC_{50} value was calculated using equation (1) and equation (2).

***In vitro* cytotoxicity assay**

Cell lines

EAC (Ehrlich's Ascites Carcinoma): Paul Ehrlich found the initial tumour for the Ehrlich's Ascites carcinoma in 1905. The ascites variant was obtained in 1932 by intraperitoneal transplantation of Ehrlich's solid adenocarcinoma.

DLA (Dalton's Lymphoma Ascites): The initial tumour for the DLA arose as a Spontaneous Carcinoma in the thymus of mice in 1947. The cell lines were obtained from Amala Cancer Research Centre, Thrissur.

Trypan blue dye exclusion technique

Any compound, which is cytotoxic to cells, inhibits the cell proliferation and kills the cells. Trypan blue[34] can penetrate into the dead cells and give it a blue colour. This method gives an exact number of dead and viable cells[35].Cells were aspirated from the peritoneal cavity of tumor-bearing mice, and it was washed three times using phosphate buffered saline (PBS). The viability of cells was checked using trypan blue(cell viability should be above 98 %).

The cell suspension was added to tubes containing various concentrations of the test compounds, and the volume was made up to 1 mL using PBS. Control tubes containing only cell suspension. These assay mixtures were incubated for 3 h at 37 °C, and then 1 mL of trypan blue was added after incubation, and the number of dead cells were counted using a haemocytometer[36]. The percentage cytotoxicity was calculated using the following equation.

$$\%\ Cytotoxicity = \frac{No.\ of\ dead\ cell}{No.\ of\ viable\ cell + No.\ of\ dead\ cells} \times 100$$

MTT assay

The ability of the cells to survive a toxic insult is the basis of most cytotoxic assays. This assay is based on the assumption that dead cells or their products do not reduce tetrazolium. The assay depends both on the mitochondrial activity per cell and number of cells present. The cleavage of 3-(4,5-dimethylthiazole-2yl); 2, 5-diphenyl tetrazolium bromide (MTT) to a blue formazan derivative by living cells is a very effective principle on which the assay is based. The principle involved is the cleavage of tetrazolium salt, 3-(4, 5-dimethyl thiazole-2yl); 2, 5-diphenyl tetrazolium bromide into a blue coloured product (formazan) by mitochondrial enzyme succinate dehydrogenase. The number of cells was found to be proportional to the extent of formazan production by the cells used[37]. Cells were seeded in a 96-well flat-bottom plate (5000 cells/well) and permitted to adhere for 24 h at 37 ^{0}C with 5 % CO_2 atmosphere. Different drug concentration was added and incubated further for 48 hrs. Before 4 h of the completion of incubation, 20 µL of MTT (5 mg/mL) was added. Dead cell percentage was determined using an ELISA plate reader set to record absorbance at 570 nm. The percentage growth inhibition was calculated using the formula given below[38].

$$\%\ Growth\ inhibition = 100\ \frac{OD\ of\ individual\ test\ group}{OD\ of\ control\ group} \times 100$$

Results

Extract yield and Preliminary qualitative phytochemical analysis

The soxhlet extraction of *P. crassicaulis* Bremek. leaf (750 g) with petroleum ether gives 12.32 g, with chloroform gives 19.99 g, with ethanol gives 75.35 g and with water gives 45 g yield.

The qualitative phytochemical analysis of *P. crassicaulis* Bremek. leaf extracts revealed that leaf petroleum ether and chloroform extracts showed nil phytoconstituents and leaf ethanolic extract confirms the presence of saponins, tannins, flavonoids, steroids/sterols, glycosides and phenols and in aqueous extract confirms the presence of saponins and glycosides (Table 1).

Table 1 — Qualitative preliminary phytochemical analysis of different leaf and flower extracts of *Pavetta crassicaulis* Bremek.

Secondary Metabolites	Type of tests	Petroleum ether crude extract	Chloroform crude extract	Ethanolic crude extract	Aqueous crude extract
		-	-	Leaf	Leaf
Alkaloids	Mayer's test	-	-	-	-
	Wagner's test	-	-	-	-
Saponins	Foam test	-	-	+	+
Tannins	Ferric chloride test	-	-	+	-
Flavonoids	Shinda test	-	-	+	-
	Zinc -HCl reduction test	-	-	+	-
	Alkaline reagent test	-	-	+	-
	Lead acetate test	-	-	+	-
Steroids	Salkowaski test	-	-	+	-
Glycosides	Keller-Killianis test	-	-	+	+
	Brown water test	-	-	+	-
	Legal test	-	-	+	-
Phenols	Ferric chloride test	-	-	+	-
	Acetic acid test	-	-	+	-
Sterols	Liebermann burchad test	-	-	+	-

+: positive result, -: Negative result

Quantitative phenol and flavonoid analysis

Total phenolic content in leaf ethanolic extract was expressed as equivalent to gallic acid (EGA) and was found to be 654.3±0.32 of dry sample, respectively. The total flavonoid content of the sample was found 385.1±0.95 of dry extract as equivalent to quercetin in samples respectively.

GC-MS analysis of leaf ethanolic crude extract

In GC-MS analysis of medicinal *P. crassicaulis* Bremek. ethanolic leaf extract revealed the presence of 36 compounds, but peak 5 is undetected and peak 33 and 34 is the same compound in different quantity, in that major percentage of compounds present were 2-Tert-Butyl-4,6-Bis(3,5-Di-Tert-Butyl-4-Hydroxybenzyl), Phenol (38.84 %), Cyclo{tetra[(5-t-butyl-2 hydroxy-1,3-phenylene)methylene]} (15.72 %), 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- (9.34 %), Bicyclo[3.3.1]nona-3,7-diene-2,9-dione (6.25 %), Methyl Ester Of Bicyclo[4.3.0]Non-1(6)-En-4,7-Dione-8-Carboxylic acid (5.66 %). The compounds such as 2-Tert-Butyl-4, 6-Bis (3, 5-Di-Tert-Butyl-4-Hydroxybenzyl) Phenol (37.44 %) has anti-oxidant and UV stabilizing properties. Cyclo{tetra[(5-t-butyl-2-hydroxy-1,3-phenylene)methylene]} (15.72 %), 4H-Pyran-4-one and 2,3-dihydro-3,5-dihydroxy-6-methyl- (9.34 %) has antioxidant properties (Table 2; Fig. 1 and 2).

