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Research Article

Antioxidant, Anti-Inflammatory and Antipyretic Activities of Ethyl Acetate Fraction of Ethanolic Extract of *Schrebera swietenioides* Roxb. Root

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ABSTRACT

Ethanolic extract of *Schrebera swietenioides* roxb. (SSE) root was prepared by soxhlation method and screened for its *in vitro* antioxidant, anti-inflammatory (acute, chronic) and antipyretic activity in wistar rats. Antioxidant assay was performed by using DPPH, ABTS and Lipid peroxidation assay methods. In antioxidant assay, % inhibition and IC_{50} was calculated. SSE produced 50% inhibition (IC50) at 1.7 µg in DPPH, 4.35µg in ABTS, 23.53µg in lipid peroxidation assay and shown more potent antioxidant activity compare to standard compound. Anti-inflammatory activity was performed by using *carrageenan* induced paw edema and complete Freund's adjuvant arthritis in rats. In these assays, SSE showed dose dependant inhibition in paw edema. Maximum inhibition was found at 300 mg /kg oral dose of SSE in rats. In Carrageenan induced paw edema SSE showed maximum 67.81% inhibition and in freund's adjuvant arthritis it showed maximum 50% inhibition at 300 mg/kg oral dose in rats, which was significantly different from control ($p<0.05$). SSE also showed dose dependant decrease in body temperature in brewer's yeast induced pyrexia in rats. SSE significantly decrease in body temperature ($p<0.05$) at 300 mg/kg compare to control. Present study showed that SSE had significant antioxidant, anti-inflammatory and antipyretic activity.

Key words: antioxidant, anti-inflammatory, antipyretic, *Schrebera swietenioides* roxb., DPPH, ABTS.

INTRODUCTION

Schrebera swietenioides roxb. (Weaver's Beam tree) belonging to family Oleaceae, is a moderate sized tree of 20 m height with thick grey bark growing in deciduous forests up to an altitude of 1200 m, throughout India. Leaves opposite, simple or imparipinnate; rachis usually winged. Infloresence a paniculate cyme. Flowers heterostylous and bisexual. Calyx campanulate, loosely enveloping the corolla, truncate or irregularly and obscurely lobed. Corolla salver-shaped, white, sometimes tinged with pink or puce; tube well developed, cylindrical; segments 6 or more, spreading to reflexed, each with a group of swollen brown to purplish hairs at the base. Stamens 2, inserted on the corolla; filaments short, anthers large, introrse. Ovary bilocular, small, truncate or obscurely bi-lobed at apex; ovules 4 in each loculus; style filiform; stigma included or excerted, subcapitate or oblong in outline. Capsule bi-valved, woody with loculicidal dehiscence; seeds produced into a long solitary subapical wing.[1, 2, 3]

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Root, bark, leaves, fruits which are used for medicinal purpose are bitter, acrid, appetizing, digestive, thermogenic, stomachic, depurative, constipating urinary astringent and anthelmintic. However, there is no scientific evidence or data to substantiate the folklore claims of this particular herb against antioxidant, anti-inflammatory and antipyretic activities.[4]

MATERIALS AND METHODS

Plant material- Roots of SSE were collected from forests of Telangana, A.P. and were authenticated by Dr. Gopal Krishna Bhatt, Udupi, Karnataka. A specimen was submitted to Manipal College of Pharmaceutical Sciences for future references. Roots of SSE were dried under shade, coarsely powdered and stored in airtight container for further use.

Chemicals and Instruments: All chemicals and solvents used in the study were analytical grade. DPPH (1, 1- Diphenyl-2-Picryl Hydrazyl) and ABTS (2, 2-Azino bis (3-ethyl Benzo Thiazoline-6-Sulphonic acid) were obtained from Sigma Chemicals (St.louis, Mo, USA). Sodium nitroprusside,

Table 1: *DPPH assay of EAF with respect to ascorbic acid as standard*

Conc	Absorbance		% inhibition	
(µg/ml)	Ascorbic acid	EAF	Ascorbic acid	EAF
1	0.266±0.008	0.246±0.008	27.1±3.70	24.5±1.86
2	0.213±0.008	0.126±0.006	41.8±2.05	61.1±2.53
4	0.15±0.005	0.083±0.003	58.96±2.57	74.4±1.35
6	0.08±0.003	0.056±0.003	77.25±0.94	82.6±0.91
8	0.056±0.003	0.053±0.003	84.56±0.58	83.6±1.22
Control	0.3661±0.008	0.326±0.006		
IC_{50}			2.98 µg/ml	1.72 µg/ml

