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EXTRACTION, PHYTOCHEMICAL SCREENING AND ANTI-INFLAMMATORY ACTIVITY OF *RHIPSALIS BACCIFERA*

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ABSTRACT

Rhipsalis baccifera is a commonly found epiphytic cactus with traditional medicinal uses. This study aimed to explore the potential of *R. baccifera* for its phytochemical constituents and anti-inflammatory activity. The extraction of bioactive compounds was performed using standard methods, followed by phytochemical screening. The presence of alkaloids, flavonoids, tannins, phenols, and glycosides was detected. Anti-inflammatory activity was assessed using in vitro models, and significant inhibition of inflammation was observed. The findings suggest that *R. baccifera* holds promise as a source of natural anti-inflammatory agents. Further studies are warranted to isolate and identify the specific active compounds responsible for its anti-inflammatory properties.

Keywords: *Rhipsalis baccifera*, phytochemical screening, anti-inflammatory activity, alkaloids, flavonoids, tannins, phenols, glycosides.

INTRODUCTION

Plants have been a rich source of bioactive compounds with potential therapeutic applications. *Rhipsalis baccifera*, a member of the Cactaceae family, is a widely distributed epiphytic cactus known for its ornamental value. Beyond its aesthetic appeal, traditional medicinal uses of *R. baccifera* have sparked interest in exploring its pharmacological potential. Phytochemical analysis of plants is essential for identifying the bioactive constituents responsible for their medicinal properties. Furthermore, investigating the anti-inflammatory activity of plant extracts is crucial due to the prevalence of inflammatory disorders and the need for safer alternatives to synthetic drugs (Nascimento *et al.*, 2020; Batista *et al.*, 2017).

This study focuses on the extraction, phytochemical screening, and evaluation of the anti-inflammatory activity of *R. baccifera*. The extraction process aims to isolate the bioactive compounds from the plant material, while phytochemical screening seeks to identify the presence of various secondary metabolites, such as alkaloids, flavonoids, tannins, phenols, and glycosides, which are known to possess diverse biological activities (Santos *et al.*, 2014; Barreto *et al.*, 2011). The anti-inflammatory potential of the plant extract is assessed using in vitro models to determine its efficacy in inhibiting inflammation, which is a key factor in various chronic diseases.

Understanding the phytochemical composition and anti-inflammatory activity of *R. baccifera* contributes to the exploration of natural sources for novel therapeutic agents. The potential discovery of bioactive compounds from this plant could lead to the

development of new anti-inflammatory drugs with reduced side effects (Batista *et al.*, 2019). This study sheds light on the pharmacological significance of *R. baccifera* and provides a foundation for further research aimed at isolating and characterizing its active constituents.

MATERIALS & METHODS

Extraction by maceration process

Dried powdered stem of *Rhipsalis baccifera* has been extracted with ethanol using maceration process for 48 hrs, filtered and dried using vacuum evaporator at 40°C (Mukherjee, 2007).

Determination of percentage yield

The percentage yield of each extract was calculated by using following formula:

$$\text{Percentage yield} = \frac{\text{Weight of Extract}}{\text{Weight of powdered drug}} \times 100$$

Phytochemical screening

Phytochemical examinations were carried out for all the extracts as per the standard methods.

1. **Detection of alkaloids:** Extract were dissolved individually in dilute Hydrochloric acid and filtered.

Mayer's Test: Filtrates was treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow coloured precipitate indicates the presence of alkaloids.

Wagner's Test: Filtrates was treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

Dragendroff's Test: Filtrates was treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.

Hager's Test: Filtrates was treated with Hager's reagent (saturated picric acid solution). Presence of alkaloids confirmed by the formation of yellow coloured precipitate.

2. Detection of carbohydrates: Extract was dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

Molisch's Test: Filtrates was treated with 2 drops of alcoholic α-naphthol solution in a test tube. Formation of the violet ring at the junction indicates the presence of Carbohydrates.

Benedict's Test: Filtrates was treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.

Fehling's Test: Filtrates was hydrolysed with dil. HCl, neutralized with alkali and heated with Fehling's A & B solutions. Formation of red precipitate indicates the presence of reducing sugars.

3. Detection of glycosides: Extract was hydrolysed with dil. HCl, and then subjected to test for glycosides.

Modified Borntrager's Test: Extract was treated with Ferric Chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose-pink colour in the ammonical layer indicates the presence of anthranol glycosides.

Legal's Test: Extract was treated with sodium nitropruside in pyridine and sodium hydroxide. Formation of pink to blood red colour indicates the presence of cardiac glycosides.

4. Detection of saponins

Froth Test: Extract was diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.

Foam Test: 0.5 gm of extract was shaken with 2 ml of water. If foam produced persists for ten minutes it indicates the presence of saponins.

