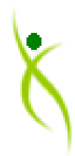
ijlpr 2022; doi 10.22376/ijpbs/lpr.2022.12.3.P70-75



International Journal of Life science and Pharma Research
ISSN 2250-0480

Research Article

Pharmacognosy & Phtochemistry

In Vitro Anti-Inflammatory Evaluation of Sesbania Procumbens Extracts

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Abstract: Plants used in the traditional healing system require a systematic evaluation that may give more promising data about their medicinal value and may be useful to meet the rising demand for novel agents to combat infections and diseases. The plant *Sesbania procumbens* is a one used traditionally by the rural population for a varietyof medicinal purposes including anti-inflammatory activity. Based on this report the plant *S. procumbens* was selected for our research and in this study different extracts of *S. procumbens* were evaluated *in vitro* for anti-inflammatory activity. The whole plant material was collected, authenticated, aerial parts were separated, shade dried for about two weeks and made into a coarse powder by using mechanical grinder. Extraction of powdered material was done by soxhlation using the different solvents of increasing polarity viz., petroleum ether, chloroform, ethyl acetate and methanol. Dried extracts thus obtained were subjected to *in vitro* anti-inflammatory evaluation by human red blood cell (HRBC) membrane stabilization in normal and different temperature (54°C and −10°C) and tonicity (hypotonic and isotonic) conditions. Six different concentrations (100, 200, 400, 600, 800, 1000µg/ml) of four extracts were subjected to evaluation with indomethacin (100µg/ml) as standard control. The data thus collected was analysed by using SPSS version 15.0. All values are expressed as mean ± SD. $P<0.05$ was considered statistically significant From the results, a dose-dependent rise in anti-inflammatory activity was found in all the tested extracts. Particularly, the methanol and ethyl acetate extracts showed significant activity in their maximum testing concentration (1000µg/ml) in all the experimental approaches. The results of this study strongly supported the claims of the anti-inflammatory property of the tested extracts. Further studies such as *in vivo* evaluations and phytochemical screening of the extracts may give more significant data which may be useful for the development of novel chemotherapeutic agents

Keywords: *Sesbania procumbens*, extraction, *in vitro* anti-inflammatory activity, HRBC membrane stabilization assay, Temperature-induced haemolysis, Tonicity induced haemolysis

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Received On **24 December, 2021**
Revised On **15 April, 2022**
Accepted On **20 April, 2022**
Published On 24 May, 2022

Funding This research did not receive any specific grant from any funding agencies in the public, commercial or not for profit sectors.

Citation V. Leslie, Gurfateh Singh, S. Aron, S. L. Harikumar , In Vitro Anti-Inflammatory Evaluation of Sesbania procumbens extracts.(2022).Int. J. Life Sci. Pharma Res.12(3), P70-75 http://dx.doi.org/10.22376/ijpbs/lpr.2022.12.3.P70-75

