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PHYTOCHEMICAL CONSTITUENTS AND ANTI INFLAMMATORY ACTIVITY OF LEAF EXTRACTS OF *SCLEROPYRUM PENTANDRUM (Dennst.) Mabb*

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ABSTRACT

Scleropyrum pentandrum (Dennst.) Mabb belonging to santalaceae family is a small tree of a maximum height of 6 to 7 meters, commonly found in the evergreen forests of Peninsular India, Western Ghats, South and Central Sahyadris and on sandy soil. It is traditionally used for it's Anti-inflammatory activity. It is used for various activities by tribal people in different parts of the world (Much works on this plant is not done till). The present study explains the phytochemical and anti-inflammatory activity of the leaves of the *Scleropyrum pentandrum*. Further study is needed to isolate and elucidate it's medicinally active components. Also necessary studies are needed to evaluate each compounds for it's pharmacological activities.

KEY WORDS

Anti-inflammatory activity, HRBC membrane stabilization, carrageenan induced rat paw oedema and *Scleropyrum pentandrum.*

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INTRODUCTION

Scleropyrum pentandrum (Dennst.) Mabb (Synonym: *Scleropyrum wallichianum Arn.)* of Santalaceaeae family grows along the divine forests of north Kerala. The plant is also distributed in Cambodia, China, Thailand, Sri Lanka and Laos. Flowering season of the plant is January to March, fruiting season is August to October[1]. It is commonly called malayammachi in kozhikkode and Naaikuli in Kasargod, Kerala and mulkirayan in Tirunelveli,

Tamilnadu[2]. It is also called irumulli and malayamukki and is used as a mechanical barrier (fencing) in dried or live condition[3]. When searched for literatures, it revealed that, a proper investigation was not done with this plant. It is used by semalai people for its contraceptive activity. The roots are boiled and the decoction is taken as a contraceptive. It is believed that women will become barren after consuming the decoction. Paste of stem bark and leaf is applied externally to treat skin diseases [4] Recently, Soundarya *et al*[5] screened antibacterial activity of methanol extract of leaves and found inhibitory efficacy was dose dependent. Extensive literature reviews revealed that much of the bioactivities of this plant remain unexplored. Anticariogenic and cytotoxic activity of methanol extract of S. pentandrum leaves were carried out by Venugopal TM *et al* [6] the extract was found to be having anticariogenic activity. Recently, five unprecedented furan-2-carbonyl C-glycosides, and two phenolic diglycosides, were isolated from leaves and twigs of *Scleropyrum pentandrum* by Tripetch Kanchanapoom *et al.*[7]. George A. Gale *et al* presented the cyclo oxygenase inhibiting, anti-malarial and anti TB activities of *scleropyrum pentandrum*[8]. Fruits and seeds of *scleropyrum pentandrum* also called *kirinda* is consumed by *Paniya*, *Kattunaikka* and *Kuruma* tribes of Wynad distrct, Kerala, India [9].

MATERIAL AND METHOD

Plant materials

The leaves of S. *pentandrum* were collected from the divine forest of Poyilkavu Durga Temple, Calicut, Kerala. The plant is identified at Centre for Medicinal Plants and Research, Kottakkal and herbarium is deposited at Botany department, Calicut University, Kerala.

Preliminary phytochemical investigation

The crude petroleum ether, chloroform, ethyl acetate, ethanolic and aqueous extracts were subjected to preliminary phytochemical analysis in order to detect the presence of various groups of phytoconstituents by carrying out the chemical analysis (Table No.1) [10, 11].

Preparation of extracts [12]

The whole plant is dried and powdered and is subjected to successive extraction with Petroleum ether, chloroform, ethyl acetate and ethanol. The crude extract were subjected to preliminary phytochemical screening and showed the presence of alkaloids, sugars and carbohydrates, steroids, tannins and flavanoids.

Acute Toxicity Studies [13, 14]

Healthy adult wistar albino rats of either sex weighing 150-200gm were used for the study. The starting dose level of the extracts was 2000mg/kg body weight. Animals were starved overnight. After dosing, the animals were closely observed for first 4 hours for any abnormal activity and intermittently for the next 24 hours. The number of animals dead was noted after 24 hours. (CPCSEA No. 254/333/2013) (Table No.2).

Anti-Inflammatory Activity [15]

The anti-inflammatory activity of the extract was determined using carrageenan induced rat paw oedema assay. The rats were divided into five groups of six rats each. Group 1, Negative Control received carrageenan 10mg/kg. The positive control group 2 was treated orally with the standard drug, diclofenac (5 mg/kg). The test groups3 and 4 received the extract in doses of 100, and 250 mg/kg. All the doses were administered 30 min before the induction of oedema by administering 0.1 ml of 1% w/v carrageenan in saline in sub plantar region of hind paw of animal. The degree of paw oedema of all the groups was measured using a plethysmometer at 0, 60, 120, and 240 min after the administration of carrageenan to each group (Table No.3 and Figure No.1).