***In vitro* antioxidant properties**

In the all the experiments leaf aqueous extract showed less anti-oxidant activity compared to the leaf ethanolic crude extracts, so in the following, only the leaf ethanolic extract was compared with pure compound and standard.

Total antioxidant and reductive capability

The total antioxidant activity of both ethanolic crude extract and the pure compound had shown significant antioxidant activity. The pure compound 2-Tert-Butyl-4,6-Bis(3,5-Di-Tert-Butyl-4-Hydroxybenzyl)Phenol activity was comparable to that of standard ascorbic acid.

However, ethanolic crude extract and pure compound have shown appreciable reductive capability when compared to standard quercetin. Like antioxidant activity, the reducing power of ethanolic crude extract and pure compound were increased with

Table 2 — Presence of metabolites in GC-MS analysis of crude ethanolic extract of *Pavetta crassicaulis* Bremek. leaf collected from Western Ghats Karnataka

S. No.	Chemical compound present	Average percentage	Properties of the compound
1	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	0.53	Food-grade flavor ingredients[39]
2	2,5-Dimethyl-4-hydroxy-3(2H)-furanone	0.52	Flavor and perfume industry[40]
3	2-Hexanone, 3-methyl-4-methylene-	1.45	Paint and paint thinner[41]
4	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	9.34	Mutagen Antimicrobial, anti-inflammatory and antioxidant capacity[42-44]
5	4-[4-Chlorophenyl]-N-[2-[1-methyl-2-pyrrolidinyl]ethyl]-6-[trichloromethyl]-2-pyrimidine	1.14	Unknown
6	2,3-Dihydro-Benzofuran -	1.35	Entactogen drug of the phenethylamine and amphetamine classes, cytotoxic[45]
7	2-Furancarboxaldehyde, 5-(hydroxymethyl)-	0.66	Food additives, Antimicrobial, Preservative, Flavoring Agents[46-47]
8	1,2,3-Propanetriol, 1-acetate	1.46	Unknown
9	6-Oxoheptanoic acid	1.53	6-Oxoheptanoic acid is a reagent to synthesise new penicillin containing keto acids as side chains. It is also used to study the various metabolic pathways of 4-hydroxypentanoate and Levulinate[48]
10	Benzaldehyde, 4-hydroxy-	0.47	flavour and fragrance agents[49]
11	2-Methoxy-4-vinylphenol	1.14	Flavoring agent, antibacterial activity, anti-inflammatory effect[50-52]
12	Phenol, 2-methoxy-4-(2-propenyl)-	0.39	flavoring agent used in the manufacture of vanillin, Anti-Infective Agents, Anti-oxidant[53-54]
13	2,4-Dimethyl-3-nitrobicyclo[3.2.1]octan-8-one	0.78	Oils obtained from myrrh and frankincense and parthenium weed have little percentage[55-56]
14	rac-2,4-Dimethyl-3-nitrobicyclo[3.2.1]octan-8-one	0.96	Unknown
15	Benzaldehyde, 2-hydroxy-6-methyl-	0.98	Pheromone of the acarid mite *Tyrophagus pernicious* and Grain Mite *Aleuroglyphus ovatus*[57-58]

(*Contd.*)

Table 2 — Presence of metabolites in GC-MS analysis of crude ethanolic extract of *Pavetta crassicaulis* Bremek. leaf collected from Western Ghats Karnataka — (*Contd*.)

S. No.	Chemical compound present	Average Percentage	Properties of the compound
16	2(3H)-Naphthalenone, 4,4a,5,6-tetrahydro-	0.77	Unknown
17	1,5-Diazocine, octahydro-1,5-dinitro-	0.40	Unknown
18	Methyl Ester Of Bicyclo[4.3.0]Non-1(6)-En-4,7-Dione-8-Carboxylic acid	5.66	Unknown
19	Acetic acid, (2-isopropenyl cyclopentylidene)-, methyl ester	0.83	Unknown
20	Bicyclo[3.3.1]nona-3,7-diene-2,9-dione	6.25	Unknown
21	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	1.32	Antimicrobial, Antioxidant, Anti-inflammatory, Analgesic[46]
22	2-Methyl-5-(4-methylphenyl)tetrazole	0.71	Unknown
23	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]-	1.12	Antimicrobial, anticancer, anti-inflammatory, diuretic, Cytotoxic, Flavoring Agents, used in the preparation of vitamins E and K1. It is also a decomposition product of chlorophyll, used in the treatment of arthritis[59-60, 43]
24	Hexadecanoic acid, methyl ester	0.29	Perfumes and Cosmetics [61]
25	Hexadecanoic acid	2.04	Perfumes, Cosmetics, Enzyme Inhibitors, Surfactants, Flavoring Agents, Adhesives and sealant chemicals, Agricultural chemicals (non-pesticidal), Fillers, Finishing agents, Intermediates, Lubricants and lubricant additives, Surface active agents, antiandrogenic flavour, hemolytic, 5-Alpha-reductase inhibitor[62-63]
26	Octanal, 7-methoxy-3,7-dimethyl-	0.37	Unknown
27	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	0.28	Anti-bacterial, Anti-inflammatory, hypocholesterolemic, cancer preventive, hepatoprotective, nematicide, insectifuge, antihistaminic, antieczemic, antiacne, 5-Alpha reductase inhibitor, anti-androgenic, anti-arthritic, anti-coronary, insectifuge[43]
28	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	0.62	Wound healing activity, Hemolytic, pesticide, flavour, antioxidant[64]
29	9,12-Octadecadienoic acid (Z,Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	0.30	Hypocholesterolemic, Nematicide, Anti-arthritic, Hepatoprotective, Anti- androgenic,Hypocholesterolemic, Nematicide, 5-Alpha reductase inhibitor, Anti-histaminic,Anticoronary Insectifuge,Anti-eczemic,Anti-acne[65]
30	2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-	0.52	Bactericide, Antifungal, Cytotoxic, Antibacterial, Antioxidant, Antitumor, Cancer preventive, Immunostimulant, Chemopreventive, Lipoxygenase-inhibitor, Perfumery, Pesticide and Sunscreen[66]
31	2-Tert-Butyl-4,6-Bis(3,5-Di-Tert-Butyl-4-Hydroxybenzyl)Phenol	38.84	Unknown
32	Cyclo{tetra[(5-t-butyl-2-hydroxy-1,3-phenylene)methylene]}	15.72	Unknown
33	Stigmast-5-en-3-ol, (3.beta.,24S)-	0.95	Antimicrobial antioxidant, Anti-inflammatory anti-arthritic, anti-asthma, diuretic[67]

increasing concentration. Hence the pure compound 2-Tert-Butyl-4,6-Bis(3,5-Di-Tert-Butyl-4-Hydroxy benzyl)Phenol, can serve as free radical inhibitors or scavengers, which are much capable as standard used (Fig. 3-6).

