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HPLC Analysis and *In Vitro* Anti-Inflammatory Activity of Ethanol Extract of *Sesbania grandiflora*

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Abstract : *Sesbania grandiflora* (family:Fabaceae) commonly known as' Sesbania', is widely used as Indian folk medicine. *Sesbania grandiflora* has the common names of Agati Ethanol extract of *Sesbania grandiflora* leaves was assessed for its anti-inflammatory activity and phytochemical screening. Phytochemical analysis of *Sesbania grandiflora* plant extracts revealed the presence of various biochemical compounds such as alkaloids, flavonoids, glycosides, triterpenoids and saponins etc. High Performance Liquid Chromatography has been used for detection and quantification of flavonoids and phenolic compounds in ethanolic leaves extract of *Sesbania grandiflora*. The standard markers like (Gallic acid, caffeic acid, rutin ,quercetin and ferulic acid) were identified by retention time and co-injected with reference standard and quantified by external standard method at 280 nm. Retention time and peaks were used as parameters to determine the presence of specific compounds. Distinct peaks and retention time were recorded, and on that basis Gallic acid (Rt=5.567), Caffeic acid (Rt=8.992), Rutin (Rt=10.992, Quercetin (Rt=12.267), Ferulic acid (Rt=23.192), and The data provided the basis for its wide uses of the therapeutic effects of this plant. The extract showed *in vitro* anti-inflammatory activity by inhibiting the heat induced albumin denaturation HRBC membrane stabilization and proteinase activity was significantly from the result, it is concluded that phytochemicals such as (tannins, flavonoids, terpenoids, phenols and saponins) present in the *Sesbania grandiflora* extract may be responsible for the anti-inflammatory activity. Aspirin was used as a standard drug for the study of anti-inflammatory activity.
Key words : *Sesbania grandiflora*, anti-inflammatory activity, aspirin, HPLC.

Introduction

Inflammation is defined as the local response of living mammalian tissues to injury due to any agent. It is a body defence reaction in order to eliminate or limit the spread of injurious agent, followed by removal of the necroses cells and tissues [12]. Inflammation is characterized in acute phase by increased blood flow and vascular permeability along with the accumulation of fluid, leukocytes and inflammatory mediators such as cytokines. In the sub acute/chronic phase it is characterized by the development of specific humoral and cellular immune responses to pathogens present at the site of tissue injury[7].

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Inflammation is the reaction of living tissues to injury, infection or irritation. Lysosomal enzymes released during inflammation produce a variety of disorders which leads to the tissue injury by damaging the macromolecules and lipid peroxidation of membranes which are assumed to be responsible for certain pathological conditions as heart attacks, septic shocks and rheumatoid arthritis etc. The extra cellular activity of these enzymes is said to be related to acute or chronic inflammation. Stabilization of lysosomal membrane is important in limiting the inflammatory response by inhibiting the release of lysosomal constituents of activated neutrophil such as bactericidal enzymes and proteases, which cause further tissue inflammation and damage upon extra cellular release or by stabilizing the lysosomal membrane [21].

HRBC or erythrocyte membrane is analogous to the lysosomal membrane [5]and its stabilization implies that the extract may as well stabilize lysosomal membranes. Stabilization of human red blood cell membrane (HRBC) by hypo tonicity induced membrane lysis can be taken as an in vitro measure of anti inflammatory activity of the drugs or plant extracts.

Medicinal plants have always been considered a healthy source of life for all people. Therapeutically properties of medical plants are very useful in healing various diseases and the advantage of these medicinal plants is being 100% natural. Nowadays people are being bombarded with thousands of unhealthy products, the level of sensibility in front of diseases is very high and that's why the use of medicinal plants can represent the best solution. Since antiquity, man has used plants to treat common infectious diseases and even long before mankind discovered the existence of microbes; the idea that certain plants had healing potential was well accepted [23].

