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Research Article

ANTI-INFLAMMATORY PROPERTIES OF *PAVETTA CRASSICAULIS* BREMEK. LEAF AND FLOWER CRUDE EXTRACTS AND ITS PURE COMPOUNDS COLLECTED FROM WESTERN GHATS, KARNATAKA, INDIA

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ABSTRACT

Objective: *Pavetta crassicaulis* Bremek. (F: Rubiaceae) plant extracts were subjected to anti-inflammatory experiment by using carrageenin-induced rat hind paw edema method.

Methods: Groups of 5 rats of both sexes (pregnant females excluded) were given a dose of the extract. After 1 h, 0.1 ml, 1% carrageenan suspension in 0.9% NaCl solution was injected into the subplantar tissue of the right hind paw. Paw volume was measured plethysmometrically at 0 h and 3 h after carrageenin injection.

Results: Anti-inflammatory experiments revealed that, the leaf and flower ethanolic extract of the *P. crassicaulis* Bremek. plant shown excellent anti-inflammatory and its extracted pure compounds, 2-tert-butyl-4,6-bis(3,5-di-tert-butyl-4-hydroxybenzyl)phenol and 4H-pyran-4-one,2,3-dihydro3,5-dihydroxy-6-methyl- is a pure compound isolated from the Pavetta crassicaulis flower extract. showed excellent anti-inflammatory activity compared with the standard.

Conclusion: *P. crassicaulis* Bremek. leaf and flower could be exploited as a valuable source of anti-inflammatory agent for the pharmaceutical industry.

Keywords: *Pavetta crassicaulis* Bremek., Western Ghats, Karnataka, Anti-inflammatory, 2-tert-butyl-4,6-bis(3,5-di-tert-butyl-4-hydroxybenzyl) phenol, 4H-pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl-.

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INTRODUCTION

Medicinal plants are the richest bioresources for many types of medicinal practices such as modern medicines, Allopathy, Homeopathy, Naturopathy, Unani, Acupuncture, Ayurveda, nutraceuticals, folk medicines, synthetic drugs and pharmaceuticals, intermediate, food supplements [1].

Indian subcontinent is a vast repository of medicinal plants that are used in traditional medical treatments which also forms a rich source of knowledge [2]. India is one of the megadiversity centers in the planet having a diverse medicinal plant species which is unexplored most of them are endemic. India shares approximately 13% of world's biodiversity, one among 17 megadiversity centers. Among the 34 hotspots in the world, India has 4 hotspots, namely, Eastern Himalaya, Indo-Burma, Western Ghats, Andaman and Nicobar Island. The various indigenous systems use several plant species to treat different ailments [3]. 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 [4].

Western Ghats is a mountain range that runs parallel to the western coast of the Indian peninsula, located entirely in India. The range starts near the border of Gujarat and Maharashtra, runs approximately 1600 km through the states of Maharashtra, Goa, Karnataka, Kerala, and Tamil Nadu ending at Kanyakumari, at the southern tip of India [5].

Experimental medicinal plant and description:
Pavetta crassicaulis Bremek. Scientific classification:
Kingdom: Plantae
Division: Magnoliophyta
Class: Magnoliopsida
Subclass: Asteridae
Family: Rubiaceae
Order: Rubiales
Genus: *Pavetta*
Species: *P. crassicaulis* Bremek.

P. crassicaulis Bremek. is an important ethnomedicinal shrub or small tree belonging to the family Rubiaceae, grows up to 4 m tall, the plant is endemic to peninsular India. The leaves are often membranous with dark bacterial nodules, has small, white, tubular flowers, sometimes salviform or funnel-shaped with 4 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 [6].

P. crassicaulis Bremek. endemic to peninsular India and distributed in many places such as Mizoram, Gujarat, Uttara Pradesh, Chhattisgarh, Orissa Maharashtra, and Karnataka [7-10].

P. crassicaulis Bremek. has medicinal properties which is used in many parts of India, *Pavetta* plant parts used in the treatment of arthritis, boils and itches, hemorrhoidal pains, visceral problems, dropsy [11], epilepsy, hemorrhoids [12], skin diseases [13], anticephalagic, fat burner, aphrodisiac [14], urinary complaints and fruits used as anthelmintic and flowers are eaten fried [15].

The preliminary qualitative phytochemical analysis of *P. crassicaulis* Bremek. methanolic crude extract revealed the presence of

alkaloids, steroids, and terpenoid with moderate antimicrobial activity [16,17].

The present study focused on the anti-inflammatory properties of *P. crassicaulis* Bremek. plant parts.

METHODS

Plant collection and authentication

The bark and leaf materials of *P. crassicaulis* Bremek. were collected from Shringeri taluk, Karnataka in April 2014 (13.4198° N, 75.2567° E) (Fig. 1). The plant was identified by Prof. K G Bhat, Udupi, and a voucher specimen was conserved under the reference number KU/AB/RN/AS/001.

Plant preparation and extraction

The samples were dried in the shade for 20–25 days, mechanically powdered, and subjected to Soxhlet extraction using petroleum ether, chloroform, and ethanol [18]. The crude extracts were collected in air-tight plastic containers and stored in cool condition.

Preliminary phytochemical screening

Air-dried and powdered leaf materials and also all crude extracts were screened for the presence of tannins, alkaloids, saponin, glycosides, flavonoids, steroids/sterols, and phenols using standard methods [19-21].

Gas chromatography and mass spectroscopy (GC-MS) analysis

Pavetta crassicaulis Bremek leaf and flower ethanolic extracts were subjected to GC-MS analysis and obtained spectra was analysed. GC model: Thermo Trace GC Ultra, MS Model: Thermo DSQ II, Ionization: Electron impact ionization (EI), chemical ionization (CI), mass range: 1–1074 m/z.

Isolation of pure compound

The stationary phase in column chromatography is silica gel and the mobile phase or eluent is the mixture of different immiscible solvents [22]. 5 g of residue is dissolve in ethanol and it will adsorbed on to the silica gel powder and of 150 g is loaded to column. The column was eluted with n-hexane 100%, flowed by n-hexane and ethyl acetate in different ratios (99:1, 90:10, 80:20, 60:40, 50:50) and followed by ethyl acetate 100% and the with mixture of ethyl acetate:ethanol (99:1, 98:2, 95:5, 90:10, 80:20). Simultaneously, the same elution is monitored by thin-layer chromatography (TLC) (silica gel and for visualization, mixture of vanillin: Sulfuric acid heated at 110°C). Each time, 5 ml were collected and TLC monitored same elute was concentrate to 5 ml stored in the refrigerator.

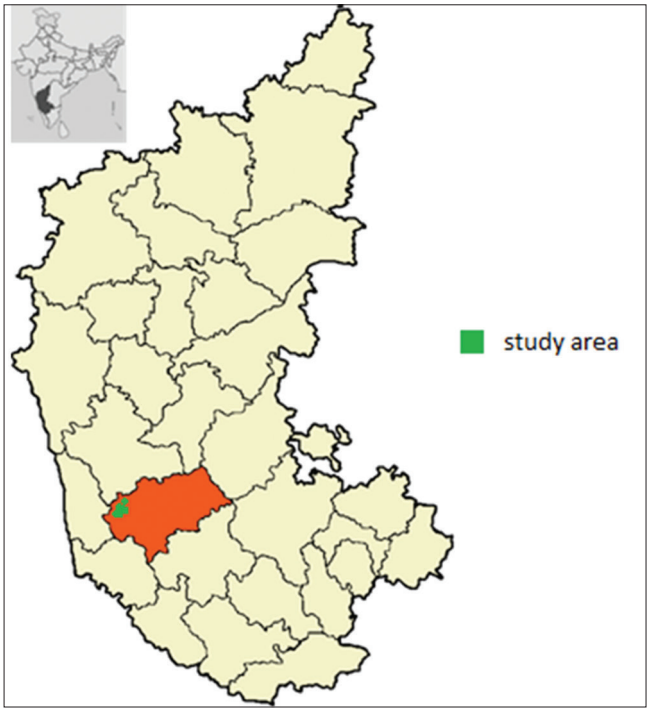

Fig. 1: Sampling site details and location

Rat maintenance

Rats (120–170 g) of either sex kept at the laboratory Animal home of the Faculty of Pharmacy, SCS College of Pharmacy, Harapanahalli, Davanagere, were used. The animals were maintained under standard environmental conditions and had free access to standard diet and water. Plant extracts were administered orally by gavage in distilled water at different dose levels. All experiments were carried out according to the Institutional Animal Ethics Committee Guidelines (Re: SCSCP/IAEC/11/12/2016-17).

Anti-inflammatory activity by carrageenin-induced rat hind paw edema method (*in vivo* model)

Anti-inflammatory activity was measured using carrageenan-induced rat paw edema assay [23,24]. Groups of 5 rats of both sexes (pregnant females excluded) were given a dose of the extract. After 1 h, 0.1 ml, 1% carrageenan suspension in 0.9% NaCl solution was injected into the subplantar tissue of the right hind paw. Paw volume was measured plethysmometrically at 0 h and 3 h after carrageenin injection [25]. Two groups of drug-treated rats and one control group were used each test day, the mean paw edema value for the test group being compared with its mean value for the control group for that day. The test compounds (50 mg/kg) were administered orally; standard group was treated with diclofenac (50 mg/kg) orally 1 h. before by injection and control group received only vehicle. Mean difference in paw volume was measured and percentage inhibition was calculated by following formula.

$$\% \text{ inhibition of edema} = \frac{Vc - Vt}{Vc} \times 100$$

Where Vt = mean paw volume of test group.
Vc = mean paw volume of control group.

Anti-inflammatory activity [26] was measured as the percentage reduction in edema level when drug was present, relative to control.

Statistical analysis

All data were validated with the help of statistical software PRISM and MINITAB; the values were expressed as mean ± standard error of the mean and results were statistically analyzed by one-way analysis of variance (ANOVA) followed by Dunnet's t-test. Symbols represent statistical significance.*p<0.05, **p<0.01, ***<0.0001 ns - not significant, as compared to control group.

RESULTS

Extract yield and preliminary 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, and with ethanol gives 75.35 g yield. The results of preliminary qualitative phytochemical screening of different extracts of *P. crassicaulis* Bremek. leaf indicate the presence of saponins, tannins, flavonoids, steroids/sterols, glycosides, and phenols in ethanolic crude extract and chloroform extract confirms the presence of phenols and sterols and petroleum extracts negative results for all the phytochemical tests.

The Soxhlet extraction of *P. crassicaulis* Bremek. flower (750 g) with petroleum ether gives 20.34 g, with chloroform gives 18.64 g and with ethanol gives 45.38 g yield. The results of phytochemical screening of *P. crassicaulis* Bremek. flower extracts indicate the presence of alkaloids, saponins, flavonoids, steroids/sterols, glycosides, and phenols in

ethanol crude extract, the chloroform crude extracts shows positive results for flavonoids. However, the petroleum ether crude extract gave negative results for all these compounds (Table 1).

