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Evaluation of anti-inflammatory potential of hydro-alcoholic extract of root of *Salacia oblonga*

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ABSTRACT

In this work, hydroalcholic extract of Salacia oblonga was assessed for it anti-inflammatory action using Carrageenan induced paw edema method in rats. The yield of the plant extract obtained from ethanol solvent was 17.37%. The results obtained from phytochemical testing of the hydroalcoholic extract of the roots of Salacia oblong revealed the presence of phenolics, tannins, flavonoids, and carbohydrates in the extract. The total phenolic content in the defatted extract was 19.34 GAE mg/g and that of the hydroalcoholic extract was 91.04 GAE mg/g. The total flavonoid content of hydroalcoholic extract of Salacia oblonga was found to be 46.42 QE mg/g. The hydroalcoholic extract of Salacia oblonga (HESO) was assessed for anti-inflammatory effect in rats carrageenan induced rat paw edema method (in vivo). HESO was able to significantly reduce inflammation in the both the doses. In the 4th hour, at dose of 100 mg/Kg, the inhibition of edema was 50.5% whereas at the dose of 200 mg/Kg, the inhibition of edema was 55.37%. The inhibition of edema was found to be dose dependent and the anti-inflammatory potential was comparable to standard drug ibuprofen which exhibited a reduction in edema by 73.18% by the end of the 4th hour.

Introduction

In the modern day, natural remedies have become an integral part of illness management [1]. Researchers have discovered various medications that have practical benefits. Various approaches have been used for centuries to control inflammation and pain, according to a literature review. While nonsteroidal anti-inflammatory drugs (NSAIDs) are useful for pain and inflammation treatment, they come

with a host of potential adverse effects, including bleeding, ulcers, seizures, and problems with urination. Consequently, there is a demand for chemicals produced from herbs that are both highly effective and economically viable, with few adverse effects [2].

Salacia species are known to elaborate anthocyanidines, catechins, quinones, friedo-oleanones, quinonemethide and related triterpenoids [3]. The major bioactive constituents are being xanthine, glucoside, mangiferin and two components with unique thiosugar structure sulfonium sulfate viz., salacinol and kotalanol [4]. Studies have shown the plant to possess anti-mutagenic, nephroprotective, hypolipidemic, anti-bacterial, anti-fungal, anti-proliferative and cardioprotective activities [5-10]. The anti-diabetic potential of the plant has also been explored in scientific studies. The objective of the present investigation was to extract the secondary metabolites present in *Salacia oblonga* using 80% methanol as the solvent and establish the total phenolic and flavonoid content in the extract and to investigate the anti-inflammatory potential of the crude extract in rat/mice.

Material and Methods

Procurement and preparation of plant material

The root of *Salacia oblonga* were procured from online platform indianjadibooti.com and were authenticated by botanist at RB Science, Bhopal wide and a specimen voucher of the same has been submitted at herbarium of the institute. The authenticated plant material were shade dried and ground to coarse powder using a slow speed motorized blender. The powder was passed through sieve no. 20 to obtain uniform size material for extraction.

Extraction of plant material

The powdered plant material were accurately weighed and filled in a cellulose thimble. The thimble was placed in the extractor of soxhlet extraction apparatus for carrying out the extraction. 98.5g of powdered seed was accurately weighed and filled in a cellulose thimble and placed in the extractor of soxhlet extraction apparatus and the assembly was set up. 350 mL of petroleum ether was flown down the extractor and heating was started to complete extraction. The marc after defatting was allowed to air dry and reweighed. The accurately weighed marc was then placed in the extractor. 87.5 g of the marc was accurately weighed and filled in a cellulose thimble and placed in the extractor of soxhlet

extraction apparatus and the assembly was set up. 300 mL of chloroform was flown down the extractor and heating was started. The extraction was carried out until the solution in siphon tube was found to be colorless. The extracts were evaporated to dryness using water bath at controlled temperature [11].

Phytochemical Screening of Extract

The extracts were analyzed qualitatively for the detecting the presence of alkaloids, glycosides, phenolics, flavonoids and other class of phytochemicals [12].