DPPH radical scavenging activity

DPPH radical scavenging activity was expressed in terms of scavenging percentage and IC_{50} values (µg/mL). By the experiment, it is revealed that leaf aqueous crude extracts show negligible scavenging activity compared with leaf ethanolic extract in all the tested concentrations. Compared the crude extracts, leaf (IC_{50}:71.09±0.39 µg/mL) ethanolic crude extract showed appreciable activity compared to the leaf aqueous (IC_{50}: 113.68±1.48 µg/mL) extract. The pure compound 2-Tert-Butyl-4,6-Bis(3,5-Di-Tert-Butyl-4-Hydroxybenzyl)Phenol (IC_{50}: 46.14±1.50 µg/mL)

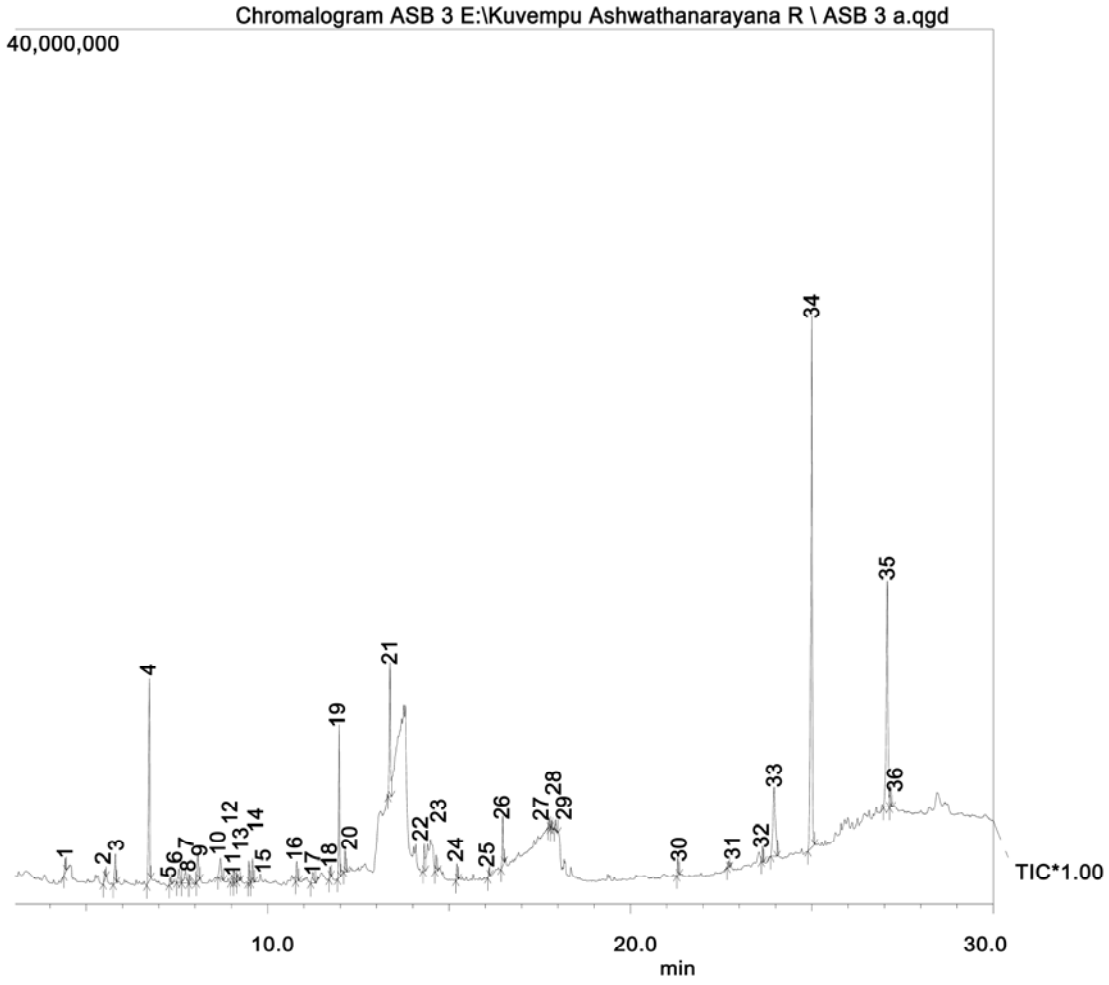

Fig. 1 — GC-MS chromatogram of *Pavetta crassicaulis* Bremek leaf ethanolic extract

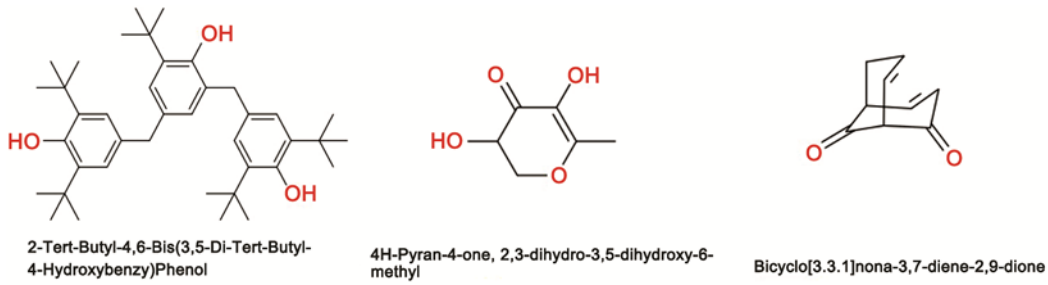

Fig. 2 — Major compounds present in ethanolic extract of *Pavetta crassicaulis* Bremek. leaf and their structure

displayed appreciable radical scavenging activity which is almost comparable to the standard Ascorbic acid (IC_{50}: DPPH: 39.48±0.02 µg/mL) used (Table 3 and Table 8).