Sesbania grandiflora (family: Fabaceae) commonly known as 'sesbania', iswidely used as Indian folk medicine. *Sesbania grandiflora* has the commonnames of Agati, Corkwood Tree and West Indian Pea. Traditionally *Sesbania gradiflora* is used alone or with other medicinal plants to treat avariety of ailments. *Sesbania grandiflora* has large red or white flowers, up to 10cm in diameters. Theplant's first outstanding feature is its extremely fastgrowth rate, especially during the first 3 or 4 years afterplanting. In Australia and in India, plantations have attained heights of 8m in under 3 years. A short-lived, quick growing, soft-wooded tree, 6-9mhigh and 0.6m in girth; leaves 15-30 cm long, abruptlypinnate; leaflets 41-61, linear-oblong, deciduous;flowers 6-10cm long with showy, fleshy white, pink orred petals; pods 30cm or more long, rather flat and somewhat 4-cornered, non-torulose, septate with swollen margins and 15-50 pale coloured seed[2], [28].It is found in Tropical Asia and North Australia. In Indiait is found at West Bengal, Assam, Karnataka and North-Eastern states. It is cultivated as an ornamental plant,grows wild in hedges and shady forests[16]. The plant is also reported to have significant antioxidant and cardioprotective effect [22], antiurolithiatic activity [16], hepatoprotective activity [19], anxiolytic and anticonvulsive activity [15], wound healing activity [14], antiulcer activity [13], antibacterial activity[18], anthelmintic activity [27]and anti-inflammatory and anti-arthritic activity [20].

Hence the present investigation is to screen the *sesbania grandiflora* leaves for HPLC analysis and invitro anti-inflamatory activity.

Materials and Methods

Collection of plant material

The plant species *Sesbania grandiflora* was collected from in and around mannargudi, Thiruvarur district, Tamil Nadu, India.

Preparation of plant powder

The plant was air dried under shade for 15 to 20 days. Then the dried material was grinded to fine powder using an electric grinder and stored in air tight bottles. The powder was used for further analysis.

Preparation of the methanolic extract

The plant materials leaf was shade dried and coarsely powdered with electrical blender.100g of *Sesbania grandiflora* was mixed with 70% ethanol and 30% water. Then it was boiled until it was reduced to one third and filtered. The filtrate was evaporated to dryness. Paste from of the extract obtained was subjected

to. The ethanol free extract was used for the preliminary phytochemicals analysis and *in vitro* anti inflammatory activity.

Qualitative phytochemical analysis

The concentrated extracts were subjected to qualitative test for the identification of various phytochemical constituents as per standard procedures [10, 11, 27, 25]

HPLC Analysis

Preparation of Extract

25g of *Sesbania grandiflora* suspended in 50 ml of 70% methanol was extracted at 80 KHz using an ultrasonic device for 30 min (twice) at 45°C. The resulting extract was collected, filtered and dried at 50°C under reduced pressure. The dried crude extract was dissolved in the 100 ml mobile phase, filtered through 0.45 mm membrane filter (Millipore) and the extract was injected into HPLC.

Preparation of Standards for HPLC

Standard stock solutions of gallic acid, ferulic acid, caffeic acid, rutin and quercetin were prepared in ethnol at concentrations of 2,4,6,8 and 10µg/ml and filtered through HPLC filter 0.45 mm membrane filter (Millipore).

Analysis of flavonoids by HPLC

The flower extract was analysed for flavonoids using a HPLC method Shimadzu corp., Kyto, consisting of a LC – 10 ATVp pump, SCL 10A system controller and a variable shimadzu SPD- N10ATVp UV VIS detector and a loop injector with a loop size of 20µl was used. The peak area ws calculated with CLASSVP software. Reverse phase chromatochraphic analysis was carried out in isocratic conditions using a C-18 reverse phase column (250×4.6 mm i, d., particle size 5µm Luna 5µ C-18; phenomenex, Torrance, CA, USA) at 250^0 C. The gradient elution of solvent A water solvent B (ethanol) had a significant effect on the resolution of compounds detection wavelength nwas 280 nm. Gallic acid, caffecic acid, ferulic acid rutin and quercetin were used as internal and external standards. Phenolic acids present in each sample identify by comparing chromatographic peaks with the retention time (Rt) of individual standards. The amount of each phenolic acid is expressed as µg /g.

***In vitro* anti-inflammatory activity**

Inhibition of Albumin (protein) activity [4]

Denaturation of proteins is a well documented cause of inflammation. As part of the investigation on the mechanism of the anti inflammation activity, ability of extract protein denaturation was studied.(Table 3). It was effective in inhibiting heat induced albumin denaturation Maximum inhibition 91% was observed from leaf extract.Aspirin, a standard anti-inflammatory drug showed the maximum inhibition 60% at the concentration of 150 µg/ml [9].

Proteinase inhibitory activity

The *Sesbania grandiflora* ethanolic extract exhibited significant antiproteinase activity. The maximum inhibition was observed from ethanolic extract (97 %). The standard aspirin (80 %) drug showed the maximum proteinase inhibitory action (Table 4)[9].