Table 2: *ABTS assay of EAF with respect to ascorbic acid as standard*

Conc	Absorbance		% inhibition	
(µg/ml)	Ascorbic acid	EAF	Ascorbic acid	EAF
1	0.473±0.012	0.393±0.003	15.86±3.4	6.13±3.06
2	0.346±0.003	0.336±0.008	38.43±1.04	19.52±4.70
4	0.273±0.012	0.216±0.003	51.38±2.86	48.33±1.13
6	0.226±0.008	0.136±0.008	59.76±1.37	67.52±0.95
8	0.143±0.008	0.076±0.008	74.49±1.94	81.64±2.36
Control	0.563±0.008	0.42±0.015		
IC_{50}			3.78 µg/ml	4.13µg/ml

ferrous sulphate, trichloroacetic acid, sodium hydroxide and potassium chloride were obtained from Ranbaxy Fine Chemicals Ltd. India. Thiobarbituric acid was obtained from Himedia Laboratories Ltd. Mumbai, India. UV spectrophotometer (Shimadzu 1650 PC) and pH meter (Elico Ltd., India) were used.

Carrageenan (Sigma Chemicals, U.S.A.), Carboxy methyl cellulose (Loba Chemie, Mumbai), Methanol (Qualigens, Mumbai), Petroleum ether (Qualigens, Mumbai), aspirin (Vikas Pharma, Mumbai), paracetamol (IPCA, Mumbai) were procured.

Preparation of extracts

Ethanol extract:

The coarsely powdered shade dried root of the plant *Schrebera swietenioides* Roxb. was extracted with absolute alcohol by hot extraction process (soxhlet) for 8 h. After completion of extraction the solvent was removed by distillation and concentrated under reduced pressure.

Ethyl acetate fraction of Ethanolic Extract:

Ethanolic extract was mixed with 250ml water and 500ml of ethyl acetate (EA) was added and shaken for 15min. The separated upper layer was removed and labelled as EA fraction (EAF). The solvent was completely removed by vacuum and semisolid mass was obtained (10.6% w/w with respect to the powdered material). Ethyl acetate fraction (EAF) was stored in refrigerator and a weighed quantity was suspended in 0.5%CMC for the experiment.

Phytochemical screening:

Preliminary phytochemical investigation was carried out for EAF. Presence of alkaloids was determined by *Mayer's*, *Dragendorff's*, *Wagner* and *Hager's* test, Flavonoids by *Shinoda*, ferric chloride and lead acetate tests, saponins by foam and haemolysis tests and sterols by *Salkowski* and *Libermann-Burchards* tests.

Animal selection

For acute toxicity and pharmacological studies Wister rats weighing between 150 g to 200 g were selected. The animals were acclimatized to standard laboratory conditions (temperature 25 ± 2^{O}C) and maintained on 12 h light, 12 h dark cycle. They were provided with regular rat feed and drinking water *ad libitum*. All the studies were conducted according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India.(Institutional Animal Ethical Committee, Manipal University, Karnataka, India; Approval No# IAEC/KMC/05/2007-2008)

Acute toxicity studies

Acute toxicity studies were conducted to determine the safe dose by staircase method[5]. The overnight fasted rats were feed with EAF of SSE suspended in 0.5% carboxy methyl cellulose (CMC) at various doses viz. 500, 1000, 2000 and 5000 mg/kg body weight. After administration the animals were observed continuously for one hour, frequently for the next four hours, then periodically after each four hour and then after 24 hours. Animals surviving till 24 hours were observed for next 14 days.

Antioxidant activity:-

1. DPPH Radical Scavenging

EAF at various concentrations was made upto 1ml with methanol, 1ml of DPPH solution 0.1 mM (0.39 mg in 10ml methanol) was added to the test tube. An equal amount of ethanol and DPPH was added to the control. Ascorbic acid was used as the standard for comparison. After 20 minutes of incubation in the dark, absorbance was recorded at 517 nm. Experiment was performed in triplicate[6, 7, 8] (Table .1)

2. ABTS Radical Scavenging

The ABTS radical cation preparation: ABTS 2mM (0.0548g in 50 ml) was prepared in distilled water. Potassium per sulphate 70mM (0.0189g in 1ml) was prepared in distilled water. 200µl of Potassium per sulphate and 50 ml of ABTS were mixed and used after 2 hrs. This solution was used for the assay.To the 0.5 ml of various concentration of ethylacetate extracts of SSE, 0.3 ml of ABTS radicalcation and 1.7 ml of phosphate buffer pH 7.4 was added. For control studies,methanol was taken and the absorbance was measured at 734 nm. The experiment was performed in triplicate.[8, 9, 10] (Table.2)

