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INVESTIGATION OF ANTINFLAMMATORY ACTION OF METHANOLIC EXTRACT OF PORTULACARIA AFRA LEAF

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ABSTRACT

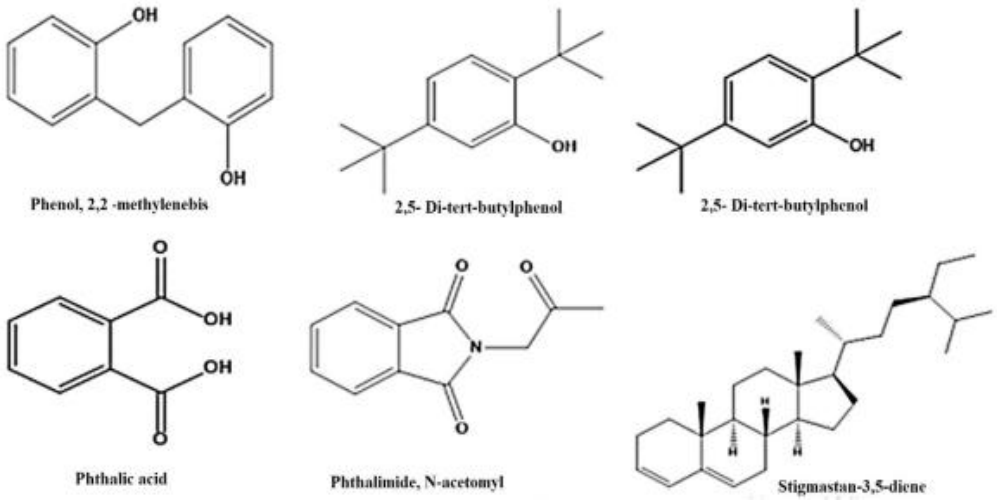
The objective of the present study was to assess the anti-inflammatory potential of methanoilc extract of the leaf of *Protulacaria afra* using the carrageenan induced rat paw edema method. Methanolic extraction using soxhlet method was carried out after defatting the leaves with petroleum ether. The extract of leaf was found to be dark brown in color and was oleo-resinous in nature. The dry weight (%) of the extract with reference to the weight of dry leaf powder was found to be 16.7%. The findings of preliminary phytochemical analysis suggest the presence of alkaloids, phenolics, terpenoids, flavonoids and carbohydrates in the methanolic extract of *Protulacaria afra.* The total phenolic content of MLPA was found to be 21.11±0.163 GAE/mg. The LD50 of MLPA was found to be more than 2000 mg/Kg. The maximum inhibition of edema by MLPA was 28.72% at the end of the 4th hour at the dose of 400 mg/kg. The methanolic extract of the plant was found possess anti-inflammatory action. Further investigations need to be carried out for determining the active principle in extract responsible of the anti-inflammatory action.

KEYWORDS: Portulacaria afra, anti-inflammatory, edema, toxicity, phytochemical.

INTRODUCTION

Portulacaria afra (jade plant) is a small-leaved succulent plant found in South Africa.[1] Jade plant isn't a major element of herbal or alternate medicine. It is recommended for warts in folk remedies. The plant is also used as a treatment for nausea, and in Africa it is used to treat epilepsy, diarrhea, corns, and to purge the intestines. In China, a variety of jade plant with pointed leaves, called the stone lotus, is used to treat diabetic symptoms. Recently several studies on P. afra have been carried out for establishing is as a potential bioactive plant.[2-8]

A number of chemical constituents have been isolated from *Portulacaria afra*.[9] The structures of a few components are presented in the figure 1.



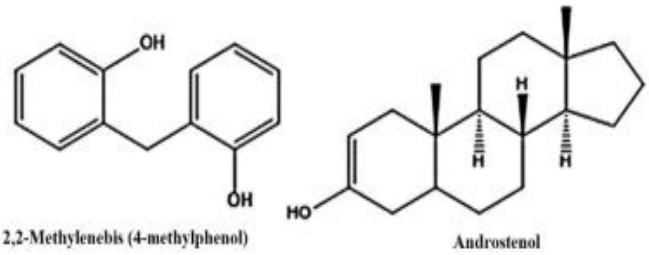


Figure 1: Structures of a few compounds isolated from *Portulacaria afra.*

Inflammation is a part of the complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells or irritants. It is characterized by redness, swollen joints, joint pain, its stiffness and loss of joint function. The ayurvedic system of medicine relies on the use of an amalgamation of several herbs to treat an ailment.