Total phenolic content determination of extracts

A small quantity of extracts (0.1g) was mixed with 8 mL of methanol and kept overnight. The suspension was filtered through a qualitative cellulose filter paper and the filtrate was diluted to 10 mL with methanol. The solution was stored at 4°C in amber color bottles and served as the stock solution (50 mg/mL) for subsequent analyses. In order to determine the total phenolic content, 200 µL of the extracted sample was mixed with 1.4 mL purified water and 100 µL of Folin-Ciocalteu reagent. After at least 30 s (but not exceeding 8 min), 300 µL of 20%Na_2CO_3 aqueous solution was added and the mixture was allowed to stand for 2 h [12]. The absorbance was measured at 765 nm using a UV-Vis spectrophotometer. Standard solutions of gallic acid (10-100 ppm) were similarly treated to plot the calibration curve. Results were expressed as milligrams of gallic acid equivalent (GAE) per 100 g of the dry sample.

Total flavonoid content determination

Determination of total flavonoids content was based on aluminium chloride method. 50 mg quercetin was dissolved in 50 ml methanol, and various aliquots of 25-150µg/ml were prepared in methanol. 0.1 g of dried extract was extracted with 10 ml methanol, filtered, and make up the volume up to 100 ml. One ml (1mg/ml) of this extract was mixed with sodium nitrite for the estimation of flavonoid. 1 ml of 2% $AlCl_3$ methanolic solution was added to 1 ml of extract or standard and allowed to stand for 60 min at room temperature; absorbance was measured at 420 nm [13].

Carageenan induced rat paw method

Test animal

Healthy Wistar rats of either sex, weighing 180-250g were used for the study. The animals were housed in cages during the course of experimental period and

maintained at 12 day and night schedule with a temperature [17-26°C] maintained at standard experimental condition. The animals were fed with standard rodent pellet feed and water *ad libitum*. The animals were fasted 12 hours before the experiment with free access to only water.

Procedure

The carageenan induced rat paw edema method was used for evaluating the anti-inflammatory activity of the hydroalcoholic extract of *Salacia oblonga (HESO)* [14]. Paw oedema was induced by subcutaneous injection of 0.1mL (1% solution) of Carrageenan into the plantar surface of the right hind paw of the rat. The test sample was administered in dose of 10 mg/kg in different groups of animals, 30 min prior to carrageenan injection. Ibuprofen (10 mg/kg i.p.) was used as a standard anti-inflammatory drug which was administered 30 min prior to carrageenan injection. Animals were divided into 4 groups (n = 6) as follows

Group -- I - Control - treated with vehicle (normal saline)

Group -- II - Standard drug – Ibuprofen

Group – III– HESO was administered in dose of 100 mg/kg.

Group – IV– HESO was administered in dose of 200 mg/kg.

Paw diameters were measured immediately before the administration of the Carrageenan and thereafter at 1, 2, 4 and 6 h using vernier caliper. The results obtained were compared with control group.

Results and Discussion

Yield and phytochemical testing

The crude extract obtained with ethanol was concentrated on water bath by evaporation of the solvent to obtain the actual yield of extraction. The yield of the plant extract obtained from ethanol solvent was 17.37%. The result of qualitative phytochemical analysis is depicted in the Table 1.

Table 1. Phytochemical analysis of hydroalcoholic extract

Chemical Tests	Inference
Alkaloids	
Mayer's reagent	-

Hager's reagent	-
Wagner's reagent	-
Dragendorff's reagent	-
Glycosides (+Ve)	
Baljet test	-
Legal's test	-
Keller-Kiliani	-
Phenols/Tannins	
Ferric chloride	+
Gelatin Solution	+
Lead acetate test	+
Flavonoids	
$FeCl_3$ test	+
Alkaline reagent test	+
Shinoda test	+
Saponins	
Foam test	-
Hemolytic test	-
Lead acetate	-
Fixed oil/Fats	

Spot	-
Saponification	-
Gums & Mucilage	
Water	-
Carbohydrates	
Molish test	-
Fehling's solution test	-
Benedict's test	+

(+) Indicates 'Presence'; (-) Indicates 'Absence'

The results obtained from phytochemical testing of the hydroalcoholic extract of the roots of *Salacia oblonga* revealed the presence of phenolics, tannins, and flavonoids, and carbohydrates in the extract.

Total Phenolic and flavonoid Content

Phenolic compounds have strong antioxidant properties and may offer health benefits, such as protection against oxidative stress and related diseases, when consumed in food or supplements. The total phenolic content in the defatted extract was 19.34 GAE mg/g and that of the hydroalcoholic extract was 91.04 GAE mg/g.

