



J. Chem. Pharm. Res., 2010, 2(5):618-625

ISSN No: 0975-7384
CODEN(USA): JCPRC5

Phytochemical investigation and anti-inflammatory activity of *Salacia reticulata*

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ABSTRACT

The pharmacological and biological properties and chemical constituents of plants from the plant Salacia Reticulata which is widely used in folk medicine. In the present study, the anti-inflammatory activity of hexane and chloroform extracts of Salacia Reticulata were studied and the activity was compared with Diclofenac sodium. The anti-inflammatory activity was found out by Carragenan induced paw edema method by using standard Diclofenac sodium. In preliminary phyto chemical investigation the hexane and chloroform extracts showed the presence of carbohydrates, glycosides, Coumarins and phytosterols. In this acute inflammation model, both extracts and the standard drugs (diclofenac sodium 10mg/kg) produced significant inhibition of paw edema as compared to the control. The chloroform extract highly significant when compared to the hexane extract. The results were found to be highly significant ($P < 0.01$) in comparison to control.

Key Words: *Salacia Reticulata*, Anti inflammatory activity.

INTRODUCTION

Salacia Reticulata is found in Southern India and Sri Lanka. Rare in evergreen forests of Western Ghats. *Salacia Reticulata* is a climbing shrub with blackish branches found in southern orissa and kerala^{1,2,3}. It has Salanisol as active principle for diabetic treatment. About 18 species of *Salacia* grow in India. A large, straggling, woody shrub with dichotomous branching. Bark is smooth, greenish grey, thin, white inside. Leaves are opposite, elliptic-oblong, base acute, apex abruptly acuminate, margin toothed with minute rounded teeth, leathery, hairless, Shiny, lateral nerves about seven pairs, prominent beneath. Flowers are bisexual, 2-8 clustered in leaf axils, greenish white to greenish yellow, calyx lobes entire, anthers dehiscing transversely. Drupes globose, tubercular, pinki, orange when ripe. Seeds are 1-4 almond like. *Salacia Reticulata*

contains Salaretin and mangiferin which reduces the sugar level and protect the body from any secondary side effect of Diabetes. Mangiferin in *Salacia Reticulata* inhibits the formation of LDL and thus maintains the blood lipid profiles. Indian Research has shown that *Salacia Reticulata* also has hepatoprotective effects^{4,5,6,7}. As such, it is also used as a liver tonic. *Salacia Reticulata* is very effective in case of Rheumatism, Menstrual disorders, Skin diseases, inflammations, spermatorrhoea. The roots are acid, bitter, thermogenic, urinary, astrigent, anodyne, anti-inflammatory. They are useful in vitiated conditions of vata diabetes, haemorrhoids, reumatism, gonorrhoea and skin diseases^{8,9}.

Collection of plant material

The plant *Salacia Reticulata* Wight was collected from Madurai and Coimbatore during the month of May-June. That was identified by Dr.Stephen M.Sc., Ph.D., Department of Botany, The American College, Madurai-02. The roots were cut off and dried in the shade for 45 days. Then the shade dried roots were made into coarse granules and was used for different investigation.

Extraction

The roots of *Salacia Reticulata* Wight were dried in the shade. Then the shade-dried roots were powdered to get a coarse granules. About 500gms of dried coarse granules of *Salacia Reticulata* roots were soaked in 3-1/2 liters of hexane for 2 days. Then it was extracted with hexane at 40°C to 60°C by continuous hot percolation method using Soxhlet apparatus. The extraction was continued for 72 hours. The hexane extract was filtered and concentrated to a dry mass by distillation (17g). A dark yellowish brown residue was obtained. The marc after the hexane extract was taken and dried to get a dry mass and it was subsequently extracted with chloroform for 72hours using Soxhlet apparatus. The chloroform extract was filtered and concentrated to a dry mass (11g). A dark brownish black residue was obtained.