ABTS free radical scavenging activity

ABTS radical scavenging activity was expressed in terms of percentage of inhibition and IC_{50}: values (µg/mL). Results revealed that leaf ethanolic crude extract shows excellent ABTS radical scavenging activity compared with the aqueous crude leaf extract. The leaf ethanolic extract (IC_{50}:171.5±3.50 µg/mL) demonstrated appreciable ABTS radical scavenging activity compared to the leaf aqueous extract (278.96±1.50 µg/mL). The pure compound 2-Tert-Butyl-4,6-Bis(3,5-Di-Tert-Butyl-4-Hydroxybenzyl)Phenol which is extracted from leaf ethanolic extract showed excellent radical scavenging activity (IC_{50}: ABTS: 88.14±0.56 µg/mL,) showed excellent activity which is comparable with the standard

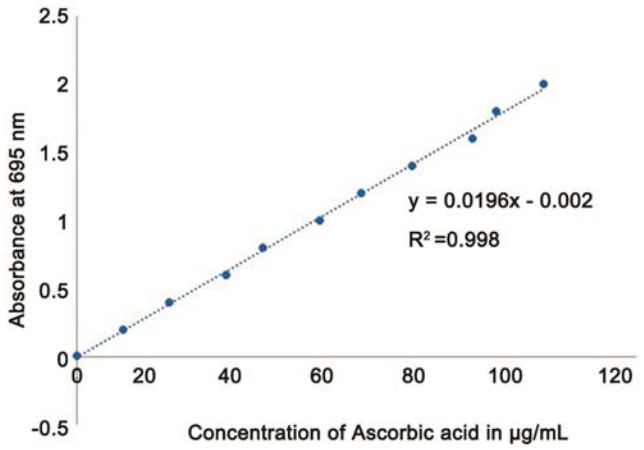

Fig. 3 — Total antioxidant activity

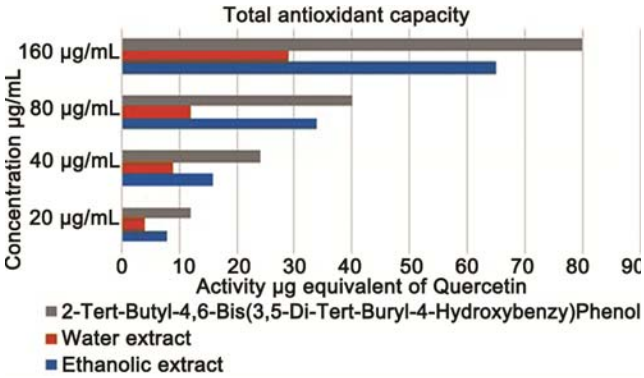

Fig. 4 — Total antioxidant properties of ethanol, water extract with pure compound

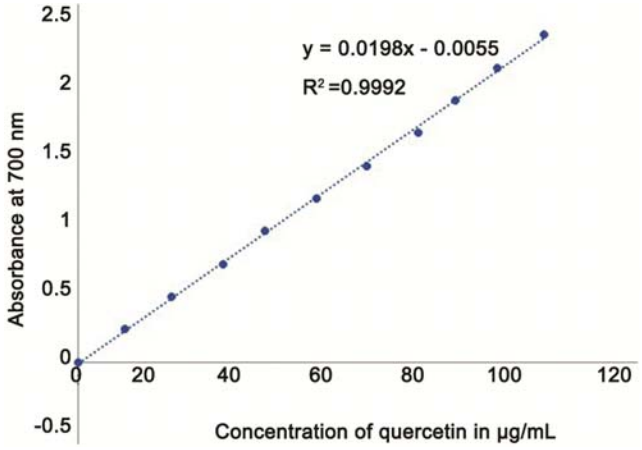

Fig. 5 — Total reductive properties

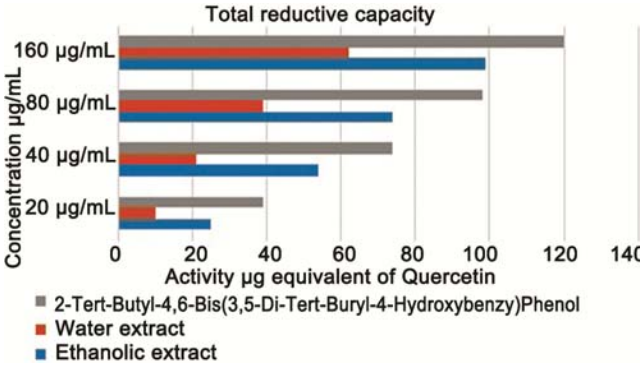

Fig. 6 — Total reductive properties of ethanol, water extract with pure compound

Butylated Hydroxylanisole (IC_{50}: ABTS: 66.92±0.36 µg/mL) (Table 3 and Table 8).

Metal chelating activity

In metal chelating activity experiment leaf ethanolic crude extracts showed (IC_{50}:448.36±1.50 µg/mL) appreciable metal chelating activity compared to the leaf aqueous crude extracts (IC_{50}:1441.87±1.15 µg/mL). The pure compound 2-Tert-Butyl-4,6-Bis(3,5-Di-Tert-Butyl-4-Hydroxybenzyl)Phenol, showed excellent metal chelating activity (IC_{50}: 307.48±3.45 µg/mL) which is lesser value than the standard EDTA (IC_{50}:213.69±0.32 µg/mL) used (Table 4 and Table 10).

Superoxide radical scavenging activity (NBT)

In Superoxide radicals scavenging NBT reduction method, both leaf and flower aqueous crude extracts shows moderate activity but compared to the aqueous extract (IC_{50}: 396.44±2.69 µg/mL) to leaf ethanolic crude extract (IC_{50}:227.42±1.32 µg/mL) showed appreciable radical scavenging activity. The pure compound 2-Tert-Butyl-4,6-Bis(3,5-Di-Tert-Butyl-4-Hydroxybenzyl)Phenol, showed excellent radical scavenging activity (IC50: 148.09±0.50 µg/mL) compared to standard Gallic acid (IC_{50}:102.17±0.49 µg/mL) (Table 4 and Table 9).

