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Pharmacological Exploration Of Saccharum officinarum Leave Extracts For Its Anti-Oxidant And Anti-Inflammatory Activity

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Abstract: Methanolic (MeSO) and Ethanolic (EtSO) extracts of *Saccharum officinarum* Linn. leaves belongs to Poaceae family were evaluated through DPPH (1, 1-diphenyl -2-picryl-hydrazyl), hydrogen peroxide scavenging and reducing power assay method to assess the antioxidant activity. MeSO and EtSO were also evaluated for *in vitro* anti-inflammatory activity by hyaluronidase inhibition assay method and *in vivo* anti-inflammatory activity by carrageenan induced rat paw edema model. EtSO & MeSO extract of leaves of *Saccharum officinarum* showed promising antioxidant activity by DPPH (79.64% and 76.53% respectively) and hydrogen peroxide (32.03% and 33.30% respectively) radical scavenging activity and also by reducing power activity. Acute toxicity studies revealed no mortality up to the dose of 2000 mg/kg body weight. An *in-vitro* anti-inflammatory study reveals that EtSO showed 66.13% and MeSO showed 47.45% inhibition of hyaluronidase enzyme at 100µg/ml concentration which is comparable to standard indomethacin. *In-vivo* anti-inflammatory study by carrageenan induced paw edema reveals that EtSO (87.23%) and MeSO (80.85%) has significant anti-inflammatory activity at 500mg/kg dose at 240 min., in comparison with control and is also comparable to standard Diclofenac. Results reveals methanolic and ethanolic extracts of Saccharum officinarum leaves on animal model have potent anti-inflammatory activity with promising *in vitro* antioxidant effects.
Key words: *Saccharum officinarum*, antioxidant, hyaluronidase inhibition, anti-inflammatory activity.

Introduction

Inflammation is a local response of living mammalian tissues to injury. It is a body defense reaction in order to eliminate or limit the spread of injurious agents. There are various components to an inflammatory reaction injury. Edema formation, leukocyte infiltration and granuloma formation represent such components of inflammation [1]. Edema formation in the paw is the result of a synergism between various inflammatory mediators that increases vascular permeability and blood flow [2]. Reactive Oxygen species (ROS) generated endogenously or exogenously are associated with the pathogenesis of various diseases such as atherosclerosis, diabetes, cancer, arthritis and aging process. Inflammation is a complex process and ROS play an important role in the pathogenesis of inflammatory diseases. Thus antioxidants which can scavenge ROS are

expected to improve these disorders. Even though various allopathic drugs like immune-suppressants, NSAIDS, corticosteroids and anti-histamine are being used till now, the potential side effects give limitations for their use. Now it is a growing concern all over for the development of new safe, potent, less toxic anti-inflammatory drug. Hence, there is a need to explore for more naturally available alternatives, so that their therapeutic values can be assessed and expanded.

Saccharum officinarum (Family-Poaceae) commonly known as Sugarcane, Noblecane is widely cultivated throughout tropic and subtropic regions. It is used as a folk medicine and also used as an antidote, antiseptic, antivenomous, bactericide, cardio-tonic, demulcent, diuretic, intoxicant, laxative, pectoral, refrigerant, and stomachic. It is a folk remedy for arthritis, bedsores, boils, cancer, colds, cough, diarrhoea, dysentery, eyes, fever, hiccups, inflammation, laryngitis, opacity, skin, sores, sore throat, spleen, tumors, and wounds [3]. Powdered sugar is used as a *drawing* agent for granulations and proud flesh [4] and in a 1:3 solution in water, for gonorrhea and vaginal discharges [5]. A decoction of the root of the race of 'tebu lanjong' is used for whooping cough; and the cane juice is given for catarrh. It is used in elephant medicine; the juice is used to 'make an elephant sagacious', and in a poultice for sprains [6]. In India, the plant as well as its juices is used for abdominal tumors. However, a detailed pharmacological screening of the *Saccharum officinarum leaves* extracts has not been reported. The present study reports the anti-inflammatory and free radical scavenging activity of *Saccharum officinarum leaves* extracts.