GC-MS analysis of ethanolic crude extract

Leaf ethanolic extract

In GC-MS analysis of medicinal *P. crassicaulis* Bremek., ethanolic leaf extract revealed the presence 34 compounds, in that major percentage of compounds present was 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%), and methyl ester of bicyclo[4.3.0]non-1(6)-en-4,7-dione-8-carboxylic acid (5.66%). The major compound 2-tert-butyl4,6-bis(3,5-di-tert-butyl-4-hydroxybenzyl)phenol (37.44%) have antioxidant properties and also used as ultraviolet stabilizer. 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%) has antioxidant properties (Table 2 and Figs. 2-4).

Flower ethanolic extract

The GC-MS analysis of *P. crassicaulis* Bremek. flower ethanolic extract revealed the presence of 39 compounds, in that, 4H pyran-4one,2,3-dihydro-3,5-dihydroxy-6 methyl- (13.82%), benzaldehyde, 2-methyl- (7.25%), benzaldehyde, 2 hydroxy-6-methyl- (6.52%), 2-furancarboxaldehyde, and 5-(hydroxymethyl)-(6.30%) in major percentage. and campesterol also (0.80%), stigmasterol (0.62%), β.Sitosterol (3.10%) in meagre percentage (Figs.5-7 and Table 3).

Column chromatography of ethanolic leaf and flower extract

Leaf ethanolic extract

Elution carried out with n-hexane in 100% concentration eluted compound 1 in small quantity and with n-hexane:ethyl acetate at the ratio 80:20 gives compound 2, but its yield is too less. Ethyl acetate was eluted mixture of many compounds and ethyl acetate:ethanol at 80:20 yielded large quantity of compound 3. The gradient of other residues was yielded mixture of compound which is confirmed by TLC therefore not subjected to further process.

Compound 3L was yielded more so, we took only compound 3L for further structural analysis.

The pure compound 3L was crystalline transparent grayish color. Further, the pure compound subjected to cNMR, hNMR, infrared (IR), mass spectral analysis, and the molecular formula was $C_{40}H_{58}O_3$, molecular weight 586.887 g/mol. From all these details, the compound name was found to be tert-butyl-4,6-bis(3,5-di-tert-butyl-4-hydroxybenzyl)Phenol (Figs. 8-11).

Table 1: Preliminary phytochemical analysis of different extract of *P. crassicaulis* Bremek. leaf and flower

Secondary metabolites	Type of tests	Petroleum ether crude extract		Chloroform crude extract		Ethanol crude extract	
		Leaf	Flower	Leaf	Flower	Leaf	Flower
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-Killiani's test	–	–	–	–	+	+
	Brown water test	–	–	–	–	+	+
	Legal test	–	–	–	–	+	+
Phenols	Ferric chloride test	–	–	+	–	+	+
	Acetic acid test	–	–	+	–	+	+
Sterols	Liebermann–Burchad test	–	–	+	–	+	+

–: Negative result, +: positive result. *P. crassicaulis: Pavetta crassicaulis*

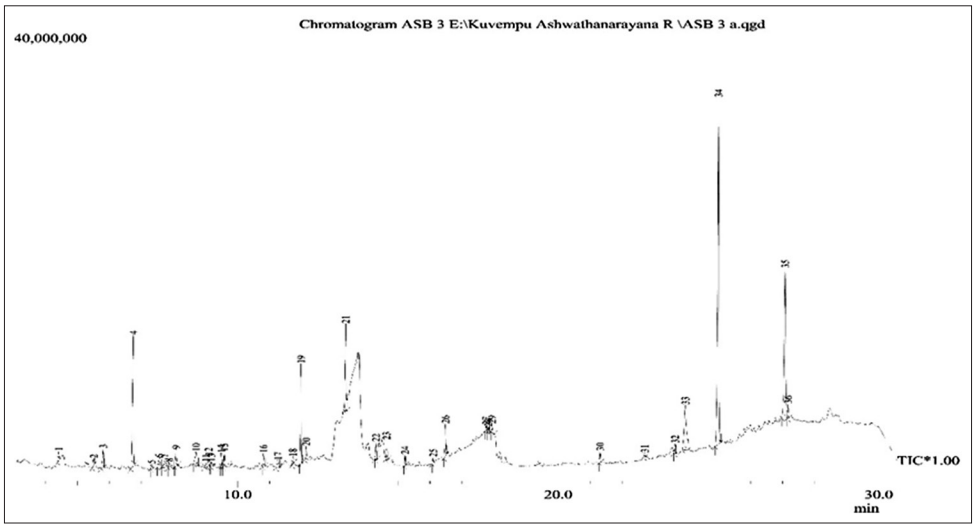

Fig. 2: Gas chromatography and mass spectroscopy chromatogram of leaf ethanolic extract of *Pavetta crassicaulis* Bremek.

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

Sl. 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 [30]
2	2,5-dimethyl-4-hydroxy-3 (2H)-furanone	0.52	Flavor and perfume industry [31]
3	2-hexanone, 3-methyl-4-methylene-	1.45	Paint and paint thinner [32]
4	4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	9.34	Mutagen antimicrobial, anti-inflammatory, and antioxidant capacity [27-29]
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 [33]
7	2-furancarboxaldehyde, 5-(hydroxymethyl)-	0.66	Food additives, antimicrobial, preservative, flavoring agents [34,35]
8	1,2,3-propanetriol, 1-acetate	1.46	Unknown
9	6-oxoheptanoic acid	1.53	6-oxoheptanoic acid is a reagent to synthesize new penicillin containing keto acids as side chains. It is also used to study the various metabolic pathways of 4-hydroxypentanoate and levulinate [36]
10	Benzaldehyde, 4-hydroxy-	0.47	Flavor and fragrance agents [37]
11	2-Methoxy-4-vinylphenol	1.14	Flavoring agent, antibacterial activity, anti-inflammatory effect [38,39]
12	Phenol, 2-methoxy-4-(2-propenyl)-	0.39	Flavoring agent used in the manufacture of vanillin, antiinfective agents, antioxidant [40,41]
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 [42,43]
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 perniciosus* and grain mite *Aleuroglyphus ovatus* [44,45]
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 [34]
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, antiinflammatory, 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 [28,46,47]
24	Hexadecanoic acid, methyl ester	0.29	Perfumes and cosmetics [48]
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 flavor, hemolytic, 5-alpha reductase inhibitor [49,50]
26	Octanal, 7-methoxy-3,7-dimethyl-	0.37	Unknown
27	9,12,15-octadecatrienoic acid, methyl ester, (Z, Z, Z)-	0.28	Antibacterial, Anti-inflammatory, hypocholesterolemic, cancer preventive, hepatoprotective, nematicide, insectifuge, antihistaminic, antieczemic, antiacne, 5-alpha reductase inhibitor, antiandrogenic, anti-arthritic, anticoronary, insectifuge [28]
28	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester	0.62	Wound healing activity, hemolytic, pesticide, flavor, antioxidant [51]
29	9,12-Octadecadienoic acid (Z, Z)-, 2-hydroxy-1-(hydroxymethyl) ethyl ester	0.30	Hypocholesterolemic, nematicide, anti-arthritic, hepatoprotective, antiandrogenic, hypocholesterolemic, nematicide, 5-alpha reductase inhibitor, anti-histaminic, anticoronary insectifuge, anti-eczemic, anti-acne [52]

(Contd...)

Table 2: (*Continued*)

Sl. No	Chemical compound present	Average percentage	Properties of the compound
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 [53]
31	2-tert-butyl-4,6-bis (3,5-di-tert-butyl-4-hydroxybenzyl) phenol	38.84	Unknown
32	Cycl {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 [54]

GC-MS: Gas chromatography and mass spectroscopy, *P. crassicaulis: Pavetta crassicaulis*

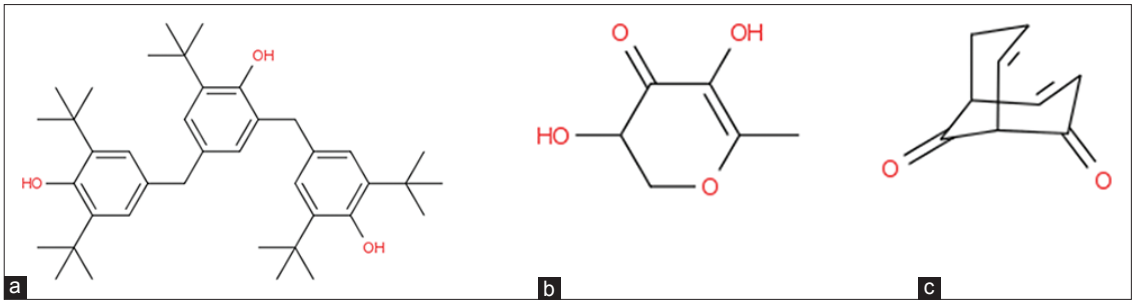

Fig. 3: Major constituents in gas chromatography and mass spectroscopy analysis of crude ethanolic extract of *Pavetta crassicaulis* Bremek. leaf. (a) 2-tert-butyl-4,6-bis(3,5-di-tert-butyl-4-hydroxybenzyl)phenol, (b) 4H-pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl-, (c) Bicyclo[3.3.1]nona-3,7-diene-2,9-dione

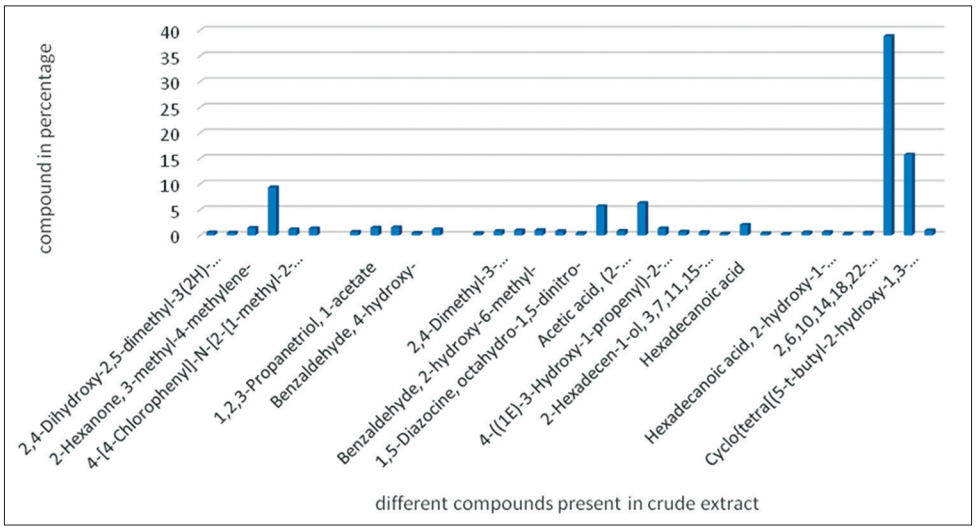

Fig. 4: Gas chromatography and mass spectroscopy of crude *Pavetta crassicaulis* Bremek. ethanolic leaf extract showing percentage of different compounds

Flower ethanolic extract

Elution carried out with n-hexane in 100% concentration eluted nil compound and with n-hexane:ethyl acetate at the ratio 90:10 gives compound 1, but its yield is too less. Ethyl acetate was eluted compound 2 and ethyl acetate:ethanol at 90:10 yielded large quantity of compound 3. The gradient of other residues was yielded mixture of compound which is confirmed by TLC therefore not subjected to further process.

