



Research Article

PHYTOCHEMICAL SCREENING AND *IN VITRO* ANTI-INFLAMMATORY ACTIVITY OF *CAPPARIS MOONII* WIGHT

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ABSTRACT

Ayurvedic system of medicine is one of the oldest systems in India. Herbs and herbal products, with their incredibly wide use throughout time and place, continue to provide real health benefits while maintaining safety profile. The conservative drug available in the marketplace to treat inflammation produces various side effects. To conquer these problems, medicinal plants play a major role to alleviate many diseases related with inflammation and analgesia. *Capparis moonii* Wight (Family - Capparidaceae) is one of the most famous timber plants in the world and is renowned for its dimensional stability, extreme durability and hard which also resists decay even when unprotected by paints and preservatives. The objective of present study was to evaluate phytochemical screening and *in vitro* anti-inflammatory activity of ethanolic extracts of *Capparis moonii* Wight fruit. *Capparis moonii* Wight fruit shows many activities such as anthelmintic, anti-itching also. The results of plant extract containing various phytochemicals like flavonoids, glycosides, alkaloids which were found to have significant ($P < 0.005$) *in vitro* anti-inflammatory activity.

Keywords: *Capparis moonii* Wight, Egg albumin, Phytochemical tests.

INTRODUCTION

Genus *Capparis* is well known for ethnomedicinal importance. *Capparis* species are reported to be utilized as contemporary ethnomedicines.¹ Species like *sepiaria*, *flexulosa*, *decidua*, *zeylanica*, *baducca*, *tomentosa*, *horrida*, *acuminata*, *pyrifolia*, *cordata*, *ovalifolia* are used in treating numerous diseases.²

Capparis moonii Wight belongs to the family Capparidaceae frequently found in the Konkan regions and grows vigorously in hot semi-arid conditions. Its worldwide distribution is restricted to only Indian Subcontinent i.e. southern India, Sri Lanka exhibiting its endemism. Capparidaceae family comprising different species of *Capparis* is distributed in tropical and sub-tropical India, whose medicinal usage has been reported in the traditional systems of medicine. Plants belonging to the Capparidaceae family have been described as a rasayana herb. *Capparis moonii* Wight is commonly known as Waghathi in Marathi and Rudanti in Sanskrit. Signs of aging (Jara Vinashnam) are delayed by use of Rudanti and are also useful in diseases which are having devastating effects on all the systems of the body (Rajyakshma Shasyate). Rudanti was widely used to get relief from asthma and cough.³ Due to significant ethnomedicinal importance of genus *capparis*, *Capparis moonii* Wight emerges as an extremely valuable plant for bio prospecting.

The ancients described inflammation by five signs, namely redness (rubor), swelling (tumour), heat (calor), pain (dolor) and loss of function (functionlaesa). Inflammation is identified to be a type of localized protective response to tissue damage and/or microbial invasion, which aids in isolation and destruction of

the harmful agent, the tissue affected and to prepare the tissue for eventual process of repair and healing. Mostly an inflammatory reaction is short-lived and gives the desired protective response. But when there is excessive or prolonged inflammation, it can lead to extensive tissue damage, organ losing its function and mortality.⁴

MATERIAL AND METHODS

Collection and Authentication

Fruits of *Capparis moonii* Wight belonging to family Capparaceae were collected in month of July from local region Solapur district, Vadgaon town, Maharashtra (India). It was authenticated by Approved botanist Dr. Surayavanshi from department of Botany, Sangola College, Sangola and District Solapur. The specimen voucher no. (CM 001 dated 05/07/2019) and herbarium sheet submitted in the college for further reference.

Preparation of drug for extraction

The authenticated fresh fruits were dried under shed and used for the preparation of extract. These fruits are extracted by ethanol solvent using Soxhlet apparatus and obtained as a semi solid mass form. The extract was stored in amber colored glass bottle in airtight container for further use.

Phytochemical Screening

Plant is selected for phytochemical investigation, either on the basis of phyto-pharmacological approach or through some other

avenue, phytochemical screening techniques can be a valuable aid.