Hydroxyl radical scavenging activity

In this experiment all the crude extracts showed appreciable activity compared to leaf aqueous extract (IC_{50}: 623.67±2.20 µg/mL) the leaf ethanolic extract (IC_{50}: 284.42±2.50 µg/mL) showed excellent scavenging activity.The pure compound "2-Tert-Butyl-4,6-Bis(3,5-Di-Tert-Butyl-4-Hydroxybenzyl)Phenol" shows excellent activity (IC_{50}: 213.48±1.45 µg/mL) nearer to the value of the standard Gallic acid (IC_{50}: 200.51±2.45 µg/mL) used (Table 5 and Table 9).

***In vitro* cytotoxic properties**

Experiment was conducting using DLA and EAC cancer cell lines. The aqueous extract was not used because the leaf ethanolic extract in preliminary phytochemical analysis showed positive confirmation results for almost all secondary metabolites and also

Table 3 — DPPH and ABTS Radical Scavenging activity of *Pavetta crassicaulis* Bremek. leaf extracts

Activity	Plant part	Extracts	Concentration in µg/mL	scavenging activity	IC_{50} value	Standard Ascorbic acid scavenging activity	IC_{50} value of Standard Ascorbic acid
DPPH radical scavenging activity	Leaf	Aqueous	25	22.54±0.2	113.68±1.48	76.23±0.23	39. 48± 0.02
			50	34.52±0.05		82.32±0.43	
			75	45.54±0.2		113.11±0.09	
			100	50.2±0.1		134.54 ± 0.91	
			125	54.5±0.05		156.43± 0.02	
			150	60.66±0.05		176.65 ± 0.34	
			175	62.34±0.1		189.41 ± 0.54	
			200	65.54±0.4		210.87±0.32	
		Ethanol	25	44.55±0.66	71.09±0.39	76.23±0.23	
			50	57.77±0.03		82.32±0.43	
			75	65.48±0.05		113.11±0.09	
			100	77.65±0.65		134.54 ± 0.91	
			125	87.32±0.1		156.43± 0.02	
			150	92.42±0.66		176.65 ± 0.34	
			175	100.23±0.35		189.41 ± 0.54	
			200	107.54±0.54		210.87±0.32	
Activity	Plant part	Extracts	Concentration in µg/mL	scavenging activity	IC_{50} value	Standard µg/mL (Butylated Hydroxyl Anisole)	IC_{50} value of Standard Butylated Hydroxyl Anisole
ABTS Radical Scavenging activity	Leaf	Aqueous	50	10.23±0.54	278.96±1.50	47.34±0.32	66.92±0.36
			100	18.32±0.12		84.65±0.05	
			150	23.43±0.6		120.43±0.36	
			200	34.22±0.05		149.68±0.1	
			250	43.54±0.3		185.65±0.3	
			300	55.67±0.45		214.76±0.62	
			350	64.87±0.42		254.36±0.06	
			400	72.34±0.6		287.98±0.6	
		Ethanol	50	25.43±0.17	171.5±3.50	47.34±0.32	
			100	34.56±0.1		84.65±0.05	
			150	49.87±0.32		120.43±0.36	
			200	55.32±0.4		149.68±0.1	
			250	67.82±0.34		185.65±0.3	
			300	86.11±0.2		214.76±0.62	
			350	94.54±0.4		254.36±0.06	
			400	111.13±0.05		287.98±0.6	

*IC_{50} value is the amount of extract needed for scavenging 50 % of the radical produced in the reaction mixture. Values are mean± standard deviation of three independent replicates.

showed excellent antioxidant properties, so, leaf extract with the pure compound tested for cytotoxic properties using DLA and EAC cells, revealed that the leaf extract showed appreciable cytotoxic properties and pure compound showed appreciable cytotoxic properties but less than the standard used.

Trypan blue dye exclusion technique

In this method leaf ethanolic crude extract and pure compound was tested for its cytotoxicity, revealed that leaf ethanolic crude extract is effective against DLA (CTC_{50}: 283.62±6.87 µg/mL) and EAC cells (CTC_{50}: 239.53±6.9 µg/mL) but compared to standard it is negligible. The pure compound 2-Tert-Butyl-4, 6-Bis(3,5-Di-Tert-Butyl-4-Hydroxybenzyl)Phenol showed moderate cytotoxic activity for both DLA (CTC_{50}: 103.48±0.5 µg/mL) and EAC cells (CTC_{50}: 271.42±3.4 µg/mL) which is not comparable to the standard curcumin (CTC_{50}: 54.31±1.5 µg/mL) (Table 6 and 11).

MTT assay

The leaf ethanolic crude extract and pure compound were tested for its cytotoxicity using MTT assay and the leaf ethanolic crude extract showed appreciable cytotoxicity against DLA (CTC_{50}:

Table 4 — Metal chelating and Superoxide radicals scavenging NBT reduction of *Pavetta crassicaulis* Bremek. leaf extracts

S. No.	Activity	Plant part	Extracts	Concentration in µg/mL	scavenging activity	IC_{50} value	Standard Ascorbic acid scavenging activity	IC_{50} value of Standard EDTA
1	Metal chelating activity	Leaf	Aqueous	200	7.65±0.17	1441.87±1.15	58.76±0.32	213.69±0.32
				400	14.34±0.12		98.34±0.03	
				600	21.34±0.06		144.65±0.32	
				800	27.98±0.62		195.76±0.45	
				1000	36.54±0.34		242.87±0.14	
				1200	42.34±0.2		283.24±0.36	
				1400	49.67±0.4		332.31±0.05	
				1800	56.75±0.05		375.52±0.82	
			Ethanol	200	37.43±0.14	448.36±1.50	58.76±0.32	
				400	55.67±0.03		98.34±0.03	
				600	71.23±0.6		144.65±0.32	
				800	95.43±0.32		195.76±0.45	
				1000	124.32±0.54		242.87±0.14	
				1200	132.45±0.42		283.24±0.36	
				1400	143.26±0.05		332.31±0.05	
				1800	165.43±0.05		375.52±0.82	
	Activity	Plant part	Extracts	Concentration in µg/mL	scavenging activity	IC_{50} value	Standard µg/mL (Butylated Hydroxyl Anisole)	IC_{50} value of Standard Gallic acid
2	Superoxide radicals scavenging NBT reduction method	Leaf	Aqueous	100	19.08±0.2	396.44±2.69	51.66±0.11	102.17±0.49
				200	24.56±0.05		95.67±0.54	
				300	39.34±0.2		148.12±0.42	
				400	50.32±0.1		199.77±1.34	
				500	62.45±0.05		247.32±0.49	
				600	78.32±0.05		282.22±0.19	
				700	89.65±0.1		341.21±0.24	
				800	90.32±0.4		395.74±0.63	
			Ethanol	100	31.32±0.32	227.42±1.32	51.66±0.11	
				200	51.22±0.03		95.67±0.54	
				300	73.23±0.32		148.12±0.42	
				400	87.65±0.45		199.77±1.34	
				500	99.43±0.14		247.32±0.49	
				600	123.65±0.36		282.22±0.19	
				700	153.22±0.05		341.21±0.24	
				800	171.76±0.82		395.74±0.63	