Compound 3F was yielded higher quantity so we took only compound 3F for further structural analysis.

The pure compound 3F was crystalline light brown colored and shiny surface. Further, it was subjected to cNMR, hNMR, IR, and mass spectral analysis and the molecular formula was found to be $C_6H_8O_4$, molecular weight 144.1253 g/mol, from all these details the compound name was found to be 4H-pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl- (Figs. 12-15).

Anti-inflammatory activity of different extract of *P. crassicaulis* Bremek

Anti-inflammatory activity of leaf crude extracts

The extracts were tested at three different dose levels to know if they were dose-dependent. From the results obtained, it is revealed

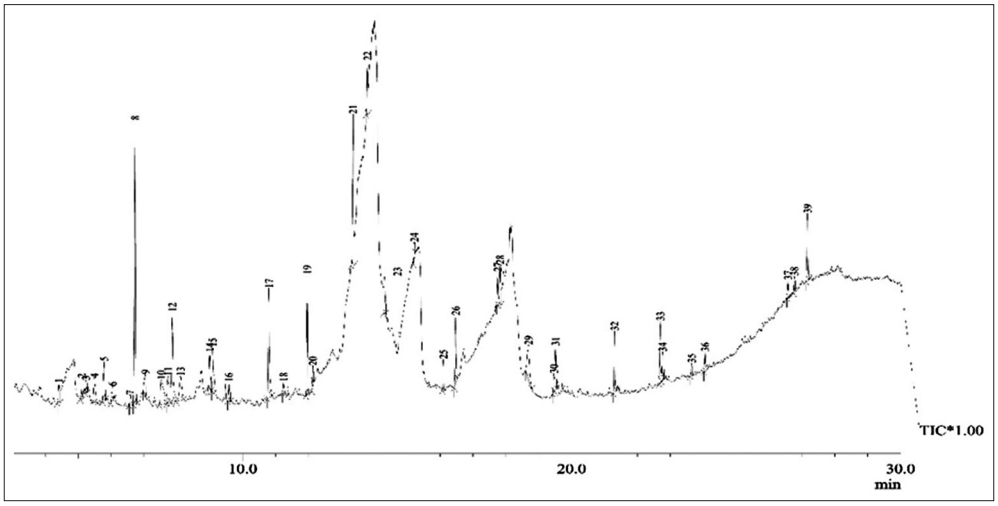

Fig. 5: Gas chromatography and mass spectroscopy chromatogram of flower ethanolic extract of *Pavetta crassicaulis* Bremek.

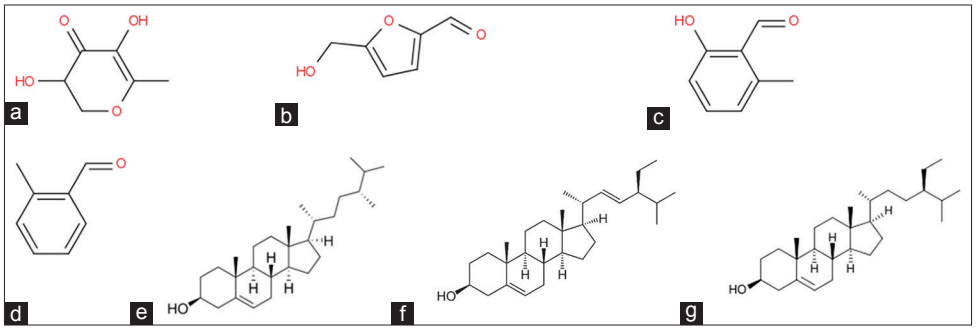

Fig. 6: Major constituents in gas chromatography and mass spectroscopy analysis of crude ethanolic extract of *Pavetta crassicaulis* Bremek. flower. (a) 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-, (b) 2-furancarboxaldehyde, 5-(hydroxymethyl)-, (c) Benzaldehyde, 2-hydroxy-6-methyl-, (d) Benzaldehyde, 2-methyl-, (e) Campesterol, (f) Stigmasterol, (g) β-Sitosterol

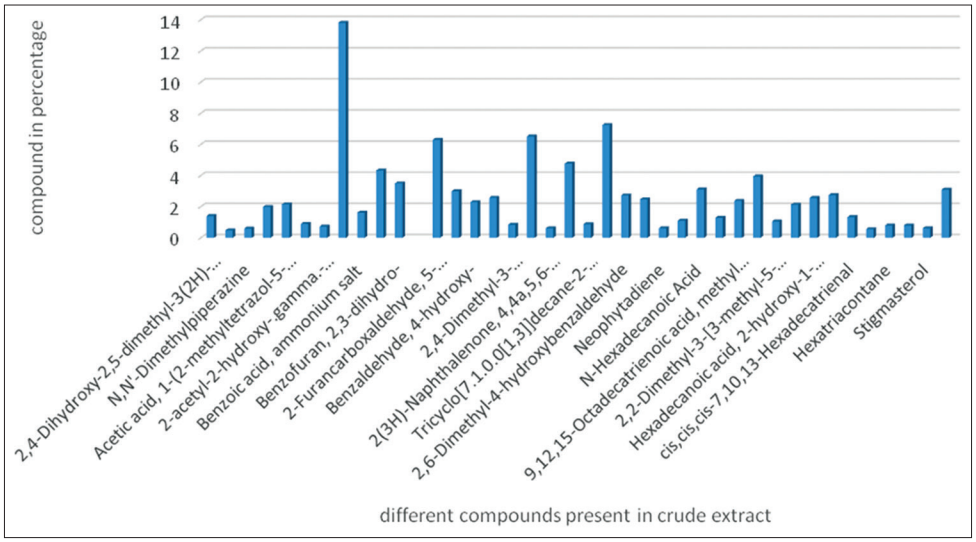

Fig. 7: Gas chromatography and mass spectroscopy of crude *Pavetta crassicaulis* Bremek. ethanolic flower extract showing percentage of different compounds

that petroleum ether and chloroform extract did not showed any anti-inflammatory activity against carrageenin-induced rat hind paw edema in all concentrations compared with the control. Leaf ethanolic extract showed appreciable anti-inflammatory in all the concentrations (100, 150, 200, 250, and 500 mg/kg concentration) by ANOVA statistical analysis obtained values were validated, which revealed that the leaf ethanolic extract of *P. crassicaulis* Bremek. was highly significant against carrageenin-induced rat hind paw edema in all concentrations in all the interval time. The leaf ethanolic extract of *P. crassicaulis* Bremek. excellent in reducing edema induced by carrageenan in all the phases. Therefore, leaf ethanolic extract of *P. crassicaulis* Bremek. crude extract definitely possess anti-

Table 3: Presence of metabolites in GC-MS analysis of crude ethanolic extract of *P. crassicaulis* Bremek. flower collected from Western Ghats Karnataka

Sl. No	Chemical compound present	Average percentage	Properties of the compound
1	2,4-dihydroxy-2,5-dimethyl-3 (2H)-furan-3-one	1.40	Food-grade flavor ingredients [30]
2	1-butanamine, 2-methyl-N-(2-methylbutylidene)-	0.48	Unknown
3	N, N′-Dimethylpiperazine	0.60	Anthelmintics, antiallergenic, antibacterial, antihistamic, antiemetic and antimigraine agents, insecticides, accelerators for rubber, urethane catalysts, and antioxidants [55]
4	2,5-dimethyl-4-hydroxy-3 (2H)-furanone	1.99	Food-grade flavor ingredients [31]
5	Acetic acid, 1-(2-methyltetrazol-5-yl) ethenyl ester	2.15	Antiarrhythmia agents, antifungal agents, carminative, antispasmodic, flavoring agents, adhesives, paint additives [56]
6	Butanedioic acid, monomethyl ester	0.90	Beverage industry, primarily as an acidity regulator [57]
7	2-acetyl-2-hydroxy-.gamma.-butyrolactone	0.73	Unknown
8	4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	13.82	Mutagen antimicrobial, anti-inflammatory and antioxidant capacity [27-29]
9	Benzoic acid, ammonium salt	1.62	Industrial preservative for paper wrappers, agent for reducing curing time in vulcanization of rubber, expectorant used for chronic bronchitis, analytical reagent for various elements, urinary anti-infective [58]
10	1,2-Benzenediol	4.33	Flavoring agents, pharmaceuticals and cosmetics, antioxidant, antibacterial [59,60]
11	Benzofuran, 2,3-dihydro-	3.50	Toxic [61]
12	2-Furancarboxaldehyde, 5-(hydroxymethyl)-	6.30	Food industry as flavoring agent [62]
13	1,2,3-Propanetriol, diacetate	3.00	As a sweetener; in the manufacture of dynamite, cosmetics, liquid soaps, candy, liqueurs, inks, and lubricants; to keep fabrics pliable; as a component of antifreeze mixtures; as a source of nutrients for fermentation cultures in the production of antibiotics and in medicine [63]
14	Benzaldehyde, 4-hydroxy-	2.30	Flavor and fragrance agents [37]
15	2-Methoxy-4-vinylphenol	2.58	Flavoring agent, antibacterial activity, anti-inflammatory effect [38, 39, 64]
16	2,4-Dimethyl-3-nitrobicyclo[3.2.1]octan-8-one	0.85	Oils obtained from myrrh and frankincense and parthenium weed have little percentage [42,43]
17	Benzaldehyde, 2-hydroxy-6-methyl-	6.52	Pheromone of the acarid mite *Tyrophagus perniciosus* and grain mite *Aleuroglyphus ovatus* [44,45]
18	2 (3H)-naphthalenone, 4,4a, 5,6-tetrahydro-	0.62	Unknown
19	Methyl Ester Of Bicyclo[4.3.0] Non-1 (6)-En-4,7-Dione-8-Carboxylic Acid	4.77	Unknown
20	Tricyclo[7.1.0.0[1,3]]decane-2-carbaldehyde	0.88	Unknown
21	Benzaldehyde, 2-methyl-	7.25	Perfumes, flavoring agents [65]
22	2,6-dimethyl-4-hydroxybenzaldehyde	2.73	Causes skin irritation, serious eye irritation, respiratory irritation, herbicides, neuropathic pain [66]
23	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	2.47	Antimicrobial, antioxidant, anti-inflammatory, analgesic [34]
24	Neophytadiene	0.62	As additive for liquid cigarette can improve aroma and evaporation rate [67]
25	Hexadecanoic acid, methyl ester	1.11	Antioxidant, hypocholesterolemic, nematicide, pesticide, anti-androgenic flavor, hemolytic, 5-alpha reductase inhibitor, surface active agents, laundry, and dishwashing products [52]
26	N-hexadecanoic acid	3.11	Anti-inflammatory, psychotropic drug, anti-oxidant, hypocholesterolemic, nematicide, pesticide, lubricant, antiandrogenic, flavor, hemolytic 5-alpha reductase inhibitor [68]
27	9,12-octadecadienoic acid, methyl ester	1.29	Anti-inflammatory, nematicide, insectifuge, hypocholesterolemic, cancer preventive, hepatoprotective, antihistaminic, anti-acne, anti-arthritic, anti-eczemic, 5-alpha reductase inhibitor, antiandrogenic, anticoronary [68,69]

(Contd...)

