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Research Article **Botany**

SCREENING OF PHYTOCHEMICALS AND ANTIINFLAMMATORY ACTIVITY OF *Pergularia daemia*

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ABSTRACT

To identify the insecticidal components of *Saraca asoca* bark were evaluated by standard protocol using the equipment Perkin-Elmer Gas Chromatography–Mass Spectrometry. The mass spectrum of the compounds found in the extract was matched with the National Institute of Standards and Technology (NIST) library. The GC-MS analysis revealed the presence of various compounds like 1-Butyl (dimethyl) silyloxypropane, 1,2,3-Benzenetriol, Squalene, Phenol, 3,4,5-trimethoxy, Hexadecanoic acid, methyl ester, Hexadecanoic acid, methyl ester, Stigmast-5-en-3-OL, (3.Beta.) and 7-Methoxy-2,3,6-Triazaphenothiazin-1(2H)-one in the ethanolic extract of *Saraca asoca*. Among the various compounds, 1,2,3-Benzenetriol, Squalene, phenol and 7-Methoxy-2,3,6-Triazaphenothiazin-1(2H)-one identified as insecticidal activity using NIST library. These findings support the use of *Saraca asoca* as insecticidal plant.

Keywords: Gas chromatography and Mass spectroscopy, *Saraca asoca*, Phytocompounds

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INTRODUCTION

Inflammation is caused by a variety of stimuli including physical damage, ultra violet irradiation, microbial invasion, and immune reactions. The classical key features of inflammation are redness, warmth, swelling, and pain. Inflammation cascades can lead to the development of diseases such as chronic asthma, rheumatoid arthritis, multiple sclerosis, inflammatory bowel disease, and psoriasis. Many of these diseases are debilitating and are becoming increasingly common in our aging society. Rheumatoid arthritis and osteoarthritis are the major inflammatory diseases affecting people worldwide. Rheumatoid arthritis is an inflammatory condition that usually affects multiple joints. It affects 0.3–1.0% of the general population and is more prevalent among women in developed countries. Persistent inflammation leads to joint destruction,. but the disease can be controlled with drugs. Osteoarthritis, which is characterized by loss of joint cartilage that leads to pain and loss of function primarily in the knees and hips, affects 9.6% of men

and 18% of women aged more than 60 years. Increases in life expectancy and aging populations are expected to make osteoarthritis the fourth leading cause of disability by the year 2020 (Woolf and Peger, 2003; Smith, 2005).

A number of natural products are used in the traditional medical systems in many countries. Alternative medicine for treatment of various diseases is getting more popular. Making medicinal plants provide relief of symptoms comparable to that obtained from allopathic medicines. The majority of clinically important medicines belong to steroidal or non-steroidal anti-inflammatory chemical therapeutic for treatment of various inflammatory diseases. Though these drugs have potent activity, they have various and severe adverse effects. Therefore, agents of natural origin with very little side effects are required as substitute of chemical therapeutics. Keeping this view the Phytochemicals and *in vitro* anti-inflammatory activity of the leaves of *Pergularia daemia*. was carried out The following aspects were analyzed to evaluate the anti-inflammatory activity.

MATERIALS AND METHODS

Plant materials:

The leaves of *Pergularia daemia* were collected in January 2017 from Manimandapam, Thanjavur, Thajavur district, Tamil Nadu, India.

Preparation of alcoholic extract

The leaves of *P. daemia* were first washed well and dust was removed from the leaves. The leaf was dried at room temperature and coarsely powdered. The powder was extracted with aqueous, methanol and 70% methanol for 24 hours. The extract was stored in refrigerator until used.

Phytochemical screening

Chemical tests were carried out on the alcoholic extract and on the powdered specimens using standard procedures to identify the constituents as described by Sofowara (1993), Trease and Evans (1989) and Harborne (1973, 1984).

