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AN EXPERIMENTAL STUDY ON PHYTOCHEMICAL SCREENING AND IN-VIVO ANTI-INFLAMMATORY ACTIVITY OF *RHIPSALIS BACCIFERA*

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ABSTRACT

The mistletoe cactus, or *Rhipsalis baccifera*, belongs to the epiphytic cactus native to Florida, the Caribbean, and Central and South America. Additionally, it may be encountered in Sri Lanka, where it is referred to as nawahandi in Sinhala, as well as the tropical regions of Africa.The only species of cactus that naturally exists outside of the Americas is this one. The purpose of this study was to investigate R. baccifera's potential for its anti-