Materials and Method

Plant material and extraction:

Leaves of *Saccharum officinarum* were collected, authenticated by the Department of Botany, Science College, Nanded, Maharashtra, India. The collected leaves were dried under shade, segregated and pulverized by mechanical grinder and the powder was passed through No. 22 sieve. The powdered material was successfully extracted with ethanol & methanol by continuous hot extraction method in soxhlet apparatus for 10 hours [7]. The extracts obtained were subjected to standardization and then utilized for evaluating *in-vitro* anti-oxidant and *in-vitro* & *in-vivo* anti-inflammatory activity.

Experimental animal:

Male Sprague Dawley rats weighing 150 to 200 g for *in vivo* anti-inflammatory activity and male Swiss albino mice were used in the present study. The experimental animals were maintained under standard laboratory conditions in an animal house of Nanded Pharmacy College approved by the Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA) (Reg No. 1613/po/a/12/CPCSEA) under 12h light/dark cycle and controlled temperature (24 ± 2°C) and fed with commercial pellet diet and water *ad libitum*. All animals were acclimatized to the laboratory environment for at least one week before the commencement of experiment. The experimental protocol for the study is followed according to the norms of Institutional Animal Ethics Committee.

Acute Oral Toxicity study:

Acute toxicity study was carried out on test extract of *Saccharum officinarum* leaves on male swiss albino mice as per OECD Guideline No. 423: Acute Oral Toxicity (Class Method). The mice were fasted overnight and the weight of each mouse was recorded just before use. Animals were divided randomly into ten treatment groups; each group consisting of three mice, each treatment group received orally the test extract of *Saccharum officinarum* leaves in a dose of 5, 50, 300, 2000 and 5000 mg/kg. For each dose two groups of animals were used. Animals were kept under close observation for 4 hours after administering the extract, and then they were observed daily for three days for any change in general behavior and/or other physical activities.

***In-vitro* Anti-oxidant activity**

Hydrogen peroxide radical scavenging activity:

A solution of H_2O_2 was prepared in phosphate buffer (pH 7.4).). 6 ml of 40 mM H_2O_2 solution was mixed with 1.0 ml of different concentration (10-100 µg/ml) of extracts leaves of *Saccharum officinarum* and standard. After incubation at 37°C for 10 minutes absorbance was measured at 230nm. Corresponding blank solutions were taken using phosphate buffer without H_2O_2. A similar procedure was repeated with distilled water instead of extract which serves as control. Ascorbic acid was used as standard. All the tests were performed in triplicate. The decrease in absorbance indicates increase in free radical scavenging activity. The % scavenging activity was measured by using formula shown below [8].

$$(\%)\ \text{Scavenging activity} = \frac{\text{Control absorbance - Test absorbance}}{\text{Control absorbance}} \times 100$$

Reducing power activity:

1mL extract of leaves of *Saccharum officinarum* and standard of various concentrations (100-1000µg/ ml) was mixed with phosphate buffer (2.5ml, pH 7.4) and potassium ferricyanide (2.5ml, 1 %). The mixture was incubated at 50°C for 20 minutes. Aliquots of trichloroacetic acid (2.5ml, 10%) were added to the mixture, then centrifuged at 3000 x g for 10min. Upper layer of the solution (2.5ml) was mixed with distilled water (2.5ml) and freshly prepared ferric chloride solution (0.5ml, 0.1%). Absorbance was measured at 700nm.Corresponding blank solutions were taken containing same solution without ferric chloride. A similar procedure was repeated with distilled water instead of extract which serves as control. Ascorbic acid was used as standard. All the tests were performed in triplicate. Increased in the absorbance of the mixture indicates increased reducing power [9].