Table 3: (*Continued*)

Sl. No	Chemical compound present	Average percentage	Properties of the compound
28	9,12,15-octadecatrienoic acid, methyl ester, (Z, Z, Z)-	2.38	Anti-inflammatory, insectifuge hypocholesterolemic, cancer preventive, nematicide, hepatoprotective, insectifuge, anti-histaminic, anti-eczemic, anti-acne, 5-alpha reductase inhibitor, anti-androgenic, anti-arthritic, anti-coronary [68]
29	Alpha.-D-glucopyranose, 4-O-. beta.-D-galactopyranosyl-	3.95	Indicator carbohydrate for intestinal permeability in Crohn's disease and malabsorption syndrome [70]
30	2,2-Dimethyl-3-[3-methyl-5-(phenylthio) pent-3-enyl] oxirane	1.06	Unknown
31	Benzoyl .beta.-d-glucoside	2.13	Unknown
32	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester	2.57	Wound healing activity, hemolytic, pesticide, flavor, antioxidant [51]
33	9,12-octadecadienoic acid (Z, Z)-, 2-hydroxy-1-(hydroxymethyl) ethyl ester	2.75	Component of cigarette smoke, hypocholesterolemic, nematicide, anti-arthritic, hepatoprotective, antiandrogenic, hypocholesterolemic 5-alpha reductase inhibitor, antihistaminic, anticoronary, insectifuge, anti-eczemic, anti-acne [53]
34	cis, cis, cis-7,10,13-hexadecatrienal	1.34	Unknown
35	2,6,10,14,18,22-tetracosahexaene, 2,6,10,15,19,23-hexamethyl-	0.56	Bactericide, antifungal, cytotoxic, antibacterial, antioxidant, antitumor, cancer preventive, immunostimulant, chemopreventive, lipoxygenase-inhibitor, perfumery, pesticide, and sunscreen [53]
36	Hexatriacontane	0.80	Drug delivery mediums for topical and oral pharmaceuticals, organic application mediums for cosmetics, cleaning materials for art conservation, as delivery mediums and/or nutrients in nutraceuticals (vitamins and supplements), particles in personal care products (shampoo, conditioner, soap, toothpaste, etc., an crystalline fat alternative in food processing [71]
37	Campesterol	0.80	Campesterol is also sometimes used to treat some specific prostate conditions, used in nutrient medicines such as nutrients, body building supplements, and food additive [72]
38	Stigmasterol	0.62	Antimicrobial, anticancer, anti-arthritic, antiasthma, diuretic, anti-inflammatory [53]
39	β.-Sitosterol	3.10	Anabolic steroids in sports, heart disease, and high cholesterol. It is also used for boosting the immune system and for preventing colon cancer, as well as for gallstones, the common cold and flu (influenza), HIV/AIDS, rheumatoid arthritis, tuberculosis, psoriasis, allergies, cervical cancer, fibromyalgia, systemic lupus erythematosus, asthma, hair loss, bronchitis, migraine headache, and chronic fatigue syndrome, anti-inflammatory, anti-cancer, antidiabetic, antipyretic, and antioxidant [73-79]

GC-MS: Gas chromatography and mass spectroscopy, *P. crassicaulis: Pavetta crassicaulis*

inflammatory secondary metabolites which was reducing the paw edema significantly (Table 4 , Figs. 16 and 17).

Anti-inflammatory activity of flower crude extracts

Like leaf ethnolic extract, the flower ethanolic extract also showed appreciated effect in suppressing inflammation. Here, also petroleum ether and chloroform showed nil effect on the tested animals in suppressing inflammation. The activity is dose dependent and in all the concentrations (100, 150, 200, 250, and 500 mg/kg concentration), the ethanolic flower extract showed excellent activity which is revealed statistically through one-way ANOVA. By this, it is proved that ethanolic leaves extract having some metabolite responsible for its anti-inflammatory activity (Table 5 , Figs. 18 and 19).

Percentage of inflammation inhibition of all leaf extracts

The percentage of inflammation inhibition of the petroleum ether and chloroform leaf crude extracts were initially showed negative values (0–1 h) after the initial hours (2–4 h), these extracts also showed positive results with little inflammation inhibition at final hour (6 h). The ethanolic crude extract showed excellent results in all the time intervals, in all the concentrations (100, 150, 200, 250, and 500 mg/kg). In all the crude extracts, ethanolic extract has excellent inflammation

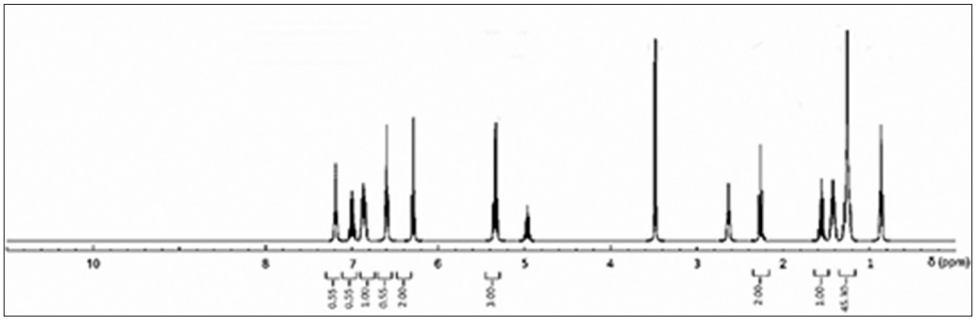

Fig. 8: 2-tert-butyl-4,6-bis(3,5-di-tert-butyl-4-hydroxybenzyl)phenol- ^{1}H NMR spectrum

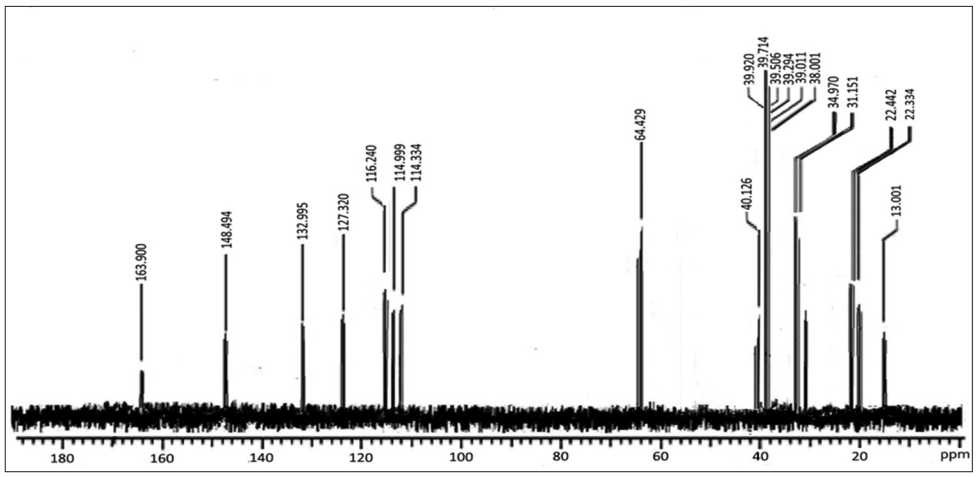

Fig. 9: 2-tert-butyl-4,6-bis(3,5-di-tert-butyl-4-hydroxybenzyl)phenol - ^{13}C NMR spectrum

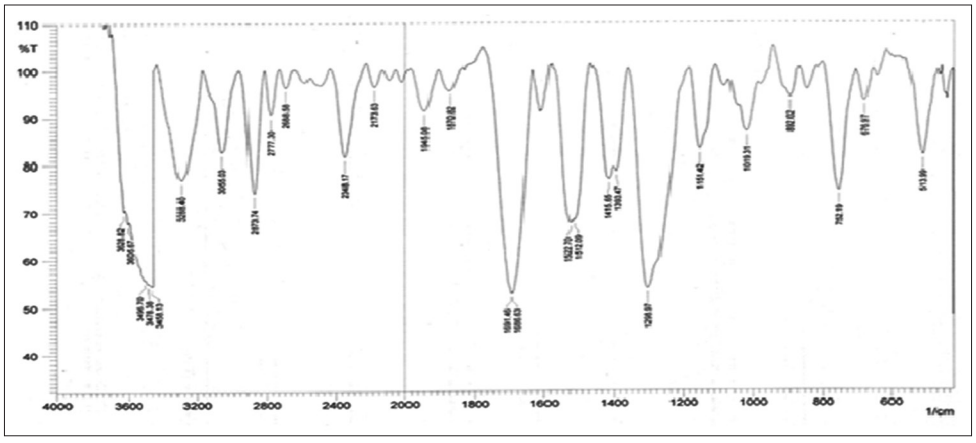

Fig. 10: 2-tert-butyl-4,6-bis(3,5-di-tert-butyl-4-hydroxybenzyl)phenol – infrared spectrum

suppression activity and rest of the extracts has negligible inflammation suppression (Table 6 , Figs. 20 and 21).

Percentage of inflammation inhibition of all flower extracts

For initial hours, petroleum ether and chloroform flower extracts were showed negative values (0–1 h) after that (2–4 h) petroleum ether and chloroform extracts also showed positive results with little inflammation inhibition. The flower ethanolic extract showed appreciable inflammation suppression activity in all the time intervals, in all the concentrations (100, 150, 200, 250, and 500 mg/kg) (Table 7, Figs. 22 and 23).