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1. INTRODUCTION

Since ancient times, medicinal plants have been widely utilized to alleviate diverse diseases and infections all over the world. Plant-based treatment approaches in various traditional healing systems have always inspired and guided the researchers to search for novel drug molecules [1, 2]. Of course, few medicines developed from natural sources, for example, the antibiotics such as penicillin, antimalarialssuch as quinine, artemisinin, hypolipidemic such as lovastatin, immunosuppressants such as cyclosporine, anti-cancer agents such as paclitaxel etc., revolutionized the therapy [3]. Some recent reports predicted that approximately two-thirds of the medicines approved globally are plant originated [4]. The organic compounds contained in the plants are responsible for their definite physiological effect. These secondary metabolites are chemically and taxonomically distinct compounds which include alkaloids, glycosides, flavonoids, essential oils, saponins, resins, phenols etc.[5, 6]. These phytochemicals are present in the different parts of the plants viz., root, stem, flower, fruit, seed and exudates which can be used to treat different types of chronic as well as infectious diseases. Plants used in the traditional healing system require a systematic evaluation that may give more promising data about their medicinal value and may be useful to meet the rising demand for novel agents to combat the infections and diseases,[7–9] With this view, *Sesbania procumbens*, a plant with ethnomedicinal importance was selected for the present study. *Sesbania procumbens* (Roxb.) Wight & Arn., belongs to the family Fabaceae, commonly found in wet places like paddy fields is prostrate or ascending herb; stem prickly, striate or six-ribbed, green-reddish. Leaves abruptly pinnate, paripinnate; leaflets 11-19 pairs, oblong, 05-2.5 x 01-1.5mm, oblique, rounded at base, entire at the margin, with bright reddish, retuse, mucronate at apex, glabrous; nerves less; petiole 0.2mm long, glabrous; rachis 2-3 long, 6-ribbed, prickle; stipules narrowly lanceolate, 5mm, truncate at base, acuminate at apex. Racemes axillary short, up to 2cm long. Flowers c. 6mm across, yellow, one to two flowered, rarely three flowered; pedicel 1mm; bract lanceolate 3.5mm; bracteoles elliptic 1.5mm, cuneate at base. Calyx was campanulate; lobe 5, short, subequal, 5mm. Petals 5, exserted; standard suborbicular, 7mm, emarginate at apex; wings oblong, 7mm, truncate at apex; keels obovate, falcate. 5mm, obtuse at apex, bluish-pink. Stamens 10, diadelphous, dorsifixed; filament 4.5mm; anther ovoid, c. 0.2mm. Ovary sessile, 05mm; two-loculed; many ovules; style 05mm long; stigma capitate, small. Pod linear, cylindric, 8cm long, flattened, septate between seed; seeds ca. 19, oblong, 2mm, glabrous [10]. The plant *S. procumbens* was used traditionally by the rural population for a variety of medicinal purposes including anti-inflammatory activity. Now, the present study was focused on the *in vitro* anti-inflammatory evaluation of *S. procumbens* extracts, an attempt to provide a direction for further studies.

2. MATERIALS AND METHODS

2.1 Plant Material Collection and Identification

The whole plant of *Sesbania procumbens* was collected from the Arapatti, the village located in the Mudukulathur block of Ramanathapuram District, Tamil Nadu, India. The collected plant was properly identified and authenticated by Dr P. Jayaraman, Retd. Professor, Presidency College, Chennai-5, Director, Institute of Herbal Science, Plant Anatomy Research Centre, West Tambaram, Chennai-45. (PARC/2019/3920)

2.2 Powdering and Extraction

Powdering of the collected material was done in reference to the previous literature [7,11,12]. Aerial parts of the collected plant material were separated, dried in shade for about two weeks, and then made into coarse powder by using a mechanical grinder and stored air tightly for further studies. Solvents of ascending order of polarity (petroleum ether, chloroform, ethyl acetate and methanol) were used for the extraction in the soxhlet apparatus assembly. For extraction, the powdered plant material (30g) was moistened with the respective solvent, loaded in the soxhlet extractor and extracted with respective solvents (500ml) individually. After each extraction, subsequent extraction was done by using the same dried marc. Each extract was filtered and distilled off to obtain the dried extract, the percentage yield of each was noted and preserved for further studies.

2.3 In Vitro Evaluation of Anti-Inflammatory Activity

All the prepared extracts were evaluated for anti-inflammatory activity *in vitro* by human red blood cell (HRBC) membrane stabilization in normal and different temperature and tonicity conditions in reference to the previous literature [13, 14]. AR grade Chemicals of Millipore Sigma were used in all the experiments. All the experiments were done in triplicate and the results were expressed as mean ± standard deviation.