3. Lipid Peroxidation Assay

Phospatidylcholine (20 mg) in 2 ml chloroform was dried under vacuum in a rotary evaporator to give a thin homogenous film and further dispersed in normal saline (5

Table 3: *Lipid peroxidation assay of EAF with respect to* α – Tocopherol as standard

Conc (µg/ml)	Absorbance		% inhibition	
	α - Tocopherol	EAF	α - Tocopherol	EAF
5	0.416±0.006	0.393±0.003	17.16±2.10	18.56±1.83
10	0.355±0.003	0.363±0.008	31.06±1.82	24.80±1.90
20	0.251±0.01	0.276±0.008	48.37±1.15	42.78±1.11
40	0.073±0.003	0.143±0.003	85.41±0.74	70.31±1.11
50	0.063±0.008	0.103±0.008	87.37±1.85	78.56±2.13
Control	0.503±0.008	0.483±0.006		
IC_{50}			20.81 µg/ml	25.15µg/ml

ml) with a vortex mixer. Lipid peroxidation was initiated by adding 0.05 mM ascorbic acid into a mixture containing 0.1 ml liposome,150 mM potassium chloride, 0.2 mM ferric chloride, extract (2 to100µg/ml). The reaction mixture as incubated at 37°C for 40 min. After incubation, the reaction was stopped by adding 1 ml of ice-cold 0.25 M sodium hydroxide containing 20 % TCA (w/v), 0.4 % TBA (w/v), and 0.05 % BHT (w/v). After incubating in a boiling water bath for 20 min, the samples were cooled to room temperature. The blank was prepared in the same manner without extract. The pink chromogen was extracted with 1ml of methanol. The absorbance was read at 532 nm. [11] (Table.3)

Anti-inflammatory activity

Carrageenan induced Paw Edema (Acute Model)

Acute inflammation was induced by injecting 1% solution of carrageenan into plantar surface of rat hind paw at the dose of 0.1ml per paw[13]. Wistar rats were divided in to five groups of six in each. A 0.5% solution of CMC at a dose of 1ml/100g/p.o was administered to group 1. The ethylacetate fraction of SSE was administered to the animals of the dose range of 100, 200 and 300mg/kg/p.o respectively to group 2, 3 and 4, against the standard drug aspirin (100mg/kg/p.o)to the group 5. After 60 minutes carrageenan solution was injected to all the groups of animals. The paw edema was measured at the intervals of 1, 2, 3 and 4 h using digital plethysmometer (UGO basile, Italy). The paw edema among the different group of animals was compared and percentage inhibition of paw edema was calculated with respect to untreated control group by using following formula.

% Inhibition of Paw Edema

$I = \{1 - .(V_t / V_c)\} \times 100$

Where,I = % inhibition of paw edema

V_t = Mean change in paw volume of drug treated rat

V_c = Mean change in paw volume of control rat

Freund's Adjuvant induced Arthritis (chronic model)

Wistar rats were divided in to five groups of six in each. Group 1served as control received 0.5 % CMC, group 5 served as standard received Aspirin (100 mg/kg p.o.) and group 2,3 and 4 received 100, 200 and 300mg/kg/p.o ethyl acetate fraction of SSE respectively. Arthritis was induced by injecting a 0.1 ml (0.5% w/v) suspension of killed *Mycobacterium tuberculosis* bacteria homogenized in liquid paraffin into the left hind paw. Drug treatment was started from the initial day i.e. from the day of injection of *Freund's* adjuvant (0 day), 30 minutes before adjuvant injection and continued till 21st day. Paw volume was measured on 1, 2, 4, 6, 8, 12, 16 and 20 day by using digital plethysmometer (UGO basile, Italy). The mean changes in injected paw edema with respect to initial paw volume, were calculated on respective days and % inhibition of paw edema with respect to untreated group was calculated using following formula[14, 15].

$I = \{1 - .(Vt / Vc)\} \times 100$

Where,I = % inhibition of paw edema

Vt = *Mean change in paw volume of drug treated rat*

Vc = *Mean change in paw volume of control rat*

Antipyretic activity

Yeast induced pyrexia

Wistar rats were divided into five groups of six in each. The normal body temperature of each rat was measured rectally and recorded. Pyrexia was induced by injecting the yeast suspension (15%W/V) by subcutaneous route in hind limbs of the rats[12]. Rats were restrained for recording rectal temperature after 18 h. A 0.5% solution of CMC at a dose of 1ml/100g/p.o was administered to group 1. The ethyl acetate fraction of SSE was administered to the animals in the dose range of 100, 200 and 300mg/kg/p.o respectively to group 2, 3 and 4, against the standard drug paracetamol (200mg/kg/p.o) to the group 5. Temperature was recorded at one hour intervals up to 3 hrs after drug administration.