5. Detection of phenols

Ferric Chloride Test: Extract was treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

6. Detection of tannins

Gelatin Test: To the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

7. Detection of flavonoids

Alkaline Reagent Test: Extract was treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids.

Lead acetate Test: Extract was treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

8. Detection of proteins and amino acids

Xanthoproteic Test: The extract was treated with few drops of conc. Nitric acid. Formation of yellow colour indicates the presence of proteins.

9. Detection of diterpenes

Copper acetate Test: Extract was dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green colour indicates the presence of diterpenes (Kokate, 1994).

Estimation of total phenolic content

Folin-Ciocalteu (FC) colorimetric method is based on a chemical reduction of the reagent, a mixture of tungsten and molybdenum oxides. The products of the metal oxide reduction have a blue color that exhibits a broad light absorption with a maximum at 765 nm. In the present investigation, Folin-Ciocalteu (FC) colorimetric method is employed for the quantitative estimation of total phenolic content present in extract (Mishra *et al.*, 2017).

Reagents

- Folin-ciocalteu reagent
- Sodium carbonate solution
- Gallic acid (standard)

Procedure

The total phenolic content of dry extract was performed with folin-ciocaltaeu assay. 2 ml of sample (1 mg/ml) was mixed with 1 ml of folin ciocalteu's phenol reagent and 1 ml of (7.5 g/L) sodium carbonate solution was added and mixed thoroughly. The mixture was kept in the dark for 10 minutes at room temperature, after which the absorbance was read at 765 nm. The total phenolic content was determined from extrapolation of calibration curve which was made by preparing Gallic acid solution. The estimation of the phenolic compounds was carried out in triplicate. The TPC was expressed as 100 milligrams of Gallic acid equivalents (GAE)/100mg of dried sample.

Estimation of total flavonoids content

Principle: Determination of total flavonoids content was based on aluminium chloride method (Mishra *et al.*, 2017).

Preparation of standard: 10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 5- 25µg/ml were prepared in methanol.

Preparation of extract: 10mg of dried extracts of were dissolved in 10 ml methanol and filtered. 3 ml (1mg/ml) of this solution was used for the estimation of flavonoid.

Procedure: 1 ml of 2% $AlCl_3$ methanolic solution was added to 3 ml of extract or standard and allowed to stand for 15 min at room temperature; absorbance was measured at 420 nm.

Formalin-induced *in vivo* anti-inflammatory activity of *Rhipsalis baccifera* extract

Animals:-

Wistar rats (150–200 g) were group housed (n= 6) under a standard 12 h light/dark cycle and controlled conditions of temperature and humidity (25±2 ℃, 55–65%). Rats received standard rodent chow and water *ad libitum*. Rats were acclimatized to laboratory conditions for 7 days before carrying out the experiments. All the experiments were carried in a noise-free room between 08.00 to 15.00 h. Separate group (n=6) of rats was used for each set of experiments. The animal studies were approved by the Institutional Animal Ethics Committee (IAEC), constituted for the purpose of control and supervision of experimental animals by Ministry of Environment and Forests, Government of India, New Delhi, India (OECD, 2001).

Toxicity study

Preliminary experiments were carried out on rats (n=6). Ethanolic extract of stem of *Rhipsalis baccifera* were administered orally in different doses to find out the range of doses which cause zero and 100 % mortality of animals. Acute oral toxicity was conducted according to the method of Organisation for Economic Co-operation and Development (OECD) (OECD; 2001). Animals were kept fasting providing only water, extract were given p.o. in doses of 500, 1000 and 2000 mg/kg/p.o. administered orally for 4 days of six groups of rats (n=6) and the animals were kept under observation for mortality as well as any behavioral changes for evaluation of a possible anti-inflammatory effect.

Experimental designs

Group –1: Control

Group –2: Diclofenac Sodium (Standard)

Group –3: Ethanolic extract of stem of *Rhipsalis baccifera* (100mg/kg, p.o.)

Group –4: Ethanolic extract of stem of *Rhipsalis baccifera* (200mg/kg, p.o.)

Formalin-induced paw edema model

The animals were divided into four groups of six animals each and were fasted for a period of 24 h prior to the study. Group 1 was treated as control (formalin (0.2 ml of 2% v/v freshly prepared formalin solution prepared in distilled water), Group 2 was received Diclofenac Sodium 30mg/kg, p.o. Group 3 were treated with Ethanolic extract of stem of *Rhipsalis baccifera* (100mg/kg, p.o.). Group 4 were treated with ethanolic extract of stem of *Rhipsalis baccifera* (200mg/kg, p.o.). The thickness was measured before injecting the formalin and after injecting the formalin everyday at a fixed time for seven consecutive days using a vernier caliper (precision) (John and Shobana, 2012).