In vitro anti- inflammatory activity by HRBC membrane stabilization method [16, 17]

Human Red Blood Corpuscles (HRBC) membrane stabilizing method was used for the determination of anti-inflammatory activity. Extract were made into dose of 1 mg/kg body weight with 2% sodium carboxy methyl cellulose. DICLOFENAC SODIUM was used as standard. The reaction mixture (4-5 ml) consist of 2ml of hypotonic saline (0.25% w/v NaCl), 1 ml of 0.15 M phosphate buffer (pH 7.4) and

1 ml of test solution (1 mg/ml) in normal saline, 0.5 ml of 10% HRBC in normal saline was added. For control, 1 ml of isotonic saline was used instead of test solution. The mixtures were incubated at 56^0C for 30 min. Then they were cooled at running tap water, centrifuged at 3000 rpm for 20 min. The absorbance of supernatant was read at 560 nm (Table No.4 and Figure No.2).

Percent membrane stabilizing activity was calculated as follows:-

% Membrane stabilization = $\frac{\text{100 – OD of Drug}}{\text{OD of test control}}$ X 100

RESULTS AND DISCUSSION

Preliminary phytochemical screening of the extract showed the presence of alkaloids, sugars and carbohydrates, steroids, tannin, flavanoids and terpenoids. Extract at 200µg/ml produced 77.81 % inhibition in hypotonicity induced HRBC membrane lysis. The maximum inhibition was produced for methanolic extract. All the extracts exhibited dose dependant response. The effect of extract was represented as follows alcoholic > aqueous> crude.

Table No.1: Phytochemical screening of *S. pentandrum*

S.No	Extracts	Alkaloids	Glycosides	Carbohydrates	Steroids	Tannin	Flavonoids	Phenols	Terpenoids
1	Pet: ether	-	-	-	-	+	-	-	-
2	Chloroform	-	-	-	-	+	+	+	+
3	Ethyl acetate	-	-	-	-	-	+	+	+
4	Alcohol	+	-	+	+	+	+	+	+
5	Water	-	-	+	-	+	-	+	-

+ Present, - Absent

Table No.2: Acute toxicity study

S.No	Concentration groups mg/kg body weight	Body weight	Symptoms shown in minutes					Latency in minute
			30	60	120	180	24 hours	
1	100	185	N	N	N	N	N	N
2	200	182	N	N	N	N	N	N
3	300	180	N	N	N	N	N	N
4	400	178	N	N	N	N	N	N
5	500	175	N	SU	DSA	DSA	N	N
6	600	177	AUS	ASD	AS	ASD	N	N
7	700	180	AUS	ASD	ADS	ASD	N	N

A – Asthenia, D – Defaecation, S – Salivation, U – Urination, N – No Symptoms

Table No.3: Anti-inflammatory activity of *S. Pentandrum* by carragenin induced paw edema method

S.No	Groups	Rat paw oedema volume (ml)				% Inhibition
		0 minutes	60 minutes	120 minutes	240 minutes	
1	1	0.14 ± 0.23	0.45 ± 0.11	0.54 ±0.09	0.69± 0.14	0
2	2	0.13 ±0.11	0.15 ±0.14	0.15 ±0.22	0.14 ±0.22	79.71
3	3	0.14 ±0.02	0.15 ± 0.05	0.18 ±0.08	0.21 ± 0.08	69.57
4	4	0.13 ± 0.03	0.14 ±0.12	0.12 ±0.04	0.11 ±0.02	84.06

Groups 1, Control. 2, Diclofenac 5 mg/kg. 3, extract 100mg/kg. 4, extract 250 mg/kg.

Table No.4: Anti-inflammatory activity of *S. Pentandrum* by HRBC method

S.No	Treatment	Conc.	Absorbance (560nm)	% Inhibition
1	Crude Powder	50 µg /ml	0.243	20.54
		100 µg /ml	0.212	22.90
		200 µg /ml	0.192	30.18
		400 µg /ml	0.162	41.09
		800 µg /ml	0.126	54.18
		1000 µg/ml	0.156	43.27
2	Alcoholic Extract	50 µg /ml	0.170	38.18
		100 µg /ml	0.094	67.27
		200 µg /ml	0.061	77.81
		400 µg /ml	0.057	79.27
		800 µg /ml	0.016	94.18
		1000 µg /ml	0.116	95.78
3	Aqueous Extract	50 µg /ml	0.068	50.18
		100 µg /ml	0.102	54.18
		200 µg /ml	0.120	56.36
		400 µg /ml	0.126	62.90
		800 µg /ml	0.137	63.63
		1000 µg /ml	0.100	75.27
4	Drug Diclofenac	200 µg /ml	0.33	88.00