*IC_{50} value is the amount of extract needed for scavenging 50% of the radicals produced in the reaction mixture. Values are mean± standard deviation of three independent replicates.

295.39±8.81 µg/mL) and EAC (CTC_{50}: 267.68±10.68 µg/mL) cells but not as good as standard. The pure compound "2-Tert-Butyl-4, 6-Bis(3,5-Di-Tert-Butyl-4-Hydroxybenzyl)Phenol" showed excellent cytotoxic activity DLA (CTC_{50}: 117.91±1.5 µg/mL) and EAC cells (CTC_{50}: 251.68±2.5 µg/mL) which is not comparable to the standard curcumin (CTC_{50}: 54.31±1.5 µg/mL) (Table 7 and 12).

Discussion

Preliminary qualitative phytochemical analysis

Preliminary quantitative phytochemical analysis of *P. crassicaulis* Bremek. petroleum ether, chloroform crude extracts showed negative results for all the tested phytochemicals, aqueous extract gives positive results for saponins and glycosides, but the ethanolic crude extract gives positive confirmation test for saponins, tannins, flavonoids, steroids/sterols, glycosides and phenols. This test confirms that the ethanol dissolves all the phytochemicals from *P. crassicaulis* Bremek. in a sufficient quantity to influence different phytochemical and pharmacological activities.

GC-MS analysis

Preliminary quantitative phytochemical analysis of *P. crassicaulis* Bremek. crude extracts give a clear picture of phytochemical dissolved in leaf ethanolic extract so, we subjected only ethanolic crude extract

Table 5 — Hydroxyl radical scavenging assay of *Pavetta crassicaulis* Bremek. leaf extracts

Activity	Plant part	Extracts	Concentration in µg/mL	scavenging activity	IC_{50} value	Standard EDTA scavenging activity	IC_{50} value Standard EDTA
Hydroxyl radical scavenging assay	Leaf	Aqueous	100	9.65±0.45	623.67±2.20	32.14±0.63	200.51±2.45
			200	16.34±0.14		58.76±0.32	
			300	22.34±0.2		76.58±0.98	
			400	28.98±0.05		98.34±0.03	
			500	37.54±0.1		119.24±0.19	
			600	49.34±0.4		144.65±0.31	
			700	55.67±0.61		172.23±0.48	
			800	68.75±0.34		195.76±0.45	
		Ethanol	100	26.43±0.32	284.42±2.50	32.14±0.63	
			200	40.32±0.05		58.76±0.32	
			300	56.43±0.36		76.58±0.98	
			400	79.11±0.1		98.34±0.03	
			500	87.54±0.3		119.24±0.19	
			600	99.26±0.62		144.65±0.31	
			700	118.34±0.06		172.23±0.48	
			800	125.43±0.6		195.76±0.45	

*IC_{50} value is the amount of extract needed for scavenging 50 % of the radicals produced in the reaction mixture. Values are mean± standard deviation of three independent replicates

Table 6 — *In-vitro* cytotoxic screening by *Pavetta crassicaulis* Bremek. leaf ethanolic extract against DLA and EAC cells by Trypan blue dye exclusion technique

S. No.	Concentration (µg/mL)	Leaf extract against DLA cells		Leaf extract against EAC cells		Standard (Curcumin)	control
		Percentage cytotoxicity	CTC_{50}	Percentage cytotoxicity	CTC_{50}		
1	10	0±0		1±1		15.4±3.3	
2	20	8.33±1.15		8.66±1.52		34.4±3.3	
3	50	12.66±2.08	283.62±6.87	15±1.73	239.53±6.9	100±0.5	0.23±1.54
4	100	17±2.64		23.33±2.08		100±0.5	
5	200	29±1		31.33±0.57		100±0.5	

Table 7 — *In-vitro* cytotoxic screening by *Pavetta crassicaulis* Bremek. leaf ethanolic extract against DLA and EAC cells by MTT assay

S. No.	Concentration (µg/mL)	Leaf extract against DLA cells			Leaf extract against EAC cells			Absorbance of standard Curcumin at 570 nm	Standard (Curcumin) % cytotoxicity	control
		Absorbance at 570 nm	Percentage cytotoxicity	CTC_{50}	Absorbance at 570 nm	Percentage cytotoxicity	CTC_{50}			
1	10	8±1.52	0.211±0.24		7.97±2.08	0.33±0.57		0.211±0.24	15.4±3.3	
2	20	7.33±0.57	0.123±0.18		7.41±3.51	7.33±1.52		0.123±0.18	34.4±3.3	
3	50	7.04±1.54	0.001±0.01	295.39±8.81	6.88±0.5	14±3	267.68±10.68	0.001±0.01	100±0.5	0.29±0.5
4	100	6.77±0.50	0.001±0.01		6.35±1.52	20.66±3.51		0.001±0.01	100±0.5	
5	200	5.79±1.50	0.001±0.01		5.71±0.57	28.66±2.08		0.001±0.01	100±0.5	

for GC-MS revealed the presence of 33 compounds (Table 2). Among all the confirmed phytochemicals 21 compounds reported for many medicinal and pharmacological properties and 12 compounds activity was not reported. Among all the phytochemicals present in the GC-MS analysis 2-Tert-Butyl-4,6-Bis(3,5-Di-Tert-Butyl-4-Hydroxybenzyl)Phenol (38.84) was the major compound, followed by Cyclo{tetra[(5-t-butyl-2-hydroxy-1,3-phenylene)methylene]}(15.72 %) not reported for its pharmacological properties. The next successive compound 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- (9.3 4 %) was reported for many pharmacological properties including antioxidant properties[42-44].