HRBC Membrane Stabilization Method

Freshly collected whole human blood from the healthy volunteers was mixed with an equal volume of the sterile Alsever solution (2% dextrose, 0.8% sodium citrate, 0.05% citric acid, and 0.42% sodium chloride/100 ml distilled water) and subjected to centrifugation (3000rpm; 10min). The packed cells thus obtained were washed and reconstituted as suspension (10%v/v) by using sterile isosaline (0.85% NaCl in H_2O sterilized by autoclaving). 1ml each of HRBC suspension and test extracts in different concentrations (100, 200, 400, 600, 800, 1000µg/ml) were taken in the individual tubes. Normal control (HRBC suspension and Alsever solution only) and standard control (Indomethacin 100µg/ml instead of test extracts) were prepared for comparative evaluation. All the prepared tubes were incubated in standard conditions (37°C; 30min), and then subjected to centrifugation. The collected supernatant was estimated for haemoglobin content by the spectrophotometric method (560 nm). The percentage of haemolysis and protection was calculated by

$$\text{Percentage of haemolysis} = \frac{\text{OD of test}}{\text{OD of control}} \times 100$$

$$Percentage\ of\ protection = 100 - \frac{OD\ of\ test}{OD\ of\ control}\ x\ 100$$

OD of test–Optical density of tested extracts; OD of control–Optical density of normal control

Temperature Induced Haemolysis

Test extracts dissolved in an isotonic phosphate buffer solution were used for the experiments. Reaction mixture (5 ml of test extracts in different concentration [100, 200, 400, 600, 800, 1000µg/ml] and 0.1ml of 10%v/v HRBC suspension), normal control tubes (saline) and the standard control tubes (100µg/ml of Indomethacin instead of test extracts) were prepared. A set of prepared tubes was kept in a regulated water bath (54°C for 20 min). Another set of tube was kept in a freezer (−10°C for 20 min). Then, all the tubes were centrifuged (3000rpm; 3min), the supernatant was collected and the haemoglobin content was found spectrophotometrically (540 nm) and the percentage inhibition of haemolysis by the tests was calculated by

$$Percentage\ inhibition\ of\ haemolysis = 1 - \frac{OD.2 - OD.1}{OD.3 - OD.1}\ x\ 100$$

OD.1–Optical density of tests cooled; OD.2–Optical density of tests heated; OD.3–Optical density of normal control heated

Tonicity Induced Haemolysis

Test extracts dissolved in hypotonic (0.2% sodium chloride) and isotonic solution (0.9% sodium chloride) were used for the experiments. Both hypotonic and isotonic reaction mixtures contain 5 ml of test extracts in different concentrations (100, 200, 400, 600, 800, 1000µg/ml) and 0.1ml of 10%v/v HRBC suspension. Normal control contains distilled water and the standard control contains Indomethacin (100µg/ml) instead of test extracts. All the prepared tubes were incubated for 1hr (37°C), then centrifuged at 3000 rpm for 3 min. The supernatant was collected and its haemoglobin content was estimated spectrophotometrically (540 nm) and the percentage inhibition of haemolysis was calculated by

$$Percentage\ inhibition\ of\ haemolysis = 1 - \frac{OD.2 - OD.1}{OD.3 - OD.1}\ x\ 100$$

OD.1–Optical density of tests in isotonic solution; OD.2–Optical density of tests in hypotonic solution; OD.3–Optical density of normal control in a hypotonic solution

3. STATISTICAL ANALYSIS

Data analysis was done by using SPSS version 15.0 (SPSS Inc. Chicago, IL. USA). All values are expressed as mean ± SD. Data were analysed by one-way ANOVA and the difference between means was assessed by Duncan's new multiple ranges. P<0.05 was considered statistically significant.

4. RESULTS AND DISCUSSION

In the present study, the aerial parts of the collected plant material of *S. procumbens* were dried, powdered and extracted. In the percentage yield calculation of dried extracts, it was found that the petroleum ether extract was 5.5gm, chloroform extract 4.3gm, ethyl acetate extract 6.4gm and methanol extract 8.5gm as the yield. In this study the solvent methanol showed maximum yield which is in accordance with the results of K. I. Anoob K *et al., 2020* [7]. Results of *in vitro* anti-inflammatory evaluation by HRBC membrane stabilization assay revealed a concentration-dependent rise of activity in all the tested extracts (Table1). Upon comparison with other tested extracts, the methanol extract showed a significant activity followed by ethyl acetate extract. Methanol extract in 1000µg/ml concentration revealed 67.14±0.72 percentage protection which is comparable with the standard drug. A similar type of outcome were found in previous literatures[7, 14].