Figure No.1: Anti-inflammatory activity of *S. Pentandrum* by paw edema method

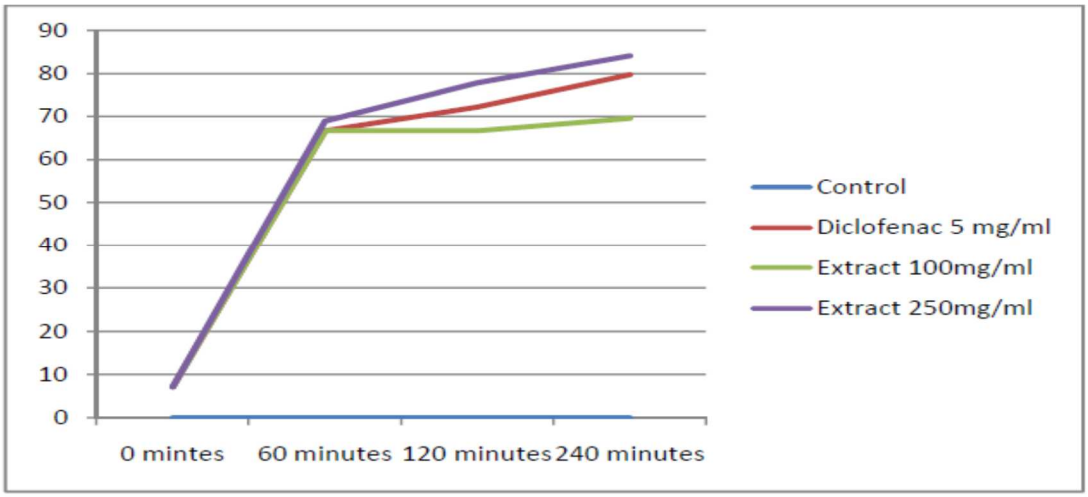
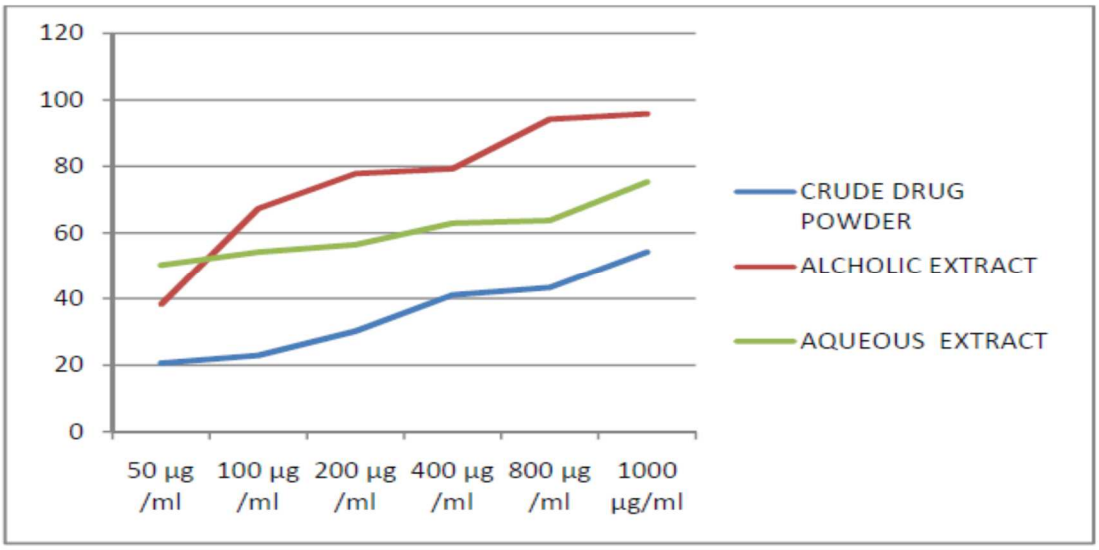


Figure No.2: Anti-inflammatory activity of *S. Pentandrum* by HRBC method



CONCLUSION

The alcoholic extract at 200mg/ml produced 77.81% inhibition in the hypotonicity induced HRBC membrane lysis shows more anti-inflammatory activity. The activity may be due to the presence of steroids. Further studies must be conducted to establish the anti-inflammatory activity of the leaf extract by different techniques and different standards as the plant is well known traditionally for its anti-inflammatory activity. Our future aim is to isolate the chemical constituents responsible for the anti-inflammatory.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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