All the other phytocompounds like Bicyclo[3.3.1]nona-3,7-diene-2,9-dione (6.25 %), Methyl Ester of Bicyclo[4.3.0]Non-1(6)-En-4,7-Dione-8 (5.66 %), Hexadecanoic acid (2.04%), 6-Oxoheptanoic acid (1.53 %), 1,2,3-Propanetriol, 1-acetate (1.46 %), 2-Hexanone, 3-methyl-4-methylene (1.45 %), 2,3-Dihydro-Benzofuran (1.35 %), 4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol (1.32 %), 4-[4-Chlorophenyl]-N-[2-[1-methyl-2-pyrrolidinyl]ethyl]-6-[trichloromethyl]-2-pyrimidine (1.14%), 2-Methoxy-4-vinylphenol (1.14 %), 2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)] (1.12 %), Benzaldehyde, 2-hydroxy-6-methyl (0.98 %),

Table 8 — DPPH and ABTS assay of pure compound "2-Tert-Butyl-4,6-Bis(3,5-Di-Tert-Butyl-4-Hydroxybenzyl)Phenol" extracted from *Pavetta crassicaulis* Bremek. leaf extract

S. No.	Test sample	Activity	Concentration in µg/mL	Scavenging activity	IC_{50} value	Standard ascorbic acid scavenging activity	IC_{50} value of standard ascorbic acid
1	2-Tert-Butyl-4,6-Bis(3,5-Di-Tert-Butyl-4-Hydroxybenzyl)Phenol	DPPH radical scavenging activity	25	33.21±0.43	46.14±1.50	76.23±0.23	39.48±0.02
			50	55.32±0.33		82.32±0.43	
			75	11.42±0.32		113.11±0.09	
			100	132.43±2.08		134.54±0.91	
			125	155.31±3.51		156.43±0.02	
			150	164.92±3		176.65 ±0.34	
			175	189.23±1.52		189.41 ±0.54	
			200	233.44±0.57		210.87±0.32	
			Concentration in µg/ml	Scavenging activity	IC50 value	Standard Butylated Hydroxylanisole scavenging activity	IC_{50} value of standard Butylated Hydroxylanisole
2		ABTS Radical Scavenging activity	50	33.45±0.23	88.14±0.56	47.34±0.32	66.92±0.36
			100	57.54±0.43		84.65±0.05	
			150	83.43±0.09		120.43±0.36	
			200	100.32±0.91		149.68±0.1	
			250	123.65±0.02		185.65±0.3	
			300	167.86±0.34		214.76±0.62	
			350	198.4±0.54		254.36±0.06	
			400	256.43±0.32		287.98±0.6	

*IC_{50} value is the amount of extract needed for scavenging 50 % of the radical produced in the reaction mixture. Values are mean± standard deviation of three independent replicates. ND- not determined

Table 9 — NBT and Hydroxyl radical scavenging assay of pure compound "2-Tert-Butyl-4,6-Bis(3,5-Di-Tert-Butyl-4-Hydroxybenzyl)Phenol" extracted from *Pavetta crassicaulis* Bremek. leaf extract

S. No.	Test sample	Activity	Concentration in µg/mL	Scavenging activity	IC_{50} value	Standard Gallic acid scavenging activity	IC_{50} value of standard Gallic acid
1	2-Tert-Butyl-4,6-Bis(3,5-Di-Tert-Butyl-4-Hydroxybenzyl)Phenol	Superoxide radicals scavenging NBT reduction method	100	31.26±0.63	148.09±0.54	51.66±0.11	102.17±0.49
			200	65.43±0.32		95.67±0.54	
			300	89.43±0.98		148.12±0.42	
			400	134.23±0.03		199.77±1.34	
			500	154.33±0.19		247.32±0.49	
			600	198.59±0.31		282.22±0.19	
			700	243.54±0.48		341.21±0.24	
			800	298.6±0.45		395.74±0.63	
			Concentration in µg/mL	Scavenging activity	IC_{50} value	Standard EDTA scavenging activity	IC_{50} value of standard EDTA
2		Hydroxyl radical scavenging assay	100	35.23±0.42	213.48±1.45	32.14±0.63	200.51±2.45
			200	66.43±0.19		58.76±0.32	
			300	96.23±0.63		76.58±0.98	
			400	123.12±0.98		98.34±0.03	
			500	124.91±0.03		119.24±0.19	
			600	150.43±0.27		144.65±0.31	
			700	182.33± 0.76		172.23±0.48	
			800	210.87±0.03		195.76±0.45	

*IC_{50} value is the amount of extract needed for scavenging 50 % of the radical produced in the reaction mixture. Values are mean± standard deviation of three independent replicates. ND- not determined

Table 10 — Metal chelating assay of pure compound "2-Tert-Butyl-4,6-Bis(3,5-Di-Tert-Butyl-4-Hydroxybenzyl)Phenol" extracted from *Pavetta crassicaulis* Bremek. leaf extract

S. No.	Test sample	Activity	Concentration in µg/mL	Scavenging activity	IC_{50} value	Standard scavenging activity (EDTA)	IC_{50} value of standard EDTA
1	2-Tert-Butyl-4,6-Bis (3,5-Di-Tert-Butyl-4-Hydroxybenzyl)Phenol	Metal chelating assay	200	34.23±0.32	307.48±2.43	58.76±0.32	213.69±0.32
			400	66.54±0.54		98.34±0.03	
			600	95.47±1.54		144.65±0.32	
			800	120.76±1.54		195.76±0.45	
			1000	165.48±2.5		242.87±0.14	
			1200	198.76±3.4		283.24±0.36	
			1400	234.54±0.43		332.31±0.05	
			1800	287.54±0.23		375.52±0.82	

*IC_{50} value is the amount of extract needed for scavenging 50 % of the radical produced in the reaction mixture. Values are mean± standard deviation of three independent replicates. ND- not determined

Table 11 — Trypan blue dye exclusion technique "2-Tert-Butyl-4,6-Bis(3,5-Di-Tert-Butyl-4-Hydroxybenzyl)Phenol" extracted from *Pavetta crassicaulis* Bremek. leaf extract