Membrane stabilization method

The HRBC membrane stabilization has been used a method to study the *in vitro* anti inflammatory activity because the erythrocyte membrane is analogous to the lysosomal membrane (Table 5)[8] and its stabilization implies that the extract may well stabilize lysosomal membranes. Stabilization of lysosomal is important in limiting the inflammatory response by preventing the release of lysosomal constituents of activated neutrophil, such as bacterial enzymes and proteases, which causes further tissue inflammation and damage upon extra cellular release. The lysosomal enzymes released during inflammation produce a various disorders. The

extracellular activity of these enzymes are said to be related to acute or chronic inflammation. The non steroidal drugs act either by inhibiting these lysosomal enzymes or by stabilizing the lysosomal membrane [21].

Results and Discussion

Qualitative Phytochemical analysis

Qualitative phytochemical analysis of ethanolic extract of *Sesbania grandiflora* was represented in Table 1.

The preliminary phytochemical analysis revealed the presence of carbohydrate, tannins,saponins, flavonoids,alkaloids,quinines,glycosides,terpenoids,phenol,coumarins,steroids and phytostroids, phlobatannins, anthraquinones in ethanolic extracts of *Sesbania grandiflora* .cardiac glycosides and triterpenoids are absent in ethanol extract of *sesbania grandiflora*.

It is evident from Table 1 that the leaves, stem and seeds of the agati was found to have higher level of carbohydrates. Similarly the presence of carbohydrates and proteins where reported in *Origanumvulgare*and *Althea officinalis* by [3] based on the preliminary phytochemical analysis carried out. Table reveals that *Sesbania grandiflora* has high content of phenols, sterols and alkaloids. The same case was reported in *Hibiscus rosa sinensis* and *Solanum nigrum*. [24]stated based on the phytochemical analysis that *Hibiscus rosa sinensis* has high content of phenols, sterols and alkaloids. It is evident from the table below that *sesbania grandiflora* was found to have high content of quinones where as saponins and glycosides were in moderate amounts. [1]stated that *Cnidosculous aconitifolius* has high content of saponins and quinones but glycosides are in moderate amounts.

Table 1: phytochemical screening of *Sesbania grandiflora*

S. No.	Name of the test	Ethanol extract
1	Carbohydrate	+
2	Tannins	+
3	Saponins	+
4	Flavonoids	+
5	Alkaloids	+
6	Quinones	+
7	Glycosides	+
8	Cardiac glycosides	_
9	Terpenoids	+
10	Triterpenoids	_
11	Phenols	+
12	Coumarins	+
13	Steroids and phytosteroids	+
14	Phlobatannins	+
15	Anthraquinones	+

(+) Indicates presence; (-) Indicates absence

HPLC Analysis

HPLC method is one of the most fast and reliable method for identification of plant phenolics. The chromategrapic separations of Gallic acid (Rt – 5.567), Caffeic acid (Rt - 8.992), Rutin (Rt – 10.992), Quercetin (Rt – 12.267) and Rerulic acid (Rt – 23.192) standard shown in Figure1. The content of each flavonoid was calculated from the corresponding calibration curve and presented as theme an of five determinations as shown in Table 2 and Figure 1.

Flavonoids and tannins are phenolic compounds and plantphenolics are a major group of compounds that act as primary antioxidants or free radical scavengers. Since these compounds were found to be present in the extracts, it might be responsible for the potent antioxidant capacity of pomegranate. These

secondary metabolites (phytochemicals) and other chemical constituents of medicinal plants account for their medicinal value. For example saponins have hypotensive and cardiodepressa[17].

Table 2: HPLC Chromatogram of *Sesbania grandiflora*

UV Detector (280nm)				
Retention Time	**Area**	**Height**	**Concentration (mg/gm)**	Name*
5.567	11328	1332	0.002	Gallic acid
8.992	21410	761	0.012	Caffeicacid
10.992	9468	339	0.002	Rutin
12.267	1311	84	0.001	Quercetin
23.192	7841	1379	0.005	Ferulicacid