The phytochemical investigation of a plant includes selection, collection, identification and authentication of plant material, extraction of the plant material. Ethanolic extract were subjected to preliminary phytochemical screening for the detection of various phytoconstituents such as alkaloids, glycosides, carbohydrates, tannins, flavonoids, saponins, sugars and proteins.

The following tests were carried out to identify the various phytoconstituents present in ethanolic extract.

Test for Carbohydrates and Glycosides

A small quantity of various extract was dissolved separately in 4 ml of distilled water and filtered. The filtrate was subjected to the following tests to detect the presence of carbohydrates and glycosides.⁵

Molisch's test

The filtrate was treated with 2-3 drops of 1% alcoholic alpha-naphthol and 2 ml of concentrated sulphuric acid was added along the sides of the test tube. Appearance of brown ring at the junction of two liquids shows the presence of carbohydrates.

Fehling's test

The filtrate was treated with each 1 ml of Fehling's solution A and B and heated on water bath. A reddish precipitate was obtained shows the presence of carbohydrates. Another portion of extracts was hydrochloric acid for few hours on a water bath and hydrolysate was subjected to the following tests to detect the presence of glycosides.

Legal's test

To the hydrolysate 1 ml of pyridine and few drops of sodium nitroprusside solution were added and then it was made alkaline with sodium hydroxide solution. Appearance of pink to red colour shows the presence of glycosides.

Bontrager's test

Hydrolysate was treated with chloroform and chloroform layer was separated. To this equal volume of dilute ammonia solution was added. Ammonia layer acquires pink colour shows the presence of glycosides.

Detection of Fixed Oils and Fats Filter paper test

Small quantities of various extracts were pressed separately between the filter paper. Appearance of oil stain on the paper indicated the presence of fixed oils.

Saponification test

Few drops of 0.5 M alcoholic potassium hydroxide were added to small quantities of various extracts along with a drop of phenolphthalein. The mixture was heated on a water bath for 1-2 hours. Formation of soap indicates the presence of fixed oils and fats.

Detection of Proteins and Free Amino Acids

Small quantities of various extracts were dissolved in few ml of water and then they were subjected to the following tests.

Million's test

The above-prepared extracts were treated with million's reagent. Red colour formed shows the presence of proteins and free amino acids.

Biuret test

To the above prepared extracts, equal volume of 5% sodium hydroxide and 1% copper sulphate solution were added. Volatile color produced shows the presence of proteins and free amino acids.

Ninhydrine test

The extracts were treated with Ninhydrin reagent. Purple colour produced shows the presence of proteins and free amino acids.

Detection of Saponins

The extracts were diluted with 20 ml of distilled water and it was agitated in a measuring cylinder for 15 minutes. The formation of 1 cm layer foam shows the presence of saponins.

Detection of Tannins and Phenolic compounds

Small quantities of the various extracts were taken separately in water and test for the presence of phenolic compounds and tannins were carried out with the following reagents.

1. 5% Ferric chloride solution – violet colour
2. 1% solution of gelatin containing 10% sodium chloride – white precipitate
3. 10% lead acetate solution – white precipitate
4. Potassium dichromate solution – red ppt

Detection of Phytosterols

Small quantities of various extracts were dissolved in 5 ml of chloroform separately. Then this chloroform solution was subjected to the following tests to detect the presence of phytosterols.

Salkowski Test

To 1 ml of prepared chloroform solution, few drops of concentrated sulphuric acid were added. Brown colour produced shows the presence of phytosterols.

Liebermann Burchard test

The above prepared chloroform solution was treated with a few drops of concentrated sulphuric acid followed by few drops of diluted acetic acid, 3 ml of acetic anhydride. A bluish green colour appeared indicates the presence of phytosterols.

Detection of alkaloids

Small quantities of various extracts were separately treated with few drops of dilute hydrochloric acid and filtered. The filtrates were used for the following tests.