DPPH (2, 2-dipheny 1, 1-picrylhydrazyl) radical scavenging activity:

The reaction mixture (3.0 ml) consists of 1 ml of 0.135mM DPPH solution in methanol was mixed with 1 ml of extract solution of *Saccharum officinarum* Linn leaves and 1.0 ml of methanol. The reaction mixture was vortexed and left in the dark at room temperature for 30 min. The various concentration of extract (10, 20, 40, 60, 80, 100µg/ml) were prepared. A reaction mixture without test sample was served as control. The absorbance was measured at 517 nm and (%) inhibition was calculated against control.

$$\%\ \text{Scavenging activity} = \frac{\text{Control absorbance - Test absorbance}}{\text{Control absorbance}} \times 100$$

***In-vitro* Anti-inflammatory activity**

The assay medium consisting of 3 - 5U hyaluronidase (from Sigma –Aldrich, Bangalore) in 100µl 20mM sodium phosphate buffer pH 7.0 with 77mM sodium chloride, 0.01% BSA was pre-incubated with different concentrations of the test compound (in Dimethyl sulfoxide; DMSO) for 15 min at 37 °C. The assay was commenced by adding 100µl hyaluronic acid (from Sigma – Aldrich, Bangalore; 0.03% in 300mM sodium phosphate, pH 5.35) to the incubation mixture and incubated for a further 45 min at 37 °C. The undigested hyaluronic acid was precipitated with 1ml acid albumin solution made up of 0.1% bovine serum albumin in 24mM sodium acetate and 79mM acetic acid, (pH 3.75).

After standing at room temperature for 10 min, the absorbance of the reaction mixture was measured at 600 nm. The absorbance in the absence of enzyme was used as the reference value for maximum inhibition. The inhibitory activity of test compound was calculated as the percentage ratio of the absorbance in the presence of test compound vs. absorbance in the absence of enzyme.

The enzyme activity was checked by control experiment run simultaneously, in which the enzyme was pre-incubated with 5µl DMSO instead, and followed by the assay procedures described above. Compound was tested in a range of 10µg -100µg in the reaction mixture. Indomethacin was used as reference standard [10].

***In-vivo* Anti-inflammatory activity**

Male Sprague Dawley rats (150 - 200 g) kept at the laboratory Animal home was used. The animals were maintained under standard environmental conditions and had free access to standard diet and water. Anti-inflammatory activity was measured using carrageenan induced rat paw edema assay [11, 12]. Groups of 6 rats were given a dose of the extract (plant extracts were dissolved in sterile distilled water and administered through the P.O. route at different dose levels). After 1h, 0.1 ml, 1% carrageenan suspension in 0.9% NaCl solution was injected into the sub-plantar tissue of the right hind paw. Paw volume was measured immediately after carrageenan injection and at 0min, 60, 120, 180, 240 min and thereafter at 24hr by using a Plethysmometer to calculate the percentage inhibition of paw edema as an indicator of anti-inflammatory activity [13].

Experimental design:

The following groups were made for the anti-inflammatory study. Six animals were taken for each group:

Group A: Control: Vehicle
Group B: Standard; 20mg/kg Diclofenac
Group C: EtSO 200mg/kg
Group D: EtSO 500mg/kg
Group E: MeSO 200mg/kg
Group F: MeSO 500mg/kg

The percent inhibition of inflammation is calculated by using following formula.

$$\textbf{\% Inhibition} = \frac{(V_t - V_o)_{Control} - (V_t - V_o)_{test}}{(V_t - V_o)_{Control}} \times 100$$

Where, V_t =Volume of paw edema at time t.

V_o = Volume of paw edema before administration of test sample (Predose).

Results

Acute Oral Toxicity:
Acute toxicity studies conducted revealed that the administration of graded doses extracts (up to a dose of 2000 mg/kg) did not produce significant changes in behaviors such as alertness, motor activity, breathing, restlessness, diarrhea, convulsions, coma and in appearance of the animals. No death was observed up to the dose of 5000 mg/kg body weight. The mice were physically active. These effects were observed during the experimental period (72 hrs). The result showed that in single dose; the plant extracts had no adverse effect, indicating that the medium lethal dose (LD_{50}) could be greater than 2000 mg/kg body weight in mice.