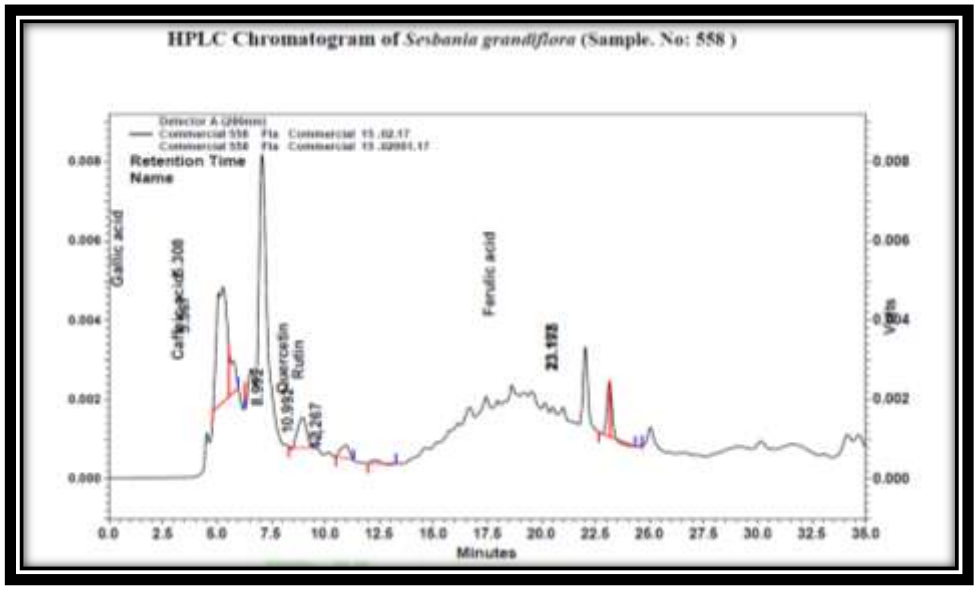

Figure 1:HPLC Chromatogram of *Sesbania grandiflora*

In Vitro anti – inflammatory activity

Inhibition of albumin denaturation

Denaturation of proteins is a well documented cause of inflammation. As part of the investigation on the mechanism of the anti inflammation activity, ability of extract protein denaturation was studied. (Table 3). It was effective in inhibiting heat induced albumin denaturation Maximum inhibition 91% was observed from leaf extract. Aspirin, a standard anti-inflammatory drug showed the maximum inhibition 60% at the concentration of 150 µg/ml [9].

Table 3:*Invitro* anti inflamatory activity of *Sesbania grandiflora* by protein activity Proteinase inhibitory activity

S.No.	Concentration (µg/ml)	Inhibition
1.	50	76% ± 0.02
2.	100	79% ± 0.02
3.	200	91% ± 0.01
4.	300	73% ± 0.08
5.	500	59% ±0.07
6.	Standard drug (Aspirin 150 µg/ml)	60% ± 0.03

The *Sesbania grandiflora* ethanolic extract exhibited significant antiproteinase activity. The maximum inhibition was observed from ethanolic extract (97 %). The standard aspirin (80 %) drug showed the maximum proteinase inhibitory action (Table 4) [9].

Table 4: *Invitro* antiinflamatory activity of *Sesbania grandiflora* by proteinase Denaturation

S.No.	Concentration (µg/ml)	Inhibition
1.	50	72%±0.052
2.	100	97%±0.090
3.	200	78%±0.056
4.	300	86%±0.055
5.	500	87%±0.056
6.	Standard drug (Aspirin 250 µg/ml)	80%±0.050

Membrane stabilization method

The HRBC membrane stabilization has been used a method to study the *in vitro*anti inflammatory activity because the erythrocyte membrane is analogous to the lysosomal membrane (Table 5) [8] and its stabilization implies that the extract may well stabilize lysosomal membranes. Stabilization of lysosomal is important in limiting the inflammatory response by preventing the release of lysosomal constituents of activated neutrophil, such as bacterial enzymes and proteases, which causes further tissue inflammation and damage upon extra cellular release. The lysosomal enzymes released during inflammation produce a various disorders. The extracellular activity of these enzymes are said to be related to acute or chronic inflammation. The non steroidal drugs act either by inhibiting these lysosomal enzymes or by stabilizing the lysosomal membrane [21].

Table 5: *Invitro* anti-inflammatory activity of *Sesbania grandiflora* by HRBC membrane stabilization method

S.No.	Concentration (µg/ml)	Inhibition
1.	50	36%± 0.045
2.	100	40%±0.040
3.	200	43%±0.055
4.	300	47%±0.0I47
5.	500	52%0.020
6.	Standard drug (Aspirin 250 µg/ml)	41%0.041

On the basis of the results obtained in the present study, It concluded that ethanol extract of *Sesbania grandiflora* has potent anti – inflammatory activity. Thus the plant extracts of *Sesbania grandiflora* may be

attributed to the presence of phenolic compounds and flavonoids etc, Therefore, further investigation is needed to isolate and identify the active compounds present in the plant extract and its efficacy.

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