Anti-inflammatory activity of pure compound 2-tert-butyl-4,6-bis(3,5-di-tert-butyl-4-hydroxybenzyl)phenol

The pure compound "2-tert-butyl-4,6-bis(3,5-di-tert-butyl-4-hydroxybenzyl)phenol" (10 mg/kg) which is extracted from ethanolic leaf crude extract of *P. crassicaulis* Bremek. has excellent anti-inflammatory activity which is almost equal to the standard diclofenac (10 mg/kg) used in all the intervals of time. By statistical tool ANOVA, it is revealed that the activity showed by the pure compound which is equivalent to the standard used(Table 4 and Figs. 17, 20, 21).

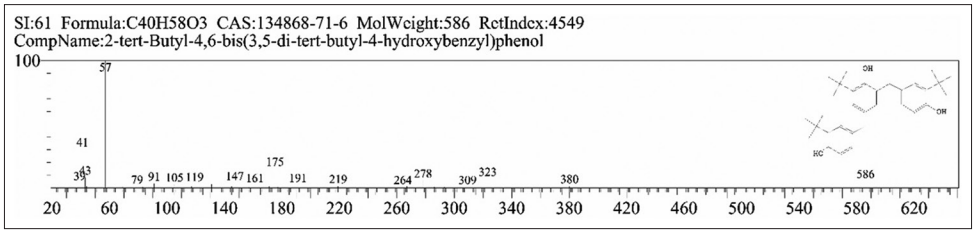

Fig. 11: 2-tert-butyl-4,6-bis(3,5-di-tert-butyl-4-hydroxybenzyl)phenol – mass spectrum

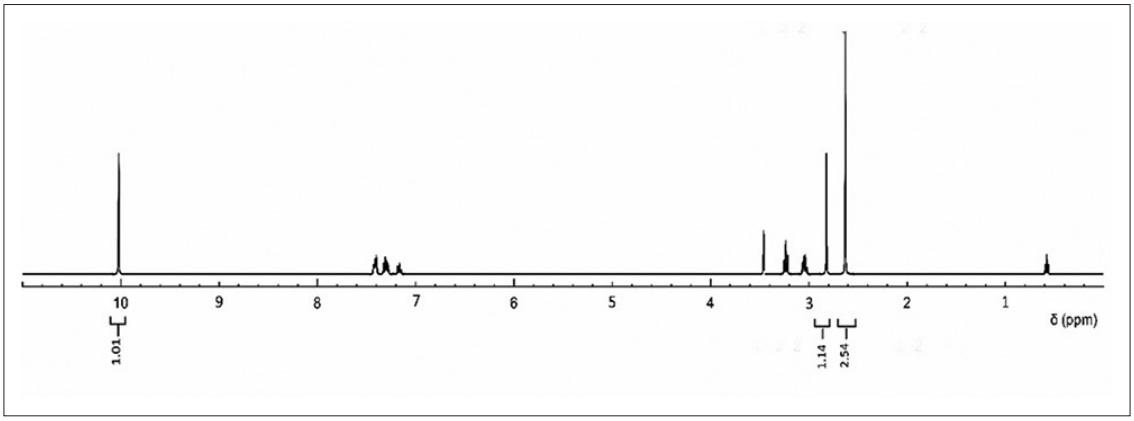

Fig. 12: 4H-pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl- ^{1}HNMR spectrum

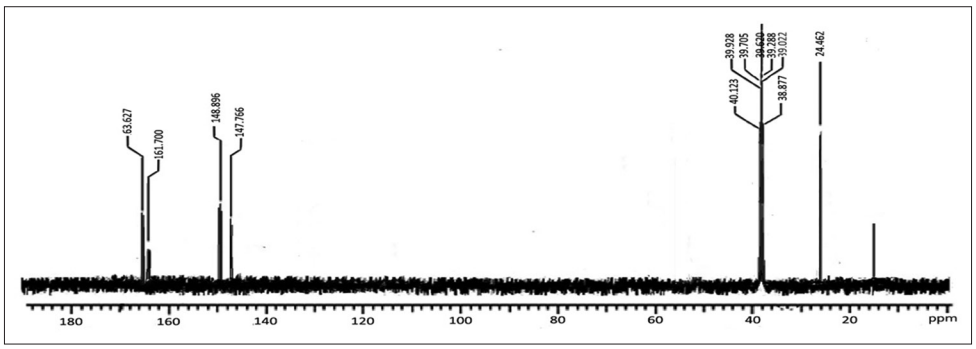

Fig. 13: 4H-pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl- ^{13}C NMR spectrum

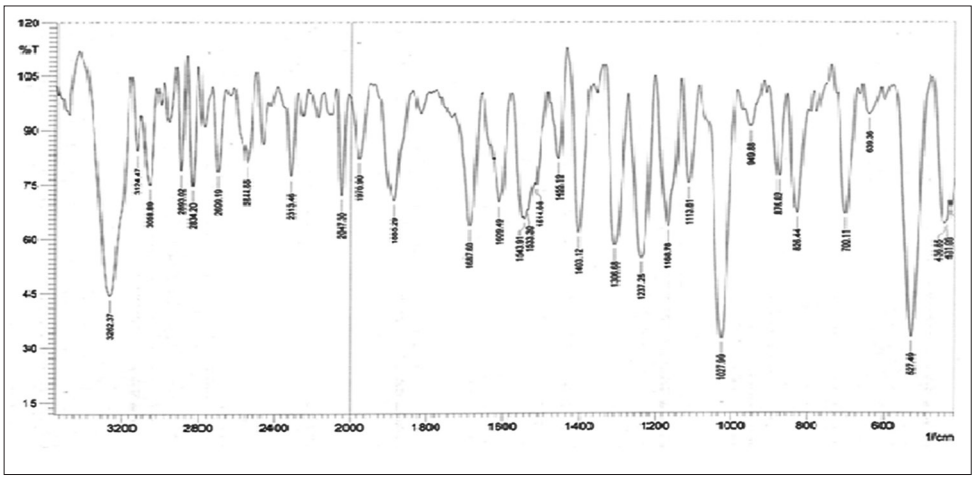

Fig. 14: 4H-pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl- infrared spectrum

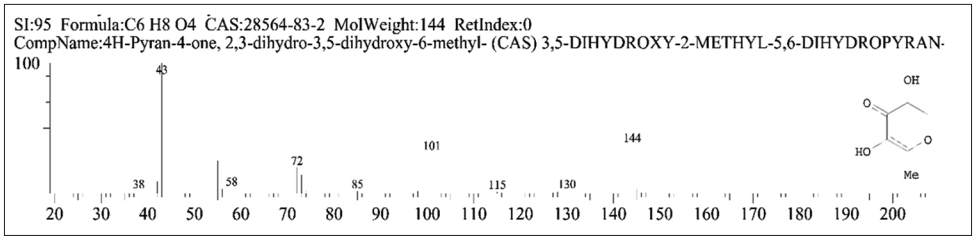

Fig. 15: 4H-pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl- mass spectrum

Table 4: Anti-inflammatory activity of different solvent extracts of *P. crassicaulis* Bremek. leaf and its pure compound at different time intervals

Extracts/pure compound/standard drug/control	Dose mg/kg	Time in hours/paw volume (ml)				
		0 h	1 h	2 h	4 h	6 h
Petroleum ether	100	2.95±0.05	2.64±0.01	2.5±0.07	2.38±0.01	2.37±0.01
	150	2.86±0.03	2.59±0.07	2.47±0.01	2.25±0.04	2.23±0.04
	200	2.78±0.02	2.55±0.05	2.43±0.04	2.17±0.01	2.17±0.01
	250	2.75±0.05	2.48±0.06	2.4±0	2.03±0.02	2.06±0.01
	500	2.71±0.04	2.38±0.06	2.38±0.01	1.98±0	1.95±0.04
Chloroform	100	2.77±0.05	2.57±0.04	2.52±0.02	2.48±0.05	2.52±0.05
	150	2.74±0.05	2.46±0.09	2.47±0.01	2.46±0	2.46±0
	200	2.62±0.01	2.44±0.04	2.43±0.02	2.44±0.04	2.42±0.05
	250	2.58±0.05	2.42±0.01	2.4±0.03	2.42±0.01	2.34±0.02
	500	2.49±0.09	2.33±0.04	2.38±0	2.4±0.02	2.3±0.09
Ethanolic extract	100	1.11±0.08***	1.49±0.09***	1.23±0.01***	1.13±0.01***	1.12±0.03***
	150	0.85±0.03***	1.06±0.04***	1.02±0.05***	0.99±0.01***	1.07±0.02***
	200	0.71±0.03***	0.95±0.05***	0.97±0.01***	0.83±0.03***	0.92±0.05***
	250	0.59±0.05***	0.85±0.02***	0.8±0.06***	0.69±0.01***	0.86±0.04***
	500	0.5±0.09***	0.71±0.08***	0.75±0.04***	0.62±0.02***	0.72±0.02***
Standard diclofenac sodium	10	0.46±0.028***	0.65±0.03***	0.62±0.03***	0.65±0.03***	0.55±0.03***
2-tert-butyl-4,6-bis (3,5-di-tert- butyl-4-hydroxybenzyl) phenol	10	0.39±0.005***	0.67±0.02***	0.67±0.02***	0.67±0.02***	0.59±0***
Control	-	2.15±0.02	2.33±0.02	2.65±0.01	2.68±0.06	2.55±0.1

The data are presented as mean±SEM, n=6. Statistical analysis were performed using one-way analysis of variance (ANOVA), followed by Dunnet's multiple comparison test. Levels of significance: *p=0.05, **p<0.01, p<***0.001 compared to control group. SEM: Standard error of the mean, *P. crassicaulis: Pavetta crassicaulis*

Table 5: Anti-inflammatory activity of different solvent extracts of *P. crassicaulis* Bremek. flower and its pure compound at different time intervals

Extracts/pure compound/standard drug/control	Dose mg/kg	Time in hours/paw volume (ml)				
		0 h	1 h	2 h	4 h	6 h
Petroleum ether	100	2.61±0	2.58±0.01	2.45±0.01	2.38±0.01	2.32±0.01
	150	2.54±0.02	2.51±0	2.39±0.01	2.31±0.01	2.26±0.01
	200	2.42±0	2.46±0.01	2.32±0.01	2.24±0.02	2.19±0
	250	2.34±0.01	2.39±0	2.26±0	2.19±0	2.07±0.01
	500	2.27±0	2.36±0	2.2±0	2.05±0.03	1.97±0.01
Chloroform	100	2.64±0.01	2.54±0.01	2.46±0	2.34±0.02	2.28±0.01
	150	2.54±0	2.47±0.01	2.39±0	2.25±0	2.16±0
	200	2.46±0	2.41±0	2.34±0	2.16±0.02	2.08±0
	250	2.35±0	2.38±0	2.27±0	2.04±0.01	1.94±0.01
	500	2.27±0	2.31±0	2.21±0	1.99±0	1.83±0.02
Ethanolic extract	100	1.87±0.01***	1.66±0.02***	1.37±0.01***	1.22±0.01***	1.11±0.01***
	150	1.75±0.01***	1.46±0.02***	1.27±0.01***	1.1±0***	1.043±0***
	200	1.67±0.01***	1.4±0.33***	1.19±0.01***	0.93±0.02***	0.88±0***
	250	1.53±0.34***	1.33±0.33***	0.86±0.06***	0.85±0.02***	0.8±0***
	500	1.46±0.01***	1.24±0.35***	0.86±0.01***	0.72±0.01***	0.71±0***
Standard diclofenac sodium	10	0.76±0.01***	0.65±0.02***	0.56±0.01***	0.55±0.01***	0.52±0***
4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	10	0.88±0.04***	0.67±0.01***	0.62±0***	0.59±0***	0.6±0***
Control	-	2.15±0.01	2.33±0.01	2.65±0	2.68±0.03	2.55±0.06