Table 1: Effect of *S. procumbens* extracts on HRBC membrane protection

Concentration (µg/ml)	% Protection			
	Pet. Ether	Chloroform	Ethyl acetate	Methanol
100	03.15±0.25	05.30±0.72	09.34±0.63	12.16±2.06
200	05.67±0.45	08.26±1.32	13.11±0.17	21.32±1.05
400	11.71±0.51	13.43±0.53	23.42±2.35	35.20±1.80
600	25.34±1.43	32.23±3.42	39.24±1.61	47.21±1.31
800	39.17±2.31	46.35±2.10	47.52±0.38	52.61±0.82
1000	48.21±1.50	52.13±1.42	59.02±0.46	67.14±0.72
100 (Indomethacin)	69.43±1.25			

Testing under different temperature conditions (54°C and -10°C) also indicated a concentration-dependent rise in the activity of tested extracts which is similar to the results of previous literatures [13-27] Particularly, in the evaluation on cold testing conditions, the methanol extract showed a notable activity again. 1000µg/ml of methanol extract in cold testing conditions showed 79.50 ± 1.20 percentage protection. The ethyl acetate extract scored the activity next to methanol extract, particularly in higher temperature conditions (Table 2). Results of this approach is slightly contradicted from the report of Anosike CA et al., 2015 and Patel D and Desai S 2016 [18, 24]. However, the results shown by the methanol and ethyl acetate extracts in higher temperature is camparable with these literatures.

Table 2: Effect of *S. procumbens* extracts on the protection of HRBC membrane in different temperature conditions

Con. (µg/ml)	% inhibition of haemolysis							
	Heat (54°C)				Cold (–10°C)			
Normal control	26.30±1.22				33.22±0.70			
Extracts	1	2	3	4	1	2	3	4
100	15.35±0.55	18.16±0.39	21.06±1.15**	35.10±1.50**	16.37±0.64	19.21±0.33	34.25±1.17**	37.41±0.23**
200	22.41±0.35	25.62±0.21	29.31±1.30**	42.31±0.71**	25.21±0.54	26.72±0.64	41.50±1.21**	43.60±0.30**
400	32.25±1.10	31.17±0.55	38.20±1.05**	51.41±0.62**	32.09±0.63	32.43±1.05	48.19±0.09**	50.85±0.61**
600	42.45±1.33	41.28±1.61	47.09±2.63**	58.31±1.10**	41.15±0.23	42.19±1.17	52.50±0.30**	62.10±0.30**
800	48.15±1.13	52.18±2.20	54.31±1.25**	67.52±0.35**	49.29±0.15	47.37±1.25	61.27±0.40**	68.42±1.31**
1000	53.51±1.15	59.10±1.05	62.29±1.10**	73.10±1.08**	52.35±1.42	54.20±1.19	69.25±1.10**	79.50±1.20**
100 (Std.)	63.10 ±0.35***				67.40±1.50***			

*Con. – Concentration; Std. – Standard (Indomethacin); 1 – Petroleum ether; 2 – Chloroform; 3 – Ethyl acetate; 4 – Methanol *** – p <0.001, ** – p<0.01, * – p<0.05 Percentage inhibition of haemolysis was calculated relative to control.*