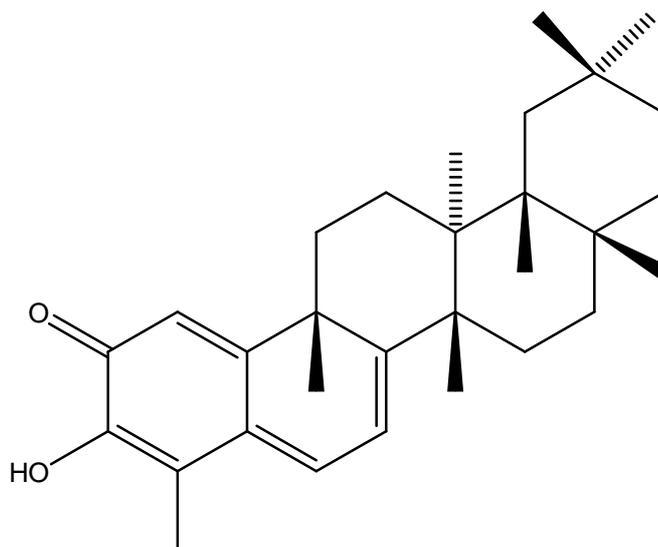
Preliminary phyto chemical investigation^{10,11}

The hexane and chloroform extracts showed the presence of carbohydrates, glycosides, coumarins and phytosterols.

Spectral determination^{12,13,14,15,16}

Compound SRA

Quinonemethide

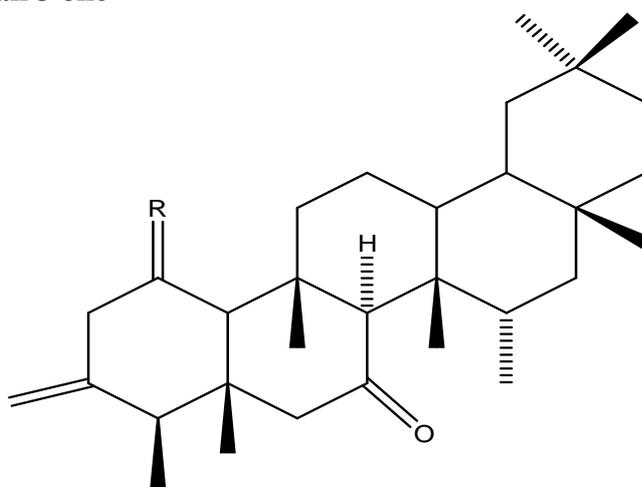


IR Spectral Data

3435 (O-H stretching), 2855 (C-H stretching), 2367 (Extended resonance), 1791 (C=O stretching), 1631 (N-H bonding), 1383(SP^3 C-H bending), 833 (C-H stretching), 764 (C-C OOpI bending).

 1H NMR Spectral Data

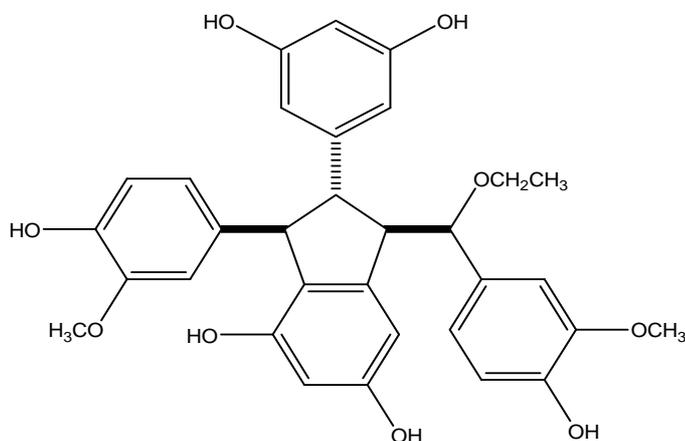
0.880-0.958(CH_3 Proton), 0.958-1.254(CH_2 Proton attached to alkyl group), 1.601-2.027 (CH Proton attached to C=C), 2.321 (CH_2 Proton attached nearer carbonyl group), 7.264-7.271(Aromatic proton).