The data are presented as mean±SEM, n=6. Statistical analysis were performed using one-way analysis of variance (ANOVA), followed by Dunnet's multiple comparison test. Levels of significance: *p=0.05, **p<0.01, ***p<0.001 compared to control group. SEM: Standard error of the mean, *P. crassicaulis: Pavetta crassicaulis*

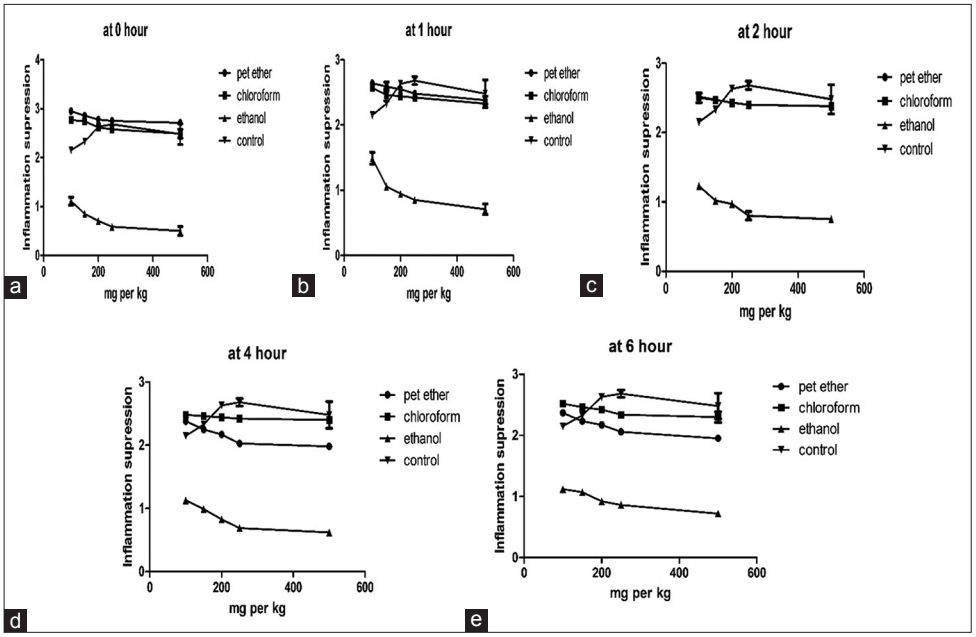

Fig. 16: (a-e) Anti-inflammatory activity of different solvent extracts of *Pavetta crassicaulis* Bremek. leaf compared with the control at different time intervals

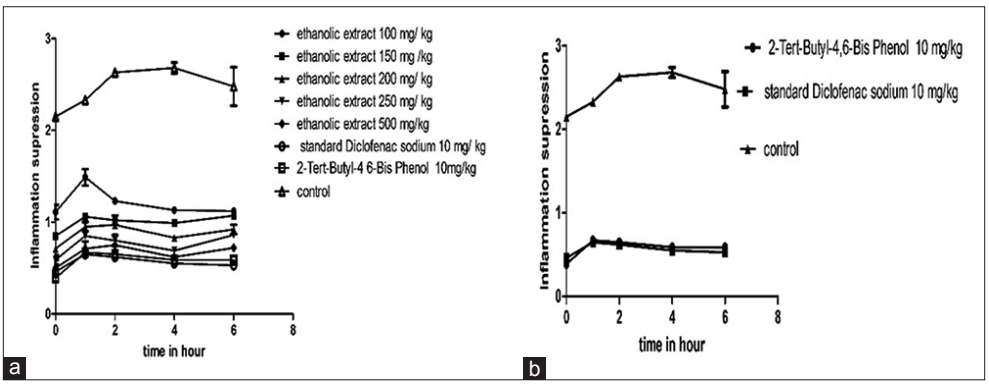

Fig. 17: (a-b) Anti-inflammatory activity of *Pavetta crassicaulis* Bremek. leaf ethanolic extracts and its pure compound compared with standard and control at different time intervals

Table 6: Percentage of edema inhibition of different solvent extracts of *P. crassicaulis* Bremek. leaf and its pure compound at different time intervals

Extracts/pure compound/standard drug/control	Dose mg/kg	Time in hours/percentage of inhibition of paw edema				
		0 h	1 h	2 h	4 h	6 h
Petroleum ether	100	−37.2	−13.3	4.94	11.19	7.05
	150	−33.02	−11.15	6.08	16.04	12.54
	200	−29.3	−9.44	7.6	19.02	14.9
	250	−27.9	−6.43	8.74	24.25	19.21
	500	−26.04	−2.14	9.5	26.11	23.52
Chloroform	100	−28.83	−10.3	4.18	7.46	1.17
	150	−27.44	−5.57	6.08	8.2	3.52
	200	−21.86	−4.72	7.6	8.95	5.09
	250	−20	−3.86	8.74	9.7	8.23
	500	−15.81	0	9.5	10.44	9.8
Ethanolic extract	100	48.37	36.05	53.23	57.83	56.07
	150	60.46	54.5	61.21	63.05	58.03
	200	66.97	59.22	63.11	69.02	63.92
	250	72.55	63.51	69.58	74.25	66.27
	500	76.74	69.52	71.48	76.86	71.76
Standard diclofenac sodium	10	78.6	72.1	76.42	75.74	78.43
2-tert-butyl-4,6-bis (3,5-di-tert-butyl-4-hydroxybenzyl) phenol	10	81.86	71.24	74.52	75	76.86
Control	-	0	0	0	0	0

Percentage of inhibition was calculated [25] with the formula % inhibition of edema =(Vc–Vt)/Vc×100. Vt=mean paw volume of test group. Vc=mean paw volume of control group. *P. crassicaulis: Pavetta crassicaulis*

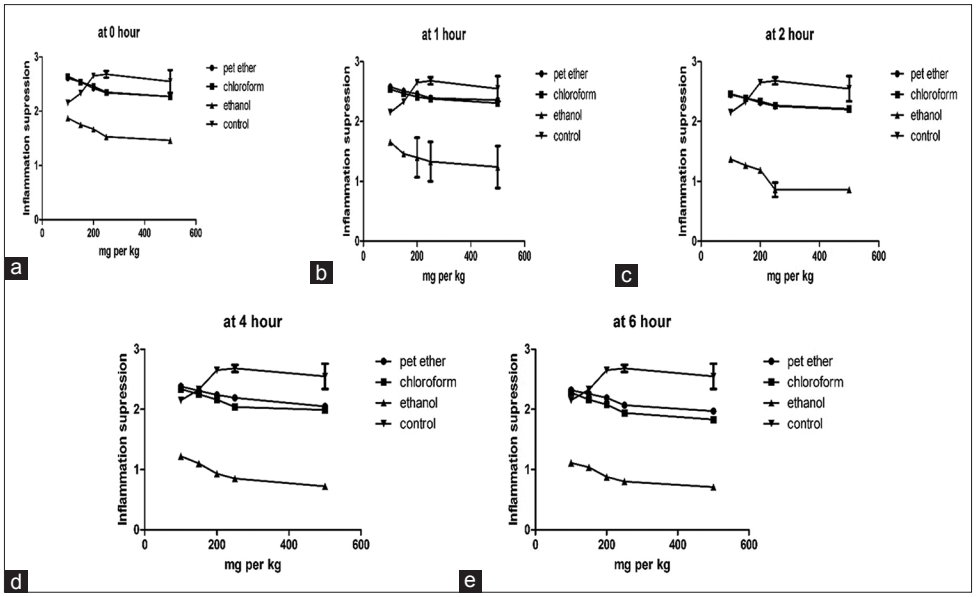

Fig. 18: (a-e) Anti-inflammatory activity of different solvent extracts of *Pavetta crassicaulis* Bremek. flower compared with the control at different time intervals

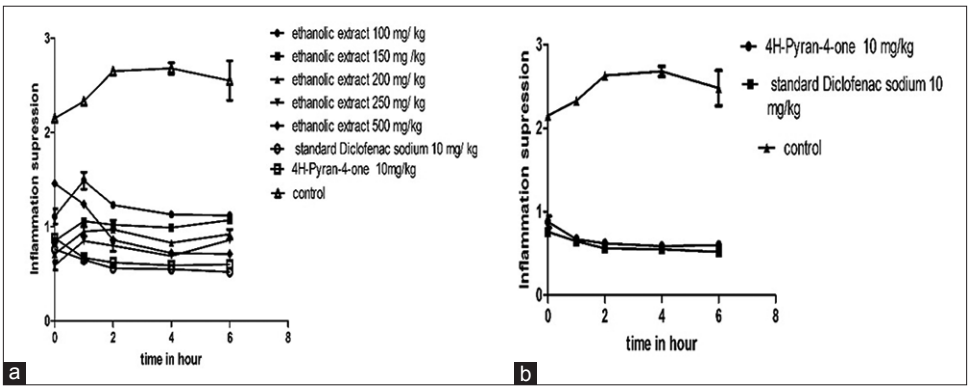

Fig. 19: (a-b) Anti-inflammatory activity of *Pavetta crassicaulis* Bremek. flower ethanolic extracts and its pure compound compared with standard and control at different time intervals