Quantitative analysis of phytochemicals

Determination of total phenols by spectrophotometric method:

Flavonoid determine by the method of Bohm and Kocipai-Abyazan (1994) .Saponin determine by the method of of Obadoni and Ochuko (2001). Total terpenoid content in the leaf extracts were assessed by standard method (Ferguson, 1956). Qualitative analysis of Vitamins (Pearson, 1976; Patel, 2005).

GC MS Analysis

GC MS analysis was carried out on Shimadzu 2010 plus comprising a AOC-20i auto sampler and gas chromatograph interfaced to a mass spectrometer instrument employing the following conditions: column RTX 5Ms (Column diameter is 0..32mm, column length is 30m, column thickness 0.50µm), operating in electron impact mode at 70eV; Helium gas (99.999%) was used as carrier gas at a constant flow of 1.73 ml /min and an injection volume of 0.5 µI was employed (split ratio of 10:1) injector temperature 270 ºC; ion-source temperature 200 ºC. The oven temperature was programmed from 40 ºC (isothermal for 2 min), with an increase of 8 ºC/min, to 150ºC, then 8ºC/min to 250ºC, ending with a 20min isothermal at 280ºC. Mass spectra were taken at 70eV; a scan interval of 0.5 seconds and fragments from 40 to 450 Da. Total GC running time is 51.25min. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. Software adopted to handle mass spectra and chromatograms was a Turbo Mass Ver 5.2.0 (Srinivasan *et al*., 2013).

Identification of components

Interpretation on GCMS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained (Dr. Dukes, 2013).

***IN-VITRO* ANTI-INFLAMMATORY ACTIVITY**

In vitro anti-inflammatory activity was carried out by the method of Sangita Chandra *et al*.,(2012). *In vitro* anti-inflammatory activity was carried out by the method of Sangita Chandra *et al*., (2012). Anti-inflammatory activity evaluated by Membrane stabilizing activity as described by Divya Singh *et al*., (2013)

Statistical analysis:

The results were presented as the mean ± SD. Data was statistically analyzed using students‘t’ test.

RESULTS AND DISCUSSION

Medicinal plants are assumes greater importance in the primary health care of individuals and communities in many developing countries. There has been an increase of demand in international trade because of very effective, cheaply available, supposedly have no side effects and used as alternative to allopathic medicines. Medicinal plants are believed to be much safer and proved elixir in the treatment of various ailments. Plants synthesize an array of chemical

compounds that are not involved in their primary metabolism. These 'secondary compounds' instead serve a variety of ecological functions, ultimately to enhance the plants survival during stress. In addition these compounds may be responsible for the beneficial effects of fruits and vegetables on an array of health related measures (Liu, 2003).

In the present study was carried out on the plant sample revealed the presence of medicinally active constituents. The phytochemical characters of the *Pargularia daemia* plant investigated and summarized in Table-1. The phytochemical screening aqueous extract of *Pargularia daemia* leaves showed that the presence of tannin, saponins, steroids, phenolics, glycosides, Flavonoids, triterpenoids, alkaloids.. Methanol extract of *Pergularia daemia* leaves showed that the presence of steroids, saponins, triterpenoids, phenolics, carbohydrate, glycosides, flavonoids, tannin and protein. Hydro-Methanolic extract of *Pergularia daemia* leaves showed that the presence of saponins, triterpenoids, phenolics, carbohydrate and glycosides, flavonoids, tannin and terpenoid., Significant amount of Flavonoids (70mg/gm) and phenol (200mg/gm).