The hydroalcoholic extract of *Salacia oblonga roots* was evaluated for quantifying the total flavonoid content. Standard curve of quercetin was plotted. The result of the total flavonoid content of the extract examined using Aluminum chloride method. The total flavonoid content of hydroalcoholic extract of *Salacia oblonga* was found to be 46.42 QE mg/g.

Anti-inflammatory action (*in vivo)*

Carrageenan-mediated inflammation is one of the most widely used test procedures for screening of anti-inflammatory action. As evident from the table 6.4, HESO was able to significantly reduce inflammation in the both the doses. In the 4^{th} hour, at dose of 100 mg/Kg, the inhibition of edema was 50.5% whereas at the dose of 200 mg/Kg, the inhibition of edema was 55.37%. The

inhibition of edema was found to be dose dependent and the anti-inflammatory potential was comparable to standard drug ibuprofen which exhibited a reduction in edema by 73.18% by the end of the 4th hour (Figure 1).

Table 2. Rat paw edema in rats

Group	Change in Paw thickness (mm)			
	1h	2h	3h	4h
Normal Saline	1.382 ± 0.0164	2.204 ± 0.0472	2.826 ± 0.0684	2.998 ± 0.0630
Ibuprofen	0.484 ± 0.0450	0.768 ± 0.0349	0.946 ± 0.0251	0.804 ± 0.0296
HESO (100 mg/kg)	1.064 ± 0.0240*	1.312 ± 0.1207**	1.444 ± 0.0907**	1.484 ± 0.0646**
HESO (200 mg/kg)	0.982 ± 0.0216**	1.110 ± 0.0833**	1.200 ± 0.0764**	1.232 ± 0.0672**

Results are reported as mean ± SD (n=6); *p<0.05, **p<0.001

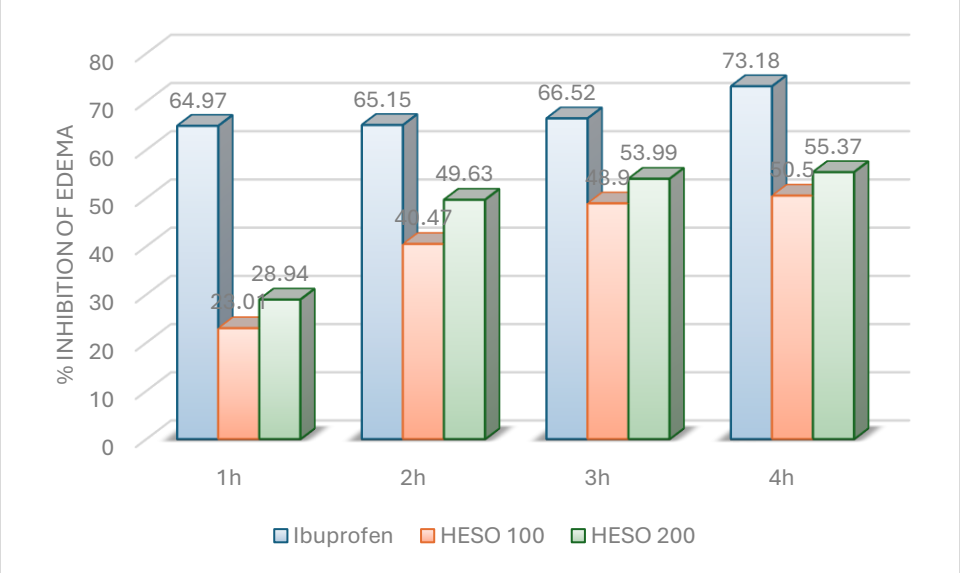

Figure 1. Comparative anti-inflammatory action of ibuprofen and HESO

Carrageenan-mediated paw edema is said to be due to cyclo-oxygenase action and hence it might be inferred that the anti-inflammatory effect of HESO on is due to

inhibition of the enzyme cyclo-oxygenase eventually causing inhibition of prostaglandin synthesis.

Conclusion

It can be concluded form the study that the hydroalcoholic extract of the roots of *Salacia oblonga* has significant analgesic effect in albumin denaturation assay and carrageenan induced rat paw edema method equivalent to the standard drug (ibuprofen). It can be assumed that the extract might be acting via the cyclooxygenase pathway. Further work related to the characterization of the active constituents as well as evaluation of the mechanism of anti-inflammatory effect will be carried out in our laboratory in the near future.

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