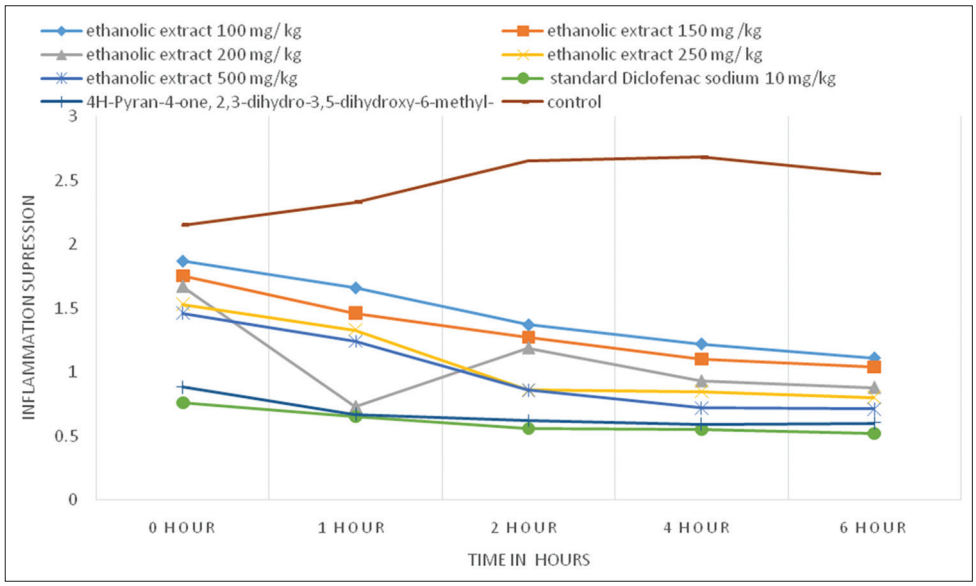

Fig. 20: Inflammation suppression in rats after treating leaf ethanolic extracts and its pure compound at different time intervals at different concentrations

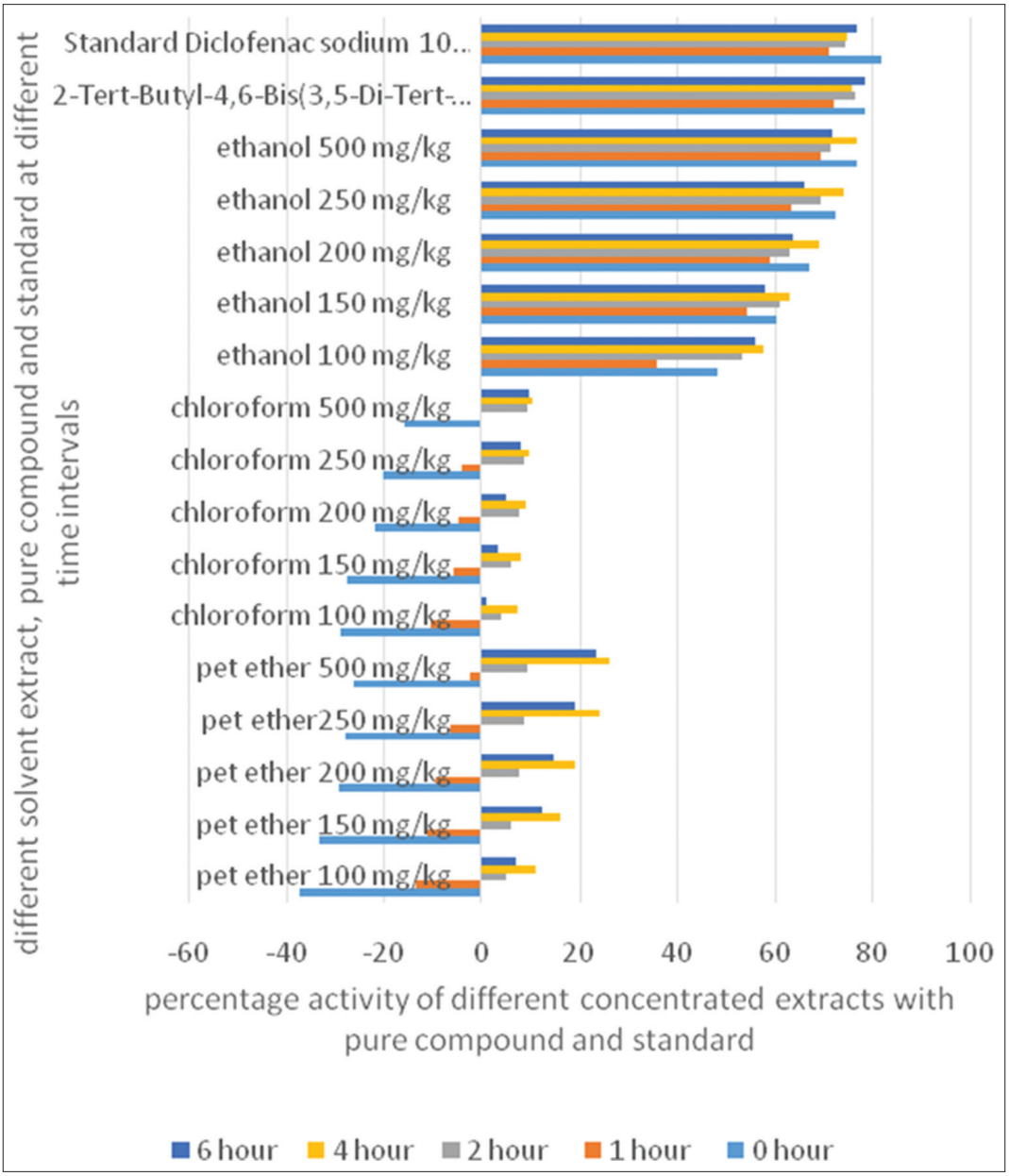

Fig. 21: Percentage inflammation inhibition of different solvent extracts of *Pavetta crassicaulis* Bremek. leaf at different concentration at different time intervals

Percentage of inflammation inhibition of pure compound 2-tert-butyl-4,6-bis(3,5-di-tert-butyl-4-hydroxybenzyl)phenol

The pure compound 2-tert-butyl-4,6-bis(3,5-di-tert-butyl-4-hydroxybenzyl)phenol (10 mg/kg) has showed excellent percentage of inflammation inhibition almost equal to the standard diclofenac (10 mg/kg) used in all the intervals of time (Table 6 and Figs. 17, 20, 21).

Anti-inflammatory activity of pure compound 4H-pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl-

The pure compound 4H-pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl- (10 mg/kg) which is extracted from ethanolic flower crude extract of *P. crassicaulis* Bremek. has excellent anti-inflammatory activity equal to the standard diclofenac (10 mg/kg) used in all the intervals of time. By statistical tool ANOVA, it is revealed that the activity showed by the pure compound which is equivalent to the standard used. The results obtained once again withstand the positive activity of the pure compound which was previously tested for its anti-inflammatory activity [27-29] (Table 5 and Figs. 19, 22, 23).

Percentage of inflammation inhibition of pure compound 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-

The pure compound 4H-pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl- (10 mg/kg) has showed excellent percentage of inflammation inhibition which is almost equal to the standard diclofenac (10 mg/kg) used in all the intervals of time (Table 7 and Figs. 19, 22, 23).

DISCUSSION

Preliminary quantitative phytochemical analysis of *P. crassicaulis* Bremek. leaf 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, flavanoids, steroids/sterols, glycosides, and phenols.

Preliminary quantitative phytochemical analysis of *P. crassicaulis* Bremek. flower petroleum ether, chloroform crude extracts showed negative results for all the tested phytochemicals aqueous extract

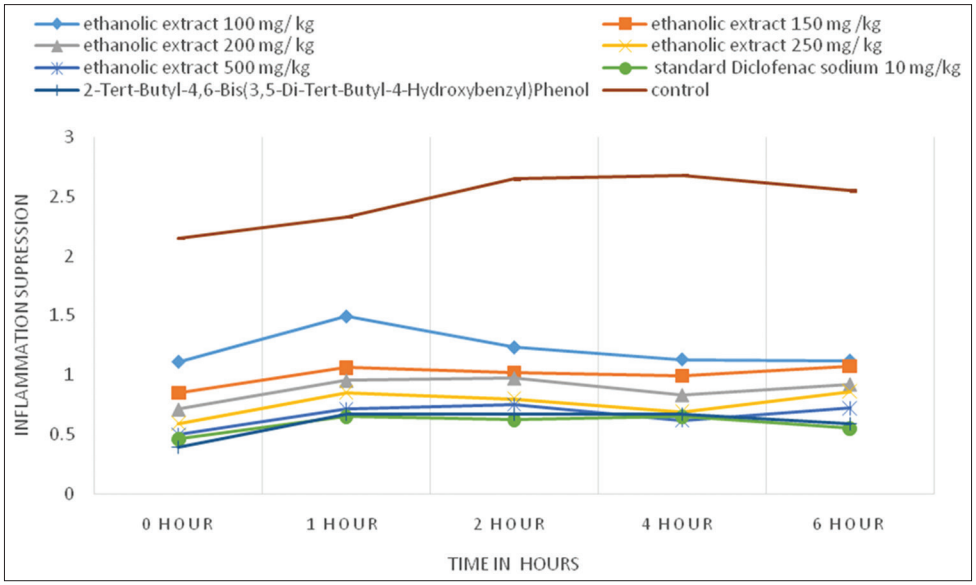

Fig. 22: Inflammation supression in rats after treating flower ethanolic extracts and its pure compound at different time intervals at different concentrations

Table 7: Percentage of edema inhibition of different solvent extracts of *P. crassicaulis* Bremek. flower and its pure compound at different time intervals

Extracts/pure compound/standard drug/control	Dose mg/kg	Time in hours/percentage of inhibition of paw edema				
		0 h	1 h	2 h	4 h	6 h
Petroleum ether	100	−21.39	−10.72	7.54	9.01	11.19
	150	−18.13	−7.72	9.81	11.37	13.8
	200	−12.55	−5.57	12.45	14.11	16.41
	250	−8.83	−2.57	14.71	18.82	18.28
	500	−5.58	−1.28	16.98	22.74	23.5
Chloroform	100	−22.79	−9.01	7.16	10.58	12.68
	150	−18.13	−6	9.81	15.29	16.04
	200	−14.41	−3.43	11.69	18.43	19.4
	250	−9.3	−2.14	14.33	23.92	23.88
	500	−5.58	0.85	16.6	28.23	25.74
Ethanolic extract	100	13.02	28.75	48.3	56.47	54.47
	150	18.6	37.33	52.07	59.21	58.95
	200	22.32	68.66	55.09	65.49	65.29
	250	28.83	42.91	67.54	68.62	68.28
	500	32.09	46.78	67.54	72.15	73.13
Standard diclofenac sodium	10	64.65	72.1	78.86	79.6	79.47
4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	10	59.06	71.24	76.6	76.47	77.98
Control	-	0	0	0	0	0

Percentage of inhibition was calculated [25] with the formula % inhibition of edema =(Vc–Vt)/Vc×100. Vt=mean paw volume of test group. Vc=mean paw volume of control group.

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 tests confirms that the ethanol dissolves all the phytochemicals from *P. crassicaulis* Bremek. in a sufficient quantity to influence different phytochemical and pharmacological activities.