Qualitative analysis of vitamins in *Pergularia daemia*

Vitamins

Vitamins are organic substances that are essential in tiny amounts for growth and activity of the body. They are obtained naturally from plant and animal foods. Organic in this definition refers to the chemistry and molecules of vitamins. The amounts of vitamins ingested from food are measured in micrograms or milligrams (Okwu, 2004). Vitamin E remains the most mysterious of vitamins. The body needs it but its lack does not lead to any known disease. Vitamin E is the most exploited vitamin in that it is sold as a cure-all and even as an anti-aging potion. Vitamin E, vitamin C, and beta carotene are antioxidants. Some studies suggest that the trio might help to strengthen the body's immune system and play a role in cancer prevention (Okwu, 2004). The best sources for vitamin C are citrus fruits, strawberries, melons, and stemy green vegetables. Vitamin C also helps to absorb and use Iron. It is important to protect the vitamins in fruits and vegetables from being destroyed; simple ways of doing this include refrigeration, washing them before cutting them, storing them in airtight containers, and avoiding high temperatures and long cooking times (Okwu, 2003). The vitamins of the *Pargularia daemia* investigated and summarized in Table-2. The vitamin analysis of *Pargularia daemia* stem showed that the presence of Vitamin C, E, A and while vitamin D was absent.

Identification of bioactive compounds in *Pergularia daemia* leaves extract by GC MS analysis

Twenty compounds were identified in *Evolvulus alsinoides* by GC-MS analysis. The active principles with their retention time (RT), molecular formula, molecular weight (MW) and concentration (%) are presented in (Table 5 and Fig 6). The prevailing compounds 3-Cumylcyclopentene, Ethyl [(2-{2-Methoxy-4-[(1-(3-Methy, 1,3Propanediamine,n,ndiethyl,{2,Bis[(Trimethylsilyl)oxy]vin,9Phosphabicyclo[4.2.1]nona-2,4,7-trien-9-ami,Trisiloxane,1,1,1,5,5,5-Hexamethyl,1,2,4-Benzenetricarboxylic acid, 1, 3-Butoxy-1,1,1,5,5,5-hexamethyl-3-(trimethyls) and 1,2,4-Benzenetricarboxylic acid were found in this plant. The presence of various bioactive compounds justifies the use of the plant for various ailments by traditional practitioners. However isolation of individual phytochemical constituents and subjecting its biological activity will definitely give fruitful results

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

There are certain problems in using animals in experimental pharmacological research, such as ethical issues and the lack of rationale for their use when other suitable methods are available or could be investigated. Hence, in the present study the protein denaturation bioassay was selected for *in vitro* assessment of anti-inflammatory property *Pargularia daemia*. Denaturation of tissue proteins is one of the well-documented causes of inflammatory and arthritic diseases. Production of auto antigens in certain inflammatory diseases may be due to *in vivo* denaturation of proteins. The mechanism of denaturation probably involves alteration in electrostatic, hydrogen, hydrophobic and disulphide bonding (Grant *et al*., 1970). Agents that can prevent protein denaturation therefore, would be worthwhile for anti-inflammatory drug development. The increments in absorbance of test samples with respect to control indicated stabilization of protein (Egg & bovine albumin) denaturation by and reference diclofenac sodium (Jagtap *et al.*, 2011). *Pergularia daemia* exhibited anti-inflammatory activities in dose dependent manner (Table 4-6).

Human Red Blood Cell (HRBC) method was selected for the in vitro evaluation of Anti-inflammatory property because the erythrocyte membrane is analogous to the lysosomal membrane and its stabilization implies that the extract may as well stabilize lysosomal membranes. Stabilization of lysosomal membrane is important in limiting the inflammatory response by preventing 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 (Chippada and Meena, 2011). The hypotonicity induced hemolysis may arise from shrinkage of the cells due to osmotic loss of intracellular electrolyte and fluid components. The extract may inhibit the processes, which may stimulate or enhance the efflux

of these intracellular components (Kumar *et al.*, 2012). The increments in absorbance of test samples with respect to control indicated stabilization of protein (Egg & bovine albumin) denaturation by and reference diclofenac sodium (Jagtap *et al.*, 2011). *Evolvulus alsinoides* exhibited anti-inflammatory activities in dose dependent manner (Table 4-6).