S. No.	Concentration (µg/mL)	Trypan blue dye exclusion technique for DLA cells		Trypan blue dye exclusion technique for EAC cells		Standard (Curcumin)	Standard CTC_{50}	Control
		Percentage cytotoxicity	CTC_{50}	Percentage cytotoxicity	CTC_{50}			
1	10	5.1±0.5		0±0		15.4±3.3		
2	20	11.3±1.5		0±0		34.4±3.3		
3	50	22.3±0.5	117.91±1.5	10.5±2.2	251.68±2.5	100±0.5	54.31±1.5	0.23±1.54
4	100	44.55±2.5		21.45±3.4		100±0.5		
5	200	77.88±1.5		43.54±1.5		100±0.5		

Table 12 — MTT assay of pure compound "2-Tert-Butyl-4,6-Bis(3,5-Di-Tert-Butyl-4-Hydroxybenzyl)Phenol" extracted from *Pavetta crassicaulis* Bremek. leaf extract

S. No.	Concentration (µg/mL)	MTT assay for DLA cells			MTT assay for EAC cells			Absorbance of standard Curcumin at 570 nm	Standard Curcumin % cytotoxicity	CTC50 of standard (Curcumin)	Control
		Absorbance at 570 nm	Percentage cytotoxicity	CTC_{50}	Absorbance at 570 nm	Percentage cytotoxicity	CTC_{50}				
1	10	7.34±1.5	8±1.5		8.10±3.4	0±0		0.211±0.24	15.4±3.3		
2	20	6.69±2.5	16.43±2.3		8±1.3	0±0		0.123±0.18	34.4±3.3		
3	50	5.18±0.5	35.21±1.2	103.48±0.5	7.60±2.5	5±1.4	271.42±3.4	0.001±0.01	100±0.5	54.31±1.5	0.31±1.5
4	100	3.41±2.5	57.43±2.3		6.24±0.01	22±1.5		0.001±0.01	100±0.5		
5	200	2.68±0.01	66.54±2.3		4.56±0.01	43±1.5		0.001±0.01	100±0.5		

rac-2,4-Dimethyl-3-nitrobicyclo[3.2.1]octan-8-one (0.96 %), Stigmast-5-en-3-ol, (3.beta.,24S) (0.95 %), Acetic acid, (2-isopropenyl cyclopentylidene)-, methyl ester (0.83 %), 2,4-Dimethyl-3-nitrobicyclo[3.2.1]octan-8-one (0.78 %),2(3H)-Naphthalenone, 4,4a,5,6-tetrahydro (0.77 %), 2-Methyl-5-(4-methylphenyl)tetrazole (0.71 %), 2-Furancarboxaldehyde, 5-(hydroxymethyl) (0.66 %), Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester (0.62), 2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one (0.53 %), 2,5-Dimethyl-4-hydroxy-3(2H)-furanone (0.52 %), 2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl (0.52 %), Benzaldehyde, 4-hydroxy- (0.47 %), 1,5-Diazocine, octahydro-1,5-dinitro- (0.4 %), Phenol, 2-methoxy-4-(2-propenyl) (0.39 %), Octanal, 7-methoxy-3,7-dimethyl (0.37 %), 9,12-Octadecadienoic acid (Z,Z)-, 2-hydroxy-1 (0.3 %), Hexadecanoic acid, methyl ester (0.29 %), 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z) (0.28 %) were present in a meager percentage have reported for many medical as well as pharmacological properties (Table 2).

The antioxidant experiments like metal chelating activity, superoxide radicals, hydroxyl radical, DPPH radicals, ABTS radical scavenging assays results concluded that leaf ethanolic extract and its extracted pure compound has appreciable antioxidant properties but not good as standards used.

Aqueous extract showed negligible activity which is indirectly confirmed in the qualitative phytochemical analysis only gives the positive results for saponins and glycosides, so its negligible activity was also expected one.

The GC-MS analysis of *P. crassicaulis* Bremek. ethanolic leaf crude extracts revealed the presence of 33 compounds, and it is evident that phenolic compounds in the plants will contribute to the antioxidant properties. The antioxidant activity of phenolic compounds is mainly due to their redox phenomenon, due to this, they play the role of adsorbing and neutralising free radicals, quenching singlet and triplet oxygen, or decomposing peroxides[68-69]. GC-MS analysis report of *P. crassicaulis* Bremek. leaf ethanolic extract clearly reveal the presence of phenolic substances like 2-Tert-Butyl-4,6-Bis(3,5-Di-Tert-Butyl-4-Hydroxybenzyl) Phenol (38.84 %), Cyclo{tetra[(5-t-butyl-2-hydroxy-1,3-phenylene) methylene]}(15.72 %), etc. may influence the antioxidant properties of ethanolic extract.

Thus, indicates that the polyphenols present in leaf ethanolic extract could be responsible for the positive effects which in turn give the positive feedback on the folklore use and withstand the use of pavetta plant parts by the tribal folklore medicinal practitioners in local India.

In-vitro cytotoxic experiments like Trypan blue dye exclusion technique and MTT assay against DLA and EAC cancer cells revealed that leaf ethanolic extract showed moderate cytotoxicity and the pure compound 2-Tert-Butyl-4,6-Bis(3,5-Di-Tert-Butyl-4-Hydroxybenzyl)Phenol showed appreciable cytotoxic activity but less than the standard curcumin used (Table 11 and Table 12).

In the crude ethanolic extract of *P. crassicaulis* Bremek. the major 2-Tert-Butyl-4,6-Bis(3,5-Di-Tert-Butyl-4-Hydroxybenzyl)Phenol may influence the cytotoxicity, or all the 33 compounds may also influence cytotoxic activity.

Conclusion

The present study shows that *P. crassicaulis* Bremek. leaf ethanolic crude extract has appreciable antioxidant and cytotoxic properties and its pure compound 2-Tert-Butyl-4,6-Bis(3,5-Di-Tert-Butyl-4-Hydroxybenzyl). Phenol has appreciable antioxidant and cytotoxic properties but not good as the standards used. These results confirm positive activity of the plant as a therapeutic agent in tribal medicine. Thus *P. crassicaulis l*eaf could be exploited as a valuable source of antioxidant and cytotoxic agent for the pharmaceutical industry.

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