Compound SRB**15 α hydroxy friedeelan 3 one****IR Spectral Data**

3421 (O-H stretching), 2854 (C-H stretching), 1710 (C=O stretching), 1649 (N-H bending), 1461 (C-H bending), 1382 (SP^3 C-H bending), 1245(C-O stretching)

 1H NMR Spectral Data

0.880-0.995(CH_3 Proton), 0.995-1.255(CH_2 Proton attached to alkyl group), 1.601-2.004 (CH Proton attached to C=C), 2.314-2.340 (CH_2 Proton attached nearer carbonyl group), 3.288-3.642 (Aromatic OH), 3.974 (Acyclic conjugated), 5.118-5.626 (CH_2 Proton attached to ethylenic double bond), 6.829-7.273 (Aromatic proton).

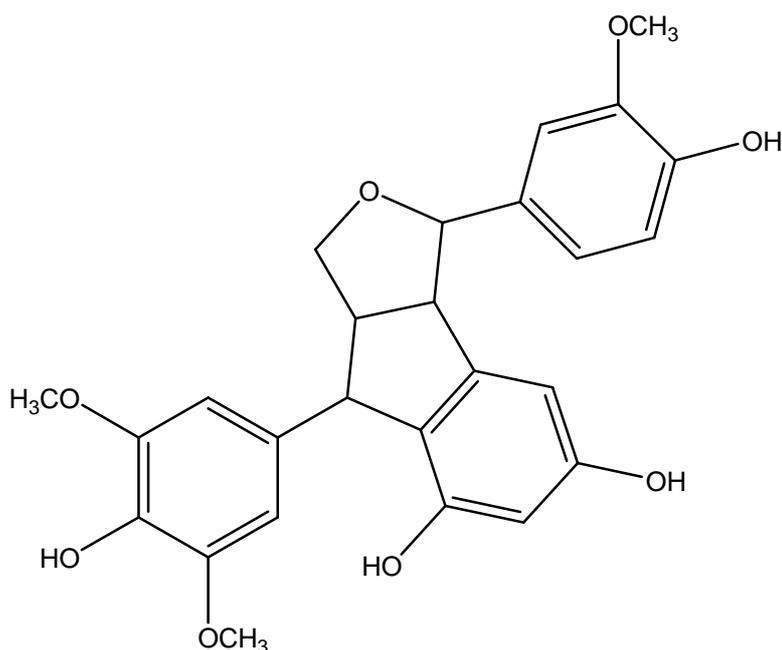
Compound SRC**Lehmbachol C**

IR Spectral Data

3442(OH Stretching), 2856 (CH Stretching), 2364 (Extended resonance), 1708 (C=O Stretching), 1653(NH bending), 1460(CH bending), 1242(CO Stretching), 1033 (SO Stretching).

¹H NMR Spectral Data

0.880-1.256(CH₃ Proton), 1.256(CH₂ Proton attached to alkyl group), 1.601-2.002 (CH Proton attached to C=C), 2.344-2.340 (CH₂ Proton attached nearer carbonyl group), 3.639 (Aromatic OH), 3.974 (Acyclic conjugated), 5.118-5.626 (CH₂ Proton attached to ethylenic double bond), 6.829-7.273 (Aromatic proton).

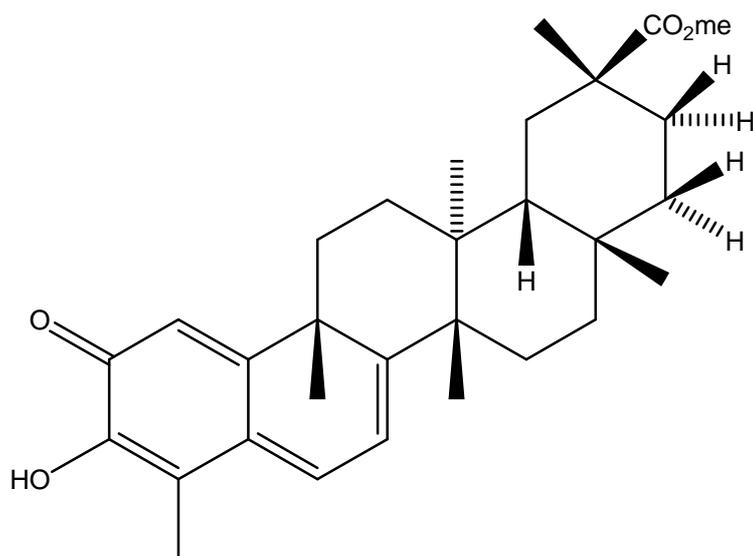
Compound SRD**Lehmbachol D****IR Spectral Data**

3537(OH Stretching), 2856 (CH Stretching), 2364 (Extended resonance), 1706 (C=O Stretching), 1608(NH bending), 1460(CH bending), 1460 (CH bending of alkanes).