1. Mayer’s reagent – cream ppt
2. Dragendroff’s reagent – orange brown ppt
3. Hager’s reagent – yellow ppt
4. Wagner’s reagent – reddish brown ppt

Detection of Gums and Mucilage

A small quantity of various extracts was added separately to 25 ml of absolute alcohol with constant stirring and filtered. The precipitate was dried in air and examined for its swelling properties. No swelling indicates the absence of gums and mucilage.

Detection of Flavonoids

1. Small quantities of various extracts were dissolved separately in aqueous sodium hydroxide. Appearance of yellow colour indicates the presence of flavonoids.
2. To the small portion of each extracts, concentrated sulphuric acid was added. Yellow orange colour was obtained shows the presence of flavonoids.
3. Shinoda’s test: Small quantities of extracts were dissolved

in alcohol. To that piece of magnesium followed by concentrated hydrochloric acid was added dropwise and heated. Appearance of magenta colour shows the presence of flavonoids.

In vitro anti-inflammatory activity by Protein denaturation method

The reaction mixture (10 mL) consisted of 0.4 mL of egg albumin (from fresh hen’s egg), 5.6 mL of phosphate buffered saline (PBS, pH 6.4) and 100 µL of plant extract in concentration (200, 400, 600, 800, 1000 µg/ml). Similar volume of double-distilled water served as control. Then the mixtures were incubated at (37°C ± 2) in an incubator for 15 min and then heated at 70°C for 5 min. After cooling, their absorbance was measured at 660 nm by using vehicle as blank. Diclofenac sodium at the concentration was used as reference drug and treated similarly for determination of absorbance.⁶⁻⁹ The percentage inhibition of protein denaturation was calculated by using the following formula,

$$\% \text{ inhibition} = \frac{C - T}{C} \times 100$$

Where, T = absorbance of test sample, C = absorbance of control

RESULT AND DISCUSSION

Table 1: Data showing the preliminary phytochemical screening of the ethanolic extract *Capparis moonii* Wight

S. No.	Constituents	Ethanolic Extract
1	Alkaloids	+
2	Glycosides	+
3	Fixed oils and fats	-
4	Protein and amino acids	+
5	Tannins	+
6	Gum and mucilage	-
7	Flavonoids	+
8	Carbohydrates	+
9	Saponins	-

+ Presence, - Absence

From observation table the results indicate the alkaloids, glycosides, protein, amino acids, tannins, flavonoids and Carbohydrate showed positive tests.

Table 2: In vitro anti-inflammatory activity of ethanol extracts of *Capparis moonii* Wight

Plant extracts	In vitro anti-inflammatory activity		IC 50 µg/ ml
	OD	% inhibition	
Control	0.92	--	499.80
Diclofenac sodium (100 µg/ml)	0.39	57.60	
200 µg/ ml	0.61	33.69	
400 µg/ ml	0.49	46.73	
600 µg/ ml	0.41	55.43	
800 µg/ ml	0.32	65.21	
1000 µg/ ml	0.21	77.17	

From observation table the results indicate the positive effect as an anti- inflammatory agent dose of 1000 µg/ml and comparatively better action at 100 µg/ml concentration of standard.

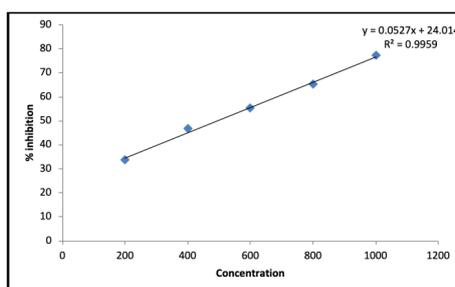


Figure 1: In vitro anti-inflammatory activity

CONCLUSION

The Ethanolic extract of *Capparis moonii* Wight (EECM) was found in a dose dependent pattern, to have positive effect as an anti-inflammatory agent at dose of 1000 µg/kg and comparatively better action at 100 µg/kg concentration of standard. This makes it possible to utilize EECM as a potential treatment drug against inflammation and fever that too from a natural source. This also brings us to the conclusion that EECM proved to be effective in pre-clinical trials and hence this can further be followed with clinical trials.

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