Overall, the *Pergularia daemia* leaves are a rich source of phytochemicals and anti-inflammatory activity that can be important in inflammatory disease prevention including arthritis. In future isolation of this molecules responsible for the activity will be carried out which may be beneficial for the development of new anti-inflammatory agent.

Table1. Phytochemical screening of *Pergularia daemia*

S.No	Phytochemical analysis	Aqueous	100% Methanol	70% Methanol	Quantitative analysis (mg/gm)
1	Tannin	-	+	+	---
2	Phlobatannins	-	---	---	---
3	Saponin	+	+	+	33
4	Flavonoids	+	++	+	168
5	Steroids	-	++	---	---
6	Terpenoids	-	---	+	50
7	Tritrpenoids	+	+	+	---
8	Alkaloids	+	---	---	-
9	Carbohydrate	-	---	+	---
10	Protein	+	++	++	---
11	Anthroquinone	+	---	+	---
12	Polyphenol	+	+	+	300
13	Glycoside	+	+	+	---

(+) Presence; (-) Absence; (++) Higher concentrations

Table: 2 Qualitative analysis of vitamins in *Pergularia daemia*

S.No	Test	Qualitative Result
1.	Vitamins A	+
2.	Vitamins C	+
3.	Vitamins D	--
4.	Vitamins E	++

(+) Presence (-) Absence (++) High concentration

Table 3. Identification of bioactive compounds in *Pergularia daemia* leaves extract by GC- MS analysis

Peak	R.Time	Area %	Height %	Molecular Formula	Name	Molecular Weight
1	11.815	18.81	10.61	$C_9H_{10}O_2$	2-Methoxy-4-vinylphenol	150
2	12.042	5.82	5.05	C_8H_{18}	Pentane, 2,2,3-Trimethyl-	114
3	13.970	20.86	6.52	$C_6H_{12}O_4$	1,5-Anhydro-l-rhamnitol	148
4	15.551	2.79	8.74	$C_9H_8O_3$	2-Acetylbenzoic acid	164
5	17.692	1.98	4.57	$C_{15}H_{22}N_2O_3S$	2-Tert-butyl-3-Methyl-1-(Toluene	310
6	18.232	3.52	9.99	$C_{14}H_{26}$	1-Tetradecyne	194
7	18.721	2.85	7.53	$C_{28}H_{44}O_4$	Phthalic acid, cis-hex-3-enyl tetradecyl ester	444
8	19.717	2.06	3.84	$C_{12}H_{14}O_4$	1,2-Benzenedicarboxylic acid, di	222
9	21.533	3.23	2.18	$C_{12}H_{22}Si_2$	Benzene, 1,4-Bis(Trimethylsilyl)-	222
10	21.700	4.29	2.71	$C_{14}H_{18}$	3-Cumylcyclopentene	186
11	22.856	2.22	4.20	$C_{25}H_{26}N_2O_7S$	Ethyl [(2-{2-Methoxy-4-[(1-(3-Methy	498
12	23.335	4.55	4.50	$C_7H_{18}N_2$	1,3-Propanediamine, n,n-diethyl-	130
13	25.451	4.65	2.51	$C_{13}H_{23}NO_2Si_2$	3-{2,2-Bis[(Trimethylsilyl)oxy]vin	281
14	26.590	4.04	4.16	$C_{12}H_{18}NP$	9-Phosphabicyclo[4.2.1]nona-2,4,7-trien-9-ami	207
15	26.708	2.06	2.71	$C_{12}H_{36}O_4Si_5$	Trisiloxane, 1,1,1,5,5,5-Hexamethyl	384
16	26.983	2.73	2.54	$C_{11}H_{10}O_6$	1,2,4-Benzenetricarboxylic acid, 1,	238
17	27.919	2.19	3.60	$C_{13}H_{36}O_4Si_4$	3-Butoxy-1,1,1,5,5,5-hexamethyl-3-(trimethyls	368
18	28.383	2.44	1.90	$C_{11}H_{10}O_6$	1,2,4-Benzenetricarboxylic acid, 1,2	238
19	28.550	2.44	3.46	$C_{13}H_{23}NO_2Si_2$	Pyridine, 3-[2,2-	281

					bis(trimethylsilyloxy)vinyl] -	
20	28.72918. 81	6.18	8.68	$C_{10}H_{15}F_5O_2$	Pentafluoropropionic acid, heptyl ester	262