¹H NMR Spectral Data

0.772-1.210(CH₃ Proton), 1.210-1.256(CH₂ Proton attached to alkyl group), 1.435-2.005 (CH Proton attached to C=C), 3.234-3.286 (OR, OH group), 3.473-3.520 (Aromatic OH), 4.181 (conjugated alkenes), 5.118-5.626 (CH₂ Proton attached to ethylenic double bond), 7.265-7.273 (Aromatic proton).

Compound SRE
Pristimerin



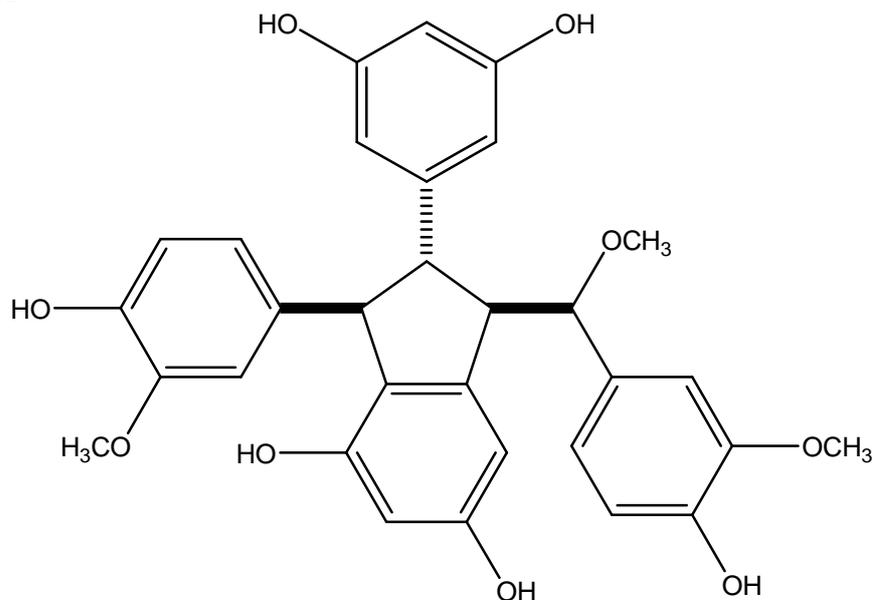
IR Spectral Data

3479(NH Stretching), 2864 (CH Stretching), 1708 (C=O Stretching), 1624(NH bending), 1460(CH bending), 1384(CH bending), 1242(CO Stretching).

¹H NMR Spectral Data

0.871-1.204(CH₃ Proton), 1.253-1.593(CH₂ Proton attached to alkyl group), 1.856-2.084 (CH Proton attached to C=C), 2.191-2.235(R-C=O), 2.235-2.405(CH₂ Proton attached to carbonyl group), 3.222-3.350(Due to OR, OH group), 3.635-4.076(CH₂ Proton attached to OR), 4.136-4.270(Conjugated alkynes), 4.310-4.622(Due to NO₂), 5.118-5.626 (CH₂ Proton attached to ethylenic double bond), 7.265-7.273 (Aromatic proton).

Compound SRH
Lehmbachol A



IR Spectral Data

3400(OH Stretching), 2856 (CH Stretching), 2364 (Extended resonance), 1743 (C=O Stretching), 1546(NH bending), 1460(CH bending), 1370 (CH bending of alkanes), 1163(Alcoholic C-O Stretching).

¹H NMR Spectral Data

0.736-1.092 (CH₃ Proton), 1.265 (CH₂ Proton attached to alkyl group), 1.607-2.064 (CH Proton attached to C=C), 2.295-2.344(3.473-3.520 (Aromatic OH), 4.181 (CH₂ Proton attached to carbonyl group), 5.118-5.626 (CH₂ Proton attached to ethylenic double bond), 7.265-7.273 (Aromatic proton).