Statistical analysis

Table 4: *Effects of EAF in carrageenan induced paw edema in rats with aspirin as standard*

Treatment	Dose (mg/kg)	Change in paw volume in ml (% inhibition in paw Edema)			
		1h	2h	3h	4h
Control (0.5%CMC)		0.39±0.01b	0.68±0.03b	0.87±0.02b	0.73 ± 0.03 b
EAF	100	0.38±0.04b (2.56%)	0.60±0.04b (11.76%)	0.63±0.05ab (27.58%)	0.53±0.08b (27.39%)
EAF	200	0.35±0.02b (10.76%)	0.36±0.04a (47.05%)	0.48±0.03ab (44.82%)	0.40±0.04a (45.26%)
EAF	300	0.33±0.05b (15.38%)	0.30±0.03a (55.88%)	0.28±0.06a (67.81%)	0.26±0.05a (64.38%)
Aspirin	100	0.18±0.03a (53.84%)	0.28±0.06a (58.82%)	0.27±0.07a (68.96%)	0.29±0.07a (60.27%)

a: $p < 0.05$ compared to control b: $p < 0.05$ compared to Aspirin (100 mg/kg), ab: more effective than control but less effective than standard at $p<0.05$

Table 5: *Effects of EAF in Freund's adjuvant induced arthritis in rats with aspirin as standard.*

Day after freund's complete adjuvant injection	Change in paw volume in ml (% inhibition in paw Edema)				
	Control (0.5%CMC)	EAF (100mg/kg)	EAF (200mg/kg)	EAF (300mg/kg)	Aspirin (100 mg/kg)
1	0.69±0.02	0.69±0.03 (0%)	0.64±0.03 (7.34%)	0.60±0.04 (13.04%)	0.59±0.03 (14.49%)
2	0.93±0.04b	0.78±0.05 (16.12%)	0.75±0.03a (19.35%)	0.73±0.04a (21.50%)	0.68±0.05a (26.88%)
4	1.07±0.03b	0.92±0.03b (14.01%)	0.85±0.04a (20.56%)	0.81±0.05a (24.29%)	0.76±0.03a (28.97%)
6	1.19±0.03b	0.87±0.04a (26.89%)	0.82±0.05a (31.09%)	0.77±0.04a (35.29%)	0.72±0.04a (39.49%)
8	0.95±0.05b	0.84±0.03 (11.57%)	0.79±0.03a (16.84%)	0.74±0.03a (22.10%)	0.73±0.03a (23.15%)
12	0.83±0.04b	0.79±0.02b (4.81%)	0.75±0.04b (9.63%)	0.63±0.04a (24.09%)	0.55±0.04a (33.73%)
16	0.84±0.05b	0.75±0.04 (10.7%)	0.68±0.03 (19.04%)	0.59±0.04a (29.76%)	0.61±0.05a (27.38%)
18	0.88±0.03b	0.71±0.03b (19.3%)	0.65±0.04a (26.13%)	0.53±0.04a (39.77%)	0.50±0.06a (43.18%)
20	0.92±0.03b	0.67±0.05ab (27.17%)	0.61±0.02a (33.69%)	0.46±0.03a (50%)	0.45±0.07a (51.08%)

a: $p < 0.05$ compared to control b: $p < 0.05$ compared to Aspirin (100 mg/kg), ab: more effective than control but less effective than standard at $p<0.05$

The experimental results are represented as Mean ± SEM (Standard Error of Mean). Statistical analysis was performed by one-way ANOVA followed by Tukey's test. $P< 0.05$ was considered significant.

RESULTS

Oral dose of ethyl acetate fraction of ethanolic extract of SSE was found to be safe up to 2.0gm /kg body weight in acute toxicity studies on wistar rats.

Phytochemical analysis

Phytochemical studies shown positive for alkaloid, steroid, saponin and glycosides in EAF.

Antioxidant activity

Antioxidant activity of the EAF was tested at different concentrations in different *in vitro* models. It was observed that free radicals were scavenged by the extract in concentration dependent manner up to the given concentration in all the models. The percentage scavenging and IC50 values were calculated for all the models. IC50 values were found to be at 1.7µg/ml in DPPH method, 4.35µg/ml in ABTS assay and 23.53µg/ml in lipid peroxidation assay (Table no. 1, 2, 3).