Table 4 *In vitro* anti-inflammatory activity of *Pergularia daemia* (Egg albumin)

S.No	Doses (µg/ml)	Plant extract	Standard (Diclofenac sodium)
1	100	18.43 ± 0.88	21.37±1.98
2	200	36.32 ± 1.04	36.45±2.37
3	300	59.48 ± 1.04	55.94±3.47
4	400	66.85 ± 1.05	79.45±4.65
5	500	80.01 ± 1.05	93.45±6.84

Values are expressed as Mean ± SD for triplicates

Table 5 *In vitro* anti-inflammatory activity of *Pergularia daemia* (Bovine serum albumin)

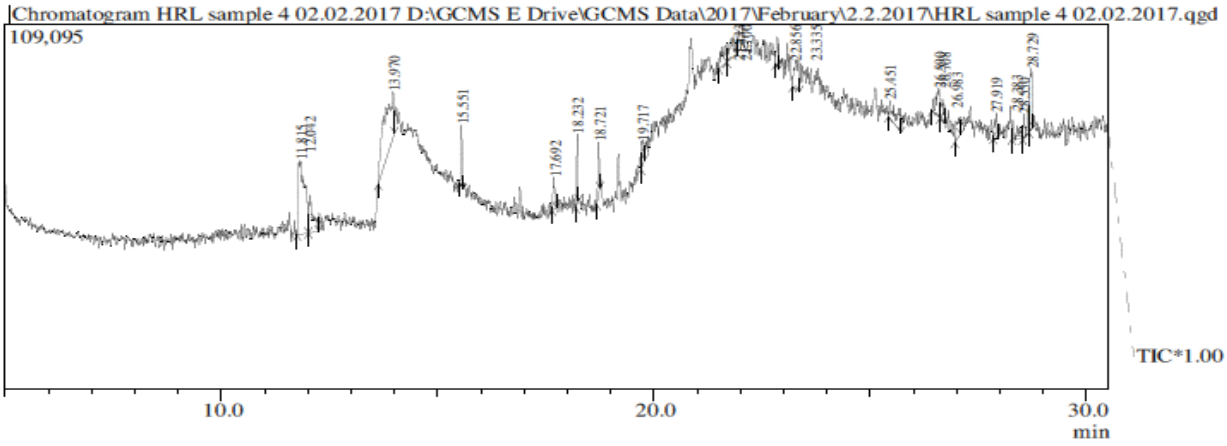
S.No	Doses (µg/ml)	Plant extract	Standard (Std.) (Diclofenac sodium)
1	100	17.36 ± 1.65	19.65±1.65
2	200	31.12 ± 0.83	32.65±2.02
3	300	55.45 ± 1.67	50.23±3.22
4	400	72.70 ± 0.30	75.65±4.47
5	500	89.22± 1.44	90.25±5.89

Values are expressed as Mean ± SD for triplicates

Table 6 *In vitro* anti-inflammatory activity of *Pergularia daemia* (HRBC Method)

S.No	Doses (µg/ml)	Plant extract	Standard (Std.) (Diclofenac sodium)
1	100	12.25 ± 1.01	23.56±2.14
2	200	39.20 ± 1.01	41.23±2.68
3	300	48.47 ± 2.22	58.89±3.97
4	400	52.39±0.58	77.56±5.12
5	500	89.36 ± 1.27	92.32±6.55

Fig 4 GC- MS analysis of leaves extract of *Pergularia daemia*



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