¹³C NMR Spectral Data

14.1, 26.63, 30.74, 31.8, 39.65, 76.4, 76.9, 77.3, 124.1, 134.83.

Anti inflammatory activity^{17,18,19}

Albino rats of wister strain (150-200gm) pf either sex were procured from the central animal house of the institute. They were housed in standard polypropylene cages and kept under controlled room temperature (24± 20°C, relative humidity 60 to 70%) in 12 hours light-dark cycle. The rats were given a standard laboratory diet and water and *ad libitum*. Food was withdrawn 12 hours before and during the experiment hours. Carrageenan (Sigma-Aldrch), Diclofenac sodium (Novartis), and chloroform and hexane extract. The animals were divided into four groups each have 6 animals. The animals were treated with standard drug and both extracts of chloroform and hexane at a dose of 1000mg/kg suspended into 1 ml of 1% CMC.Group-1 served as normal control, received 10 ml/kg normal saline through orally.Group-2 served as positive control, received 10mg/kg of diclofenac sodium administered through intraperitoneally. Group-3 served as treatment control, received 1000mg/kg of hexane extract of *salacia reticulata*. One hour after treatment with both extract and standard, acute inflammation was produced by subplanatar injection of 0.1ml of 1% suspension of Carrageenan in the right hind paw of the rats. The paw volume was measured plethysomometrically by the method of chattopadhyay *et al* at zero and four hours after the Carrageenan injection. Mean increase in paw volume was measured and the percentage of inhibition was calculated. Statistical analysis was done by unpaired student “t” test. A.P<0.01 was considered as significant.

RESULTS

In preliminary phyto chemical investigation the hexane and chloroform extracts showed the presence of carbohydrates, glycosides, Coumarins and phytosterols Table no.1. The results of the animal experiments are shown in Table no.2. In this acute inflammation model, both extracts and the standard drugs (diclofenac sodium 10mg/kg) produced significant inhibition of paw edema as compared to the control. The chloroform extract highly significant when compared to the hexane extract. The results were found to be highly significant (P< 0.01) in comparison to control.

Table 2 Anti inflammatory activity of *salacia reticulata* wight carrageenan induced paw edema method

S. No	Dose	Increase in paw volume in ml (Mean ± SEM)	% Inhibition of paw volume
1	Normal saline 10ml/kg	7.0 ± 0.30	-
2	Diclofenac sodium 10ml/kg	2.30 ± 0.009	67.1%
3	Hexane extract 1000 mg/kg	4.6 ± 0.26	34.28 %
4	Chloroform extract 1000mg/kg	3.08 ± 0.15	56.0 %

Values are expressed as mean ± SEM. Values are significantly different from control (P < 0.01).

Table 1: Preliminary phytochemical screening of the root extracts of *salacia reticulata* wight

Name of the Constituents	Hexane extract	Chloroform extract
Fixed oil	-	--
Carbohydrates	+	+
Protein	-	-
Tannin	-	-
Alkaloids	-	-
Phytosterol	+	+
Flavanoids	-	-
Glycoside	+	+
Saponins	-	-

+ -- Indicates positive test results.

- -- Indicates negative test results.

DISCUSSION

Carrageenan – induced hind paw oedema is the standard experimental model of acute inflammation. Carrageenan is the phlogistic agent of choice for testing anti-inflammatory agent drugs as it is not known to be antigenic and is devoid of apparent systemic effects. The experimental model exhibits a high degree of reproducibility. Carrageenan-induced edema is a biphasic response. The first phase is mediated through the release of histamine, serotonin, through and kinenin's whereas the second phase is related to the release of prostaglandin and slow reacting substances which peak a 3 hours. The both extracts produced significant inhibition of Carrageenan-induced paw edema. The inhibition was however less than that of the standard drug (diclofenac sodium). The result of the present study suggested that both hexane and chloroform extract in dose of 1000mg/kg orally, significantly suppressed Carrageenan induced paw edema in rats. The chloroform extract was found to be highly significant when compared with the hexane extract.

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