The objective of the present investigation was to extract the secondary metabolites present in *Portulacaria afra* whole plant using methanol as the solvent and establish the total phenolic content present in the extract followed by investigation of the anti-inflammatory potential of the crude extract in animal.

MATERIAL AND METHODS

Collection and Identification of the Plant

The plants of *Portulacaria afra* were purchased from Shubham Nursery, Bhopal, Madhya Pradesh and the preliminary identification of the plant was done at MFP-PARC, Bhopal for authentication.

Preparation of the plant material for extraction

The leaves of the plant were washed with distilled water and dried in shade (preventing from direct sunlight). The dried leaf has been powdered using slow speed blender and is kept in closed airtight container.

Extraction of leaf[10-13]

The extraction was carried out using ethanol as the solvent by hot continuous extraction method using a soxhlet apparatus. Briefly, 100 g of powder was packed in a thimble and the thimble was placed in the extractor of the soxhlet apparatus. 200 mL of pure methanol was flown down the extractor in to the round bottom flask. The flask was heated at 75°C to carry out the extraction process. The extraction was carried out for 11 hours (complete extraction of contents was confirmed by the clear solution in the siphon tube of the extractor). The extract was filtered hot through Whatman filter paper in order to remove any impurity. The extract was then concentrated on boiling water bath to obtain the oleo-resinous residue. The oleo-resinous extract was collected and placed in desiccators to remove the excessive moisture**.** The dried extract was weighed and stored in desiccator for further analysis.

Phytochemical Screening of the extract[11,14]

The methanolic extract was tested for the presence of alkaloids, glycosides, tannins, phenolics, sterols and saponins using the reported methods.

Acute Toxicity Study[15]

A total of three animals were used which received a single oral dose (2000 mg/kg) of extract. Animals were observed individually at least once during the first 30 min after dosing, periodically during the first 24 h and daily thereafter for a period of 14 days. Once daily observations were made for changes in skin and fur, eyes and mucous membrane (nasal) and also respiratory rate, circulatory (heart rate and blood pressure), autonomic (salivation, perspiration, urinary incontinence, and defecation) and central nervous system (drowsiness, tremors and convulsion) changes. Mortality, if any, was also observed over the period of 2 weeks.

Anti-inflammatory action

Animals

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. The protocol was approved by the institutional ethical committee.

Carageenan induced rat paw edema method

The carageenan induced rat paw edema method was used for evaluating the anti-inflammatory activity of the methanolic leaf extract of *Portulacaria afra* (MLPA).

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– MLPA was administered in dose of 200 mg/kg.

Group – IV– MLPA was administered in dose of 400 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. The percentage inhibition of paw inflammation exhibited by each group was calculated by using following formula.

% inhibition = C-T/ C x 100

C= Paw volume (mm) in vehicle treated group (control)
T= Paw volume (mm) in drug treated group

RESULTS AND DISCUSSION

Plant material

The leaf of bright green in color, grow opposite on the stem and egg shaped (elephant ear shape), fleshy without a clear petiole.

Extraction yield

The extract of leaf was found to be dark brown in color and was oleo-resinous in nature. The dry weight (%) of the extract with reference to the weight of dry leaf powder was found to be 16.7%.

Phytochemical Screening

A small fraction of the dried extracts were subjected to the phytochemical screening for detecting the presence alkaloids, glycosides, tannins, saponins, flavonoids and terpenoids. The MLPA was found to contain alkaloids, tannins, saponins, flavonoids, terpenoids and carbohydrates. Proteins and glycosides were not found to be extracted using methanol as the solvent.

Total Phenolic Content

MLPA was evaluated for quantification of the total phenolic content concentration in extract, using gallic acid as the standard. The total phenolic content in extracts, expressed as gallic acid equivalents. The total phenolic content of MLPA was found to be 21.11±0.163 GAE/mg.