Anti-inflammatory activity

Carrageenan induced Paw Edema

EAF at the dose of 100, 200 and 300 mg/kg p.o showed significant reduction in paw edema ($P<0.001$) after carrageenan administration. It was observed that EAF at the dose of 300mg/kg/p.o produced 67.81% inhibition of paw edema (Table-4) after 3 hr of drug administration, whereas, 68.96% was produced by aspirin after the same duration.

Freund's adjuvant induced arthritis

In chronic inflammation induction model, the EAF reduced the arthritis maximum by 50% at the doses of 300mg/kg p.o. on 20th day compared to the standard drug aspirin (100 mg/kg p.o.) which reduced the arthritis by 51.08% (Table-5).

Antipyretic activity

Different doses of EAF showed significant ($p<0.05$) antipyretic activity and reduction of the elevated rectal temperature started after 3 hr of treatment (Table 6). EAF was found to decrease the rectal temperature by 1.1^{0}C at 300mg/kg p.o. as compared to paracetamol treated group which shown a decrease of 1.65^{0}C at 200mg/kg p.o.

Discussion

There is an extensive evidence to implicate free radicals in

Table 6: *Effects of EAF in Brewer's yeast induced pyrexia in rats with paracetamol as standard*

Treatment	Dose (mg/kg)	Rectal temperature (ºC)		Rectal temperature after administration of drug (ºC)		
		Normal (A)	18 hr after yeast administration(B)	1h	2h	3h
Control (0.5%CMC)	0.5 ml	35.10±0.18	36.70±0.15	36.65±0.16b	36.60±0.16b	36.53± 0.15
EAF	100	35.15±0.19	36.60±0.18	36.54±0.18b	36.36±0.17b	36.10±0.15b
EAF	200	35.20±0.16	36.81±0.16	36.51±0.14b	36.43±0.17b	35.75±0.12ab
EAF	300	35.20±0.19	36.53±0.15	36.33±0.17b	36.10±0.12b	35.43±0.13a
Paracetamol	200	35.15±0.12	36.7±0.18	35.2±0.12a	35.06±0.19a	35.05±0.16a

a: $p < 0.05$ compared to control b: $p < 0.05$ compared to paracetamol (200 mg/kg), ab: more effective than control but less effective than standard at $p<0.05$

the development of degenerative diseases. Free radicals have been implicated in the causation of ailments such as diabetes, liver cirrhosis, nephrotoxicity, cancer etc. Together with other derivatives of oxygen, they are inevitable by products of biological redox reactions. Reactive oxygen species such as superoxide anions, hydroxyl radical, and nitric oxide inactivate enzymes and damage important cellular components causing tissue injury through covalent binding and lipid peroxidation. [8, 16, 17, 18, 19]

In DPPH assay it may be postulated that EAF reduces the radical moderately to the corresponding hydrazine when it reacts with the hydrogen donors in the antioxidant principles.[20, 21]

In ABTS the antioxidant activity of EAF by this assay implies that the action may be either inhibiting or scavenging radicals since both inhibition and scavenging properties of antioxidant towards this radical have been reported in earlier studies.[22]

In lipid peroxidation assay, malondialdehyde and other aldehydes are produced, that react with the thiobarbituric acid to give pink coloured species. The aldehyde products are responsible for DNA damage, generation of cancer and aging related diseases. The decrease in the concentration of the malondialdehyde levelled with the increase in the concentration of EAF indicate the antioxidant role of the extract.[23]

Yeast-induced pyrexia is called pathogenic fever and its etiology involves production of prostaglandins. EAF showed significant antipyretic activity and the effects are comparable to standard drug (Paracetamol), that may be due to inhibition of prostaglandin synthesis. Again, many extracts containing alkaloids, glycosides, flavonoids and steroids, have been reported antipyretic potential in various studies.[24, 25] Therefore, the activity may be due to the presence of the above group of phytoconstituents.

Results also indicate acute and chronic antiinflammatory activity of EAF against carrageenan and complete Freund's adjuvant arthritis. Significant inhibition in paw edema induced by carrageenan in the second and third phase indicate much more role on kinins and prostaglandin and little effect in inhibition of serotonin and histamine (first phase) in its mechanism. [26]

In Freund's adjuvant arthritis, the effect shown by EAF may be due to altered levels of free radicals and antioxidant enzymes to a considerable extent. This has been supported by its antioxidant property.

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