Testing of the anti-inflammatory efficacy of *S. procumbens* extracts under different tonicity conditionsalso revealed a concentration-dependent rise of activity. Previous literaures [13-27] documented similar type of outcome. In this testing approach also the methanol extract showed significant activity particularly in isotonic testing conditions as compared to other extracts tested and the standard drug used for the comparative evaluation (Table 3). The ethyl acetate extract also showed significant activity. The results indicated that the test extracts showed a maximum activity in isotonic testing conditions compared with the one that is hypotonic which is slightly contradicted with the report of Anosike CA et al., 2015 and Patel D and Desai S 2016 [18, 24] However, the results of hypotonicity induced hemolysis of the present study is comparable with the findings of [18, 24]. From the results, it was found that all the tested extracts revealed a dose-dependent rise in activity. Generally, it is well known that the cell membrane plays a crucial role in cell viability. Exposure to different adverse conditions such as heat, cold and hypotonicity encourage the formation of free radicals which in turn trigger the lipid peroxidation that facilitates the susceptibility of the cell to secondary damage and the released inflammatory mediators enhance the cell membrane permeability that causes the escape of serum proteins and fluids into the cells. But the stabilization of the cell membrane can prevent it. In this study, the methanol and ethyl acetate extracts of *S. procumbens* extracts showed a significant RBC membrane stabilization compared with the standard drug Indomethacin. It may be due to the arrest of inflammatory mediators and lytic enzyme release. Previous literature [13, 14, 19]. stated that the saponins and flavonoids of plants have a significant effect on lysosomal membrane stabilization in both *in vitro* and *in vivo* evaluation. Detailed phytochemical screening of the extracts in the future may support the claim. In short, the results of this study would be beneficial for further studies which may give significant data.

Table 3: Effect of *S. procumbens* extracts on the protection of HRBC membrane in tonicity variation

Con. (µg/ml)	% inhibition of haemolysis							
	Hypotonic solution				Isotonic solution			
Normal control	07.10±0.24				05.61±0.43			
Extracts	1	2	3	4	1	2	3	4
100	06.13±0.21	09.51±0.34	10.27±0.15**	12.02±0.75**	08.15±0.73	13.05±0.21	15.16±0.24**	17.21±0.40**
200	11.21±0.42	14.30±0.43	18.34±0.09**	23.16±0.34**	15.24±0.64	20.17±0.18	24.62±0.42**	25.52±0.61**
400	16.10±0.20	19.13±0.52	26.52±0.23**	31.51±0.42**	21.32±0.46	26.51±0.15	35.33±0.25**	37.23±0.46**

600	22.35± 0.09	21.15± 0.73	33.21± 0.08**	44.23± 0.05**	26.09± 0.19	32.52± 0.14	42.26± 0.52**	49.16± 0.53**
800	24.41± 0.51	25.08± 1.30	41.50± 0.20**	49.10± 0.20**	32.40± 0.35	40.10± 0.21	51.15± 0.31**	61.24± 0.32**
1000	29.31± 0.27	31.20± 1.63	48.33± 0.24**	54.25± 0.44**	41.20± 0.08	49.62± 0.19	59.27± 0.26**	72.17± 0.80**
100 (Std.)		71.25±0.40***				86.8±0.50***		

Con. – Concentration; Std. – Standard (Indomethacin); 1 – Petroleum ether; 2 – Chloroform; 3 – Ethyl acetate; 4 – Methanol
****** – p <0.001, ** – p<0.01, * – p<0.05 Percentage inhibition of haemolysis was calculated relative to control.***

5. CONCLUSION

In this study, *Sesbania procumbens*, a plant with ethnomedicinal importance was selected for the evaluation. In the *in vitro* anti-inflammatory evaluation by HRBC membrane stabilization in diverse temperature and tonicity conditions, the tested extracts revealed a dose-dependent rise of activity particularly the methanol and ethyl acetate extracts showed significant (P<0.05) activity upon comparing with other tested and standard drugs. Our future studies directed toward the detailed phytochemical screening and *in vivo* evaluations may give significant results.

6. AUTHOR'S CONTRIBUTION STATEMENT

V. Leslie carried out the whole experiment with the assistance of Dr S. L. Harikumar. Dr Gurfateh Singh designed the whole research work, and Dr S. Aron carried out the supervision of the experiments.

7. ACKNOWLEDGEMENT

We would like to thank Mr J. Kumaran, M. Pharm., (Pharmaceutical Biotechnology), for his assistance in the preparation of this manuscript.

8. CONFLICTS OF INTERESTS

Conflict of interest declared none.

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