Acute toxicity

The acute toxicity test was performed by using the dried MLPA at concentration of 2000 mg/kg to the test animal, administered orally. No animal died and hence the dose of upto 2000 mg/Kg was considered to be safe. As none of the animals died, the LD_{50} was considered to be more than 2000 mg/Kg and any dose less than 2000 mg/Kg would be considered for evaluation of anti-inflammatory action.

Anti-inflammatory activity

MLPA at lower dose was able to reduce the inhibition only after the fourth hour significantly ($p<0.01$). The maximum inhibition of edema by MLPA at 200 mg/kg dose was 13.75% at the end of the 4th hour while that with 400 mg/kg dose was 28.72%. The inhibition of edema was found to be dose dependent and at every time point the inhibition of the higher dose was better than that of the lower dose (Figure 2).

MLPA exhibited a dose dependent inhibition of edema in the rats. At the dose of 200 mg/kg, MLPA was not significant in inhibiting edema. The anti-inflammatory effect of MLPA was visible at the dose of 400 mg/kg with significant inhibition of paw edema. Carrageenan-induced paw edema model in rats is known to be sensitive to cyclo-oxygenase inhibitors and has been used to evaluate the effect of non-steroidal anti-inflammatory agents, which primarily inhibit the cyclo-oxygenase involved in prostaglandin synthesis. Therefore, it can be inferred that the inhibitory effect of MLPA on carrageenan-induced inflammation may be due to inhibition of the enzyme cyclo-oxygenase leading to inhibition of prostaglandin synthesis.

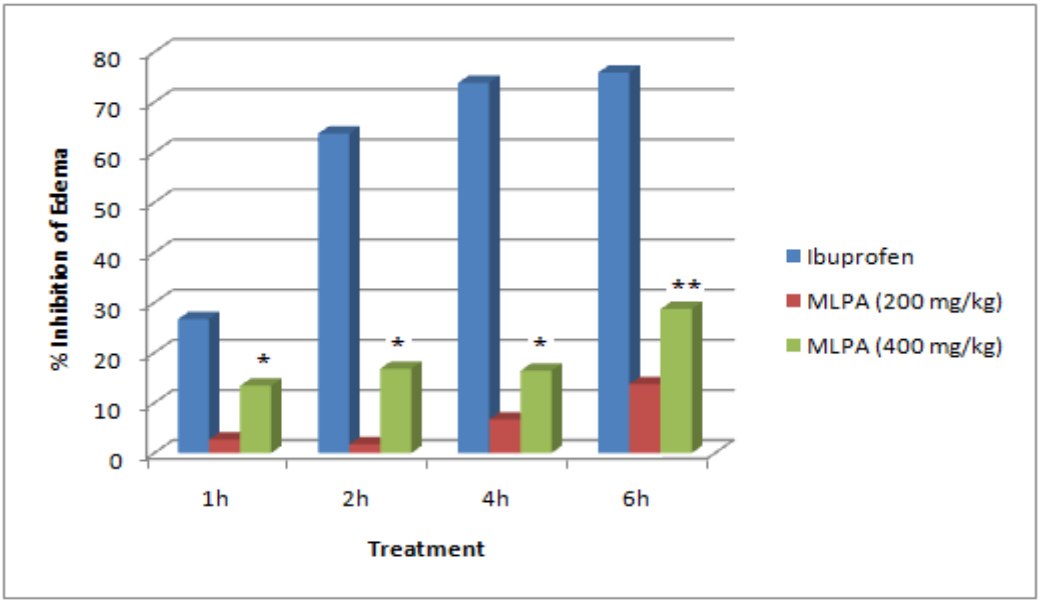


Figure 2: Comparative anti-inflammatory effect of ibuprofen and MLPA.

CONCLUSION

The objective of the present study was to assess the anti-inflammatory potential of methanoilc extract of the leaf of *Protulacaria afra* using the carrageenan induced rat paw edema method. The methanolic extract of the plant was found possess anti-inflammatory action. Further investigations need to be carried out for determining the active principle in extract responsible of the anti-inflammatory action.

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