Leaf ethanolic crude extract was subjected to GC-MS revealed the presence of 33 compounds (Table 2 and Fig. 2). Among all the confirmed phytochemicals, 21 compounds reported for many medicinal and pharmacological properties and 12 compounds activity was not reported. Among the 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- (9.34%) was reported for many pharmacological properties including antioxidant properties [26-28].

All the other phytocompounds such as 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%), 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-

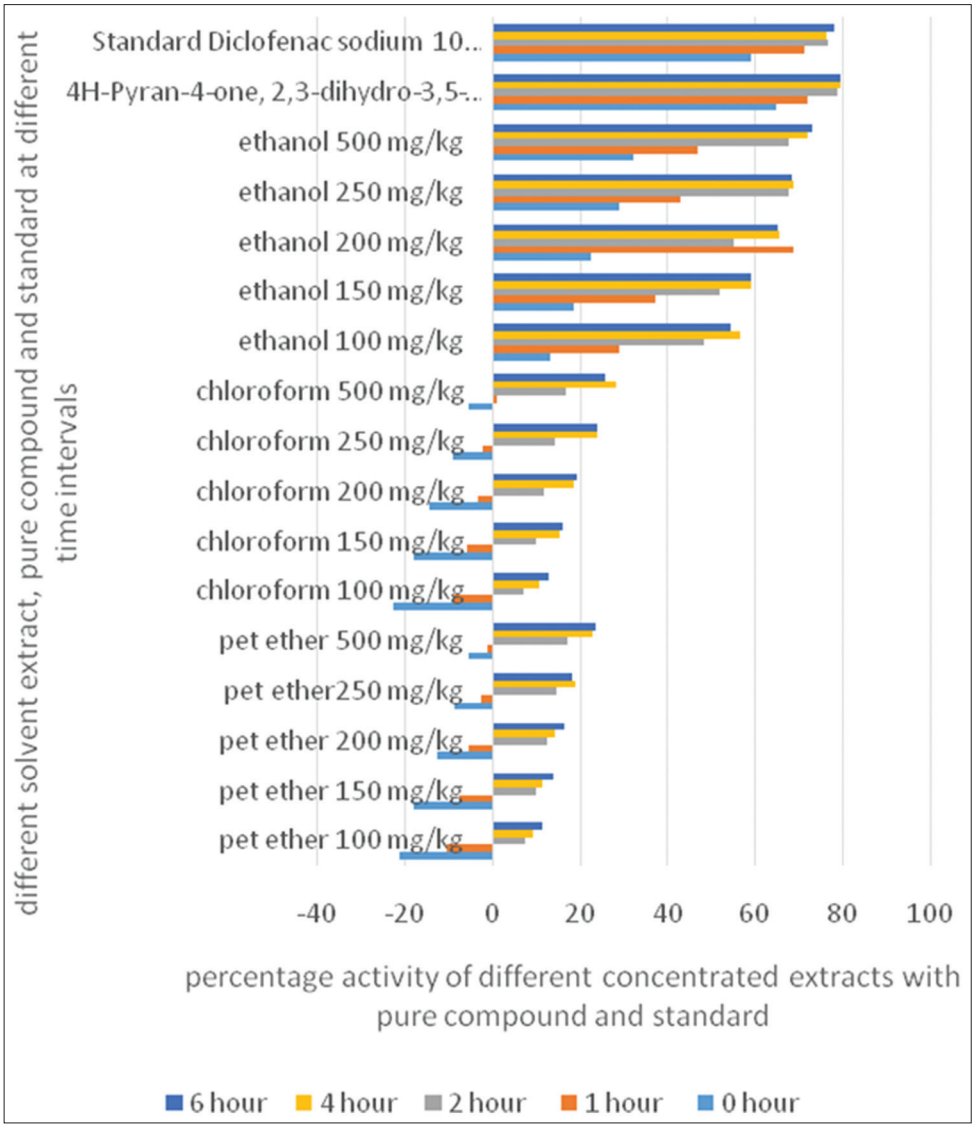

Fig. 23: Percentage inflammation inhibition of different solvent extracts of *Pavetta crassicaulis* Bremek. flower at different concentration at different time intervals

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 and Fig. 2).

Flower ethanolic crude extract was subjected to GC-MS revealed the presence of 39 compounds (Table 3 and Fig. 5). Among all the confirmed phytochemicals, 31 compounds reported for many medicinal and pharmacological properties and 8 compounds activity was not reported.

Among the phytochemicals present in the flower ethanolic extract, 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- (13.82%), Benzaldehyde, 2-methyl- (7.25%), Benzaldehyde, 2-methyl-6 hydroxy (6.52%), 2-Furancarboxaldehyde, 5-(hydroxymethyl)- (6.3%), were the major compounds. The major compound 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-was previously report for its anti-oxidant and other pharmacological properties [27-29], Benzaldehyde, 2-methyl-, Benzaldehyde, 2-methyl- 6 hydroxy, 2-Furancarboxaldehyde, 5-(hydroxymethyl)- were commonly used in food and perfume industry as a flavoring agent [44,45,62].

The rest of the compound present in the GC-MS analysis of flower ethanolic extract was Methyl Ester Of Bicyclo[4.3.0]Non-1(6)-En-4,7-Dione-8- (4.77%), 1,2-Benzenediol (4.33%), Alpha.-D-Glucopyranose, 4-O-.beta.-D-galactopyranosyl- (3.95%), Benzofuran, 2,3-dihydro- (3.5%), N-Hexadecanoic Acid (3.11%), β.-Sitosterol (3.1%), 1,2,3-Propanetriol, diacetate (3%), 9,12-Octadecadienoic acid (Z,Z)-, 2-hydroxy-1- (2.75%), 2,6-Dimethyl-4-hydroxybenzaldehyde (2.73%), 2-Methoxy-4-vinylphenol (2.58%), Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester (2.57%), 4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol (2.57%), 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)- (2.38%), Benzaldehyde, 4-hydroxy- (2.3%), Acetic acid, 1-(2-methyltetrazol-5-yl)ethenyl ester (2.15%), Benzoyl. beta.-d-glucoside (2.13%), 2,5-Dimethyl-4-hydroxy-3(2H)-furanone (1.99%), Benzoic acid, ammonium salt (1.62%), 2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one (1.4%), cis,cis,cis-7,10,13-Hexadecatrienal (1.34%), 9,12-Octadecadienoic acid, methyl ester (1.29%), Hexadecanoic acid, methyl ester (1.11%), 2,2-Dimethyl-3-[3-methyl-5-(phenylthio)pent-3-enyl]oxirane (1.06%), Butanedioic acid, monomethyl ester (0.9%), Tricyclo[7.1.0.0[1,3]]decane-2-carbaldehyde

(0.88%), 2,4-Dimethyl-3-nitrobicyclo[3.2.1]octan-8-one (0.85%), Hexatriacontane (0.8%), Campesterol (0.8%), 2-acetyl-2-hydroxy-.gamma.-butyrolactone (0.73%), 2(3H)-Naphthalenone, 4,4a,5,6-tetrahydro- (0.62%), Neophytadiene (0.62%), Stigmasterol (0.62%), N,N'-Dimethylpiperazine (0.6%), 2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl- (0.56%), 1-Butanamine, 2-methyl-N-(2-methylbutylidene)- (0.48%) in meager quantity has many pharmacological properties were reported (Table 3 and Fig. 5).

The pure compounds showed excellent inflammation suppression activity in that, 2-tert-butyl-4,6-bis(3,5-di-tert-butyl-4-hydroxybenzyl) phenol showed 75.88 % average edema suppression and 4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl- 72.24% average edema suppression respectively which is almost equal to the 75.94% average edema suppression.

Some of the researchers used different methods of anti-inflammatory activity such as croton oil-induced ear edema in rats [80,81], protein denaturation bioassay [82-86], membrane stabilization assay using human red blood cell [87,88], but most of the researchers followed carrageenan-induced rat paw edema [89-93] to evaluated anti-inflammatory activity.

The results obtained from the above experiments, the ethanolic leaf and flower extracts of *P. crassicaulis* Bremek. showed excellent inflammation suppression activity, in that leaf ethanolic extract showed good inflammation suppression compared with the flower ethanolic extract. GC-MS analysis both ethanolic crude extracts revealed the presence of 33 and 39 compounds, respectively, in that major compounds were 2-Tert-Butyl-4,6-Bis(3,5-Di-Tert-Butyl-4-Hydroxybenzyl)Phenol (38.84%) and 4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl- (13.82%) respectively.

The extracted pure compounds 2-tert-butyl-4,6-bis(3,5-di-tert-butyl-4-hydroxybenzyl)phenol and 4H-pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl- showed excellent anti-inflammation activity almost comparable to the standard diclofenac used.

The pure compound extracted from flower ethanolic extract 4H-pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl- [27-29] was previously confirmed for its positive inflammation suppression activity and the pure compound extracted from leaf ethanolic extract 2-tert-butyl-4,6-bis(3,5-di-tert-butyl-4-hydroxybenzyl)Phenol is the new report in anti-inflammatory activity.

2-tert-butyl-4,6-bis(3,5-di-tert-butyl-4-hydroxybenzyl)Phenol isolated from leaf is excellent inflammation suppressor in compared with the 4H-pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl- extracted from flower ethanolic extract and the crude extracts were also showed the same activity which was also influence by the quantity of compound present in both ethanolic extracts (2-tert-butyl-4,6-bis(3,5-di-tert-butyl-4-hydroxybenzyl)phenol - 38.84% and 4H-pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl- 13.82% in GC-MS analysis).

In the crude extracts minor compound in little quantity may also influence the activity, if major compound confirms the positive effect, then its influence is more in the activity. In our experiments, both the extracted pure compound showed positive effect in suppressing inflammation.

After administration of crude extracts in tested animals were showed effective in suppressing the inflammation from 0 h and in next successive hours, these extracts maintain its effectiveness till the last testing hour (6 h).

CONCLUSION

The present study shows that the leaf and flower ethanolic crude extracts have remarkable anti-inflammatory activity compared to both petroleum ether and chloroform extracts. The pure compound 2-tert-butyl-4,6-bis(3,5-di-tert-butyl-4-hydroxybenzyl)phenol extracted from leaf ethanolic crude extract and 4H-pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl- extracted from flower ethanolic extract has excellent anti-inflammatory activity which is almost equal to the standards diclofenac sodium used. These results confirm positive activity of the plant as therapeutic agent in tribal medicine. Thus, *P. crassicaulis* Bremek. leaf and flower parts could be exploited as a valuable source of anti-inflammatory agent for the pharmaceutical industry.

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AUTHOR'S CONTRIBUTIONS

Ashwathanarayana R has collected the data, conducted the experiment, and drafted the article. Dr. Raja Naika, professor, has supervised the experiment and reviewed the article.

CONFLICT OF INTEREST

None.

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