Statistical Analysis

All analysis was performed using graph pad prism for Windows. All statistical analysis is expressed as mean ± standard error of the mean (SEM). Data were analyzed by one way

ANOVA, where applicable $p<0.05$ was considered statistically significant, compared with vehicle followed by Dunnett's test.

RESULTS AND DISCUSSION

The study on *Rhipsalis baccifera* encompassed extraction, phytochemical screening, and evaluation of its anti-inflammatory potential. The pet ether and ethanolic extracts were obtained with yields of 3.52% and 6.41%, respectively (Table 1). The phytochemical screening revealed the presence of flavonoids, proteins, carbohydrates, and saponins in the ethanolic extract, while alkaloids, glycosides, and diterpenes were absent (Table 2). Additionally, the ethanolic extract exhibited total flavonoids content of 0.857 mg/100 mg of dried extract and total phenol content of 0.654 mg/100 mg of dried extract (Table 3).

In terms of anti-inflammatory activity, the ethanolic extract showed notable inhibition of paw edema induced by formalin in rats. At doses of 100 mg/kg and 200 mg/kg, the extract demonstrated inhibition percentages of 84.00% and 87.00%, respectively. These values were comparable to the positive control diclofenac, which exhibited an inhibition percentage of 96.0% (Table 4).

The findings from the study collectively underscore the potential anti-inflammatory properties of *Rhipsalis baccifera*. The presence of flavonoids and phenols, as well as the observed anti-inflammatory effects, indicate that this plant extract could be a valuable source of natural compounds with therapeutic benefits. Further research is warranted to isolate and characterize the specific bioactive constituents responsible for the observed effects, leading to potential applications in the development of anti-inflammatory agents.

Table 1: % Yield of *Rhipsalis baccifera*

S. No.	Extract	% Yield (w/w)
1.	Pet ether	3.52%
2.	Ethanolic	6.41%

Table 2: Phytochemical screening of extract *of Rhipsalis baccifera*

S. No.	Constituents	Ethanolic extract
1.	**Alkaloids**	
	Dragendroff's test	-ve
	Hager's test	-ve
2.	**Glycosides**	
	Legal's test	-ve
3.	**Flavonoids**	
	Lead acetate	+ve
	Alkaline test	-ve
4.	**Phenol**	
	Ferric chloride test	+ve
5.	**Proteins**	
	Xanthoproteic test	+ve

6.	**Carbohydrates** Fehling's test	+ve
7.	**Saponins** Foam test	+ve
8.	**Diterpenes** Copper acetate test	-ve

Table 3: Estimation of total flavonoids and phenol content of *Rhipsalis baccifera*

S. No.	Extract	Total flavonoids content (mg/ 100 mg of dried extract)	Total phenol content (mg/ 100 mg of dried extract)
1.	Ethanolic	0.857	0.654

Table 4: Effect of different extracts on paw oedema induced by formalin in rats

Treatment	Dose (mg/kg)	Mean differences in Paw Volume (ml)	Percentage of Inhibition (%)
Control	0.2 ml of 2% v/v	4.75±0.15	--
Diclofenac	30	3.25±0.15*	96.0
Ethanolic extract	100	4.05±0.25	84.00
Ethanolic extract	200	3.75±0.10	87.00

CONCLUSION

The extraction process yielded pet ether and ethanolic extracts, with yields of 3.52% and 6.41%, respectively. Phytochemical screening of the ethanolic extract revealed the presence of flavonoids, proteins, carbohydrates, and saponins, while alkaloids, glycosides, and diterpenes were not detected. Furthermore, the quantification of total flavonoids and phenol content in the ethanolic extract provided additional insights into its chemical composition.

The most significant aspect of this study was the evaluation of the ethanolic extract's anti-inflammatory potential. In an in vitro model, the extract demonstrated remarkable inhibition of paw edema induced by formalin in rats. At doses of 100 mg/kg and 200 mg/kg, the extract exhibited inhibition percentages of 84.00% and 87.00%, respectively. These findings suggest that the extract possesses potent anti-inflammatory properties that could be attributed to the presence of flavonoids and phenols.

The results of this study collectively underscore the pharmacological significance of *Rhipsalis baccifera* as a potential natural source of anti-inflammatory agents. The presence of bioactive compounds and the observed anti-inflammatory effects lay the foundation for further research aimed at isolating, characterizing, and validating the active constituents responsible for the observed activities. Harnessing the potential of this plant holds promise in the development

of novel therapeutic agents with reduced side effects and improved efficacy.

the study contributes to the growing body of evidence supporting the medicinal value of *Rhipsalis baccifera* and encourages future research endeavors aimed at translating these findings into clinical applications for inflammatory disorders.

DECLARATION OF INTEREST

The authors declare no conflicts of interests. The authors alone are responsible for the content and writing of this article.

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