Hydrogen peroxide radical scavenging activity:
EtSO & MeSO showed anti-oxidant potential. EtSO having 32.03 percent scavenging activity, MeSO 33.30 percent Scavenging activity, while Ascorbic acid showed 77.36 percent Scavenging activity at 100µg/ml concentration respectively. Both the extract has almost same anti-oxidant potential (Table 1).

DPPH (2, 2-dipheny 1, 1-picrylhydrazyl) radical scavenging activity
EtSO & MeSO 76.53 percent showed anti-oxidant potential. EtSO having 79.64 percent Scavenging activity, MeSO Scavenging activity, while Ascorbic acid showed 97.66 percent Scavenging activity at 100µg/ml concentration respectively. Both the extract has almost same anti-oxidant potential (**Table 1**).

Reducing power activity:
EtSO & MeSO extract of leaves of *Saccharum officinarum* showed anti-oxidant potential. EtSO having 0.4402±0.041 absorbance at 100µg/ml concentration, MeSO having 0.4640±0.006 absorbance at 100µg/ml concentration, while Ascorbic acid showed 0.7942±0.004 at 100µg/ml absorbance measured at 700nm. Both the extract showed almost same absorbance at 100µg/ml concentration, having almost similar anti-oxidant potential. Increased in the absorbance of the extract indicates increased reducing power, which showed that EtSO &MeSO extract of leaves of *Saccharum officinarum* has anti-oxidant activity.

***In-vitro* Anti-inflammatory activity**
EtSO showed 66.13 percent inhibition of hyaluronidase enzyme at 100µg/ml concentration, MeSO showed 47.45 percent inhibition of hyaluronidase enzyme at 100µg/ml concentration, while standard indomethacin showed 79.30 percent inhibition of hyaluronidase enzyme at mere 10 µg/ml concentration. EtSO extract of leaves of Saccharum officinarum showed better activity than MeSO and thus, EtSO has anti-inflammatory potential (Table 2; Figure 1).

***In-vivo* Anti-inflammatory activity**
EtSO has significant anti-inflammatory activity in comparison with control and in relation with Diclofenac. In a given dose of EtSO (200mg/kg) at 240 min showed 59.57 % inhibition, EtSO (500mg/kg) at 240 min showed 87.23% inhibition, MeSO (200mg/kg) at 240 min showed 55.31% inhibition, MeSO (500mg/kg) showed 80.85% inhibition which is comparable to Diclofenac (20mg/kg) at 240 min showed 91.48 % inhibition. Percent inhibition at 24hr is for test extracts is significant. All the data subjected to statistical analysis by students't' test and by ANOVA as shown in Table 3.

Table 1: Radical scavenging activity of *Saccharum officinarum* leaves extract

Method	Extract	% Scavenging activity					
		Concentrations(µg/ml)					
		10 µg/ml	20 µg/ml	40 µg/ml	60 µg/ml	80 µg/ml	100 µg/ml
H_2O_2	EtSO	2.08	9.48	10.66	17.44	30.79	32.03
	MeSO	3.84	9.60	11.92	20.41	31.49	33.30
	Standard Ascorbic acid	30.79	44.12	53.29	60.87	69.71	77.36
DPPH	EtSO	61.53	65.57	69.61	72.88	76.84	79.64
	MeSO	55.94	61.30	64.64	69.15	72.57	76.53
	Standard Ascorbic acid	72.46	81.81	88.96	93.55	95.64	97.66

Each values represents as mean ± SEM. ±; n=3

Table 2: Percentage inhibition of hyaluronidase enzyme by *Saccharum officinarum* leaves extract

Sample	Test concentration (in µg)	O.D. at 600nm	% inhibition
EtSO	10	0.001	0.13
	50	0.142	19.09
	100	0.492	66.13
MeSO	10	0.003	0.40
	50	0.123	16.53
	100	0.353	47.45
Indomethacin	10	0.59	79.30
	50	0.761	*
	100	0.783	*

* Beyond measurable range: Much higher activity

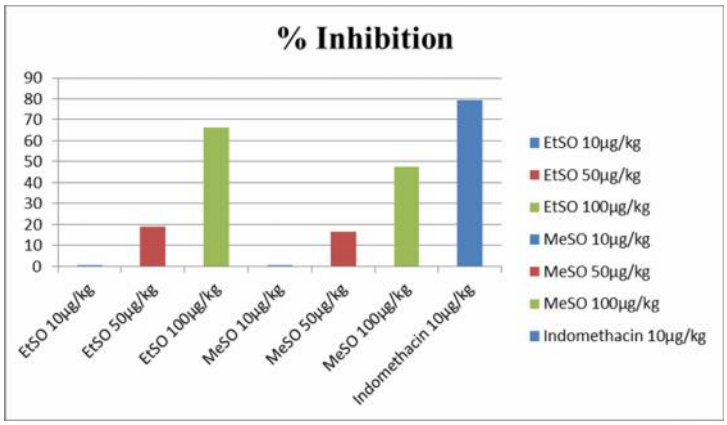

Figure 1: Percentage inhibition of hyaluronidase enzyme by *Saccharum officinarum* leaves extract

Table 3: *In-Vivo* anti-inflammatory activity of *Saccharum officinarum* leaves extract in carageenan induced rat paw edema

Experi-mental Groups	Mean Paw Volume (ml) ± S.E.M					
	0 min	60 min	120 min	180 min	240 min	24hr
Control	0.39 ± 0.0096	0.58 ± 0.0263	0.69 ± 0.0126	0.74 ± 0.0024	0.76 ± 0.0024	0.47 ± 0.0079
Diclofenac (20mg/kg)	0.35 ± 0.0103	0.4 ± 0.0096** (58.62)	0.36 ± 0.0105** (80)	0.33 ± 0.0099** (88.88)	0.32 ± 0.0103** (91.48)	0.3 ± 0.0061** (88.88)
EtSO (200mg/kg)	0.35 ± 0.009	0.62 ± 0.009* (17.24)	0.69 ± 0.004 (2.50)	0.63 ± 0.0123** (22.22)	0.47 ± 0.0049** (59.57)	0.34 ± 0.0101**a (88.88)
EtSO (500mg/kg)	0.42 ± 0.0104	0.39 ± 0.0107**a (65.51)	0.39 ± 0.0166**a (75)	0.36 ± 0.0147**a (84.44)	0.35 ± 0.0066**a (87.23)	0.31 ± 0.0022**a (88.88)
MeSO (200mg/kg)	0.37 ± 0.0051	0.56 ± 0.014**a (68.96)	0.64 ± 0.0117* (11.11)	0.67 ± 0.0245* (15.55)	0.5 ± 0.0102** (55.31)	0.37 ± 0.0047* (12.50)
MeSO (500mg/kg)	0.41 ± 0.016	0.39 ± 0.0195**a (62.06)	0.42 ± 0.0183** (65)	0.39 ± 0.0129**a (77.77)	0.37 ± 0.012**a (80.85)	0.31 ± 0.0056**a (83.33)

Each values represents as mean ± SEM; n=6; *: Significant difference (P<0.05 or less) & **: highly significant difference (P<0.001) when compared with control; **a**: No significant difference when compared with standard. Values in parenthesis represent percent inhibition of paw oedema. All the extracts when compared with control by ANOVA showed significant difference (P<0.05)

Discussion

Inflammation is the integral part of the body's defense mechanism. Acute inflammation is characterized by vasodilatation, exudation of plasma, release of various inflammatory mediators, cytokines, growth factors and emigration of leukocytes. While the features of chronic inflammation includes infiltration of mononuclear cells, proliferation of fibroblasts, blood vessels and increased connective tissue formation. Tissue infection is a prototype of inflammatory response. Anti-inflammatory drugs inhibit different stages of inflammation.

The screening studies for antioxidant properties of medicinal and food plants have been performed increasingly for the last few decades with the hope of finding an effective remedy for several present-day diseases and means to delay aging symptoms [14]. The disorders related to the excessive oxidation of cellular substrates (oxidative stress) include type II diabetes, neurodegenerative diseases, and some types of cancer [15] Furthermore, there is also a huge demand for natural antioxidants in food and related industries, for replacing the synthetic preservatives [16].

Secondary metabolites from medicinal plants function as small molecular weight antioxidants through direct antiradical, chain-breaking of the free radical propagation and interaction with transition metals. Other mechanisms include the inhibition of ROS-generating enzymes such as xanthine oxidase, inducing nitric oxide synthase, and improving the endogenous cellular antioxidant mechanisms such as the up-regulation of the activity of SOD [17]. Furthermore, phenolic compounds function as high-level antioxidants because they possess the ability to adsorb and neutralize free radicals as well as quench reactive oxygen species.

Flavonoids, as one of the most diverse and widespread groups of natural compounds, are also probably the most natural phenolics capable of exhibiting *in-vitro* and *in-vivo* antioxidant activities. In addition, plant flavonoids which show an antioxidant activity *in-vitro* also function as antioxidants in-vivo. Again, a strong relationship between the total phenolic content and antioxidant activity in fruits, vegetables, grain products, and plant subjects of ethno pharmacological treatments has also been reported [16].

The extracts are then evaluated for *in-vitro* anti-oxidant activity; EtSO & MeSO showed potential anti-oxidant activity. In hydrogen peroxide radical scavenging activity EtSO having 32.03% and MeSO having 33.30% activity, while Ascorbic acid showed 77.36 % scavenging activity at 100µg/ml concentration respectively. Both the extract has almost same anti-oxidant potential.

In reducing power activity of both the extract i.e. EtSO & MeSO extract of *Saccharum officinarum* Linn leaves showed good anti-oxidant potential. EtSO having 0.4402±0.04, MeSO having 0.4640±0.006 in comparison to Ascorbic acid 0.7942±0.004 absorbance at 100µg/ml measured at 700nm. Both the extract showed almost same absorbance at 100µg/ml concentration, having almost similar anti-oxidant potential. Increased in the absorbance of the extract indicates increased reducing power, which showed that EtSO & MeSO extract of *Saccharum officinarum* Linn leaves has good anti-oxidant activity.

In DPPH radical scavenging activity, both the extract i.e. EtSO (79.64 %) & MeSO (76.53%) showed significant anti-oxidant activity while Ascorbic acid showed 97.66 percent scavenging activity at 100µg/ml concentrations. Both the extract has almost same anti-oxidant potential.

In-vitro Anti-inflammatory studies reveals that EtSO showed 66.13% and MeSO showed 47.45% inhibition of hyaluronidase enzyme at 100µg/ml concentration, while standard indomethacin showed 79.30%t inhibition at 10 µg/ml concentration. EtSO extract of *Saccharum officinarum* leaves showed better activity than MeSO.

In-vivo Anti-inflammatory study (Carrageenan induced paw edema) reveals that EtSO and MeSO have significant anti-inflammatory activity in comparison with control and in relation with Diclofenac. In a given dose of EtSO 200mg/kg showed 59.57% inhibition, EtSO 500mg/kg showed 87.23% inhibition at 240 min, MeSO 200mg/kg showed 55.31% inhibition, MeSO 500mg/kg showed 80.85% inhibition at 240 min, while Diclofenac (20mg/kg) at 240 min showed 91.48 percent inhibition. Present study confirms the anti-inflammatory and antioxidant potential of plant which not only rationalizes the ethanobotanical claims but also identified a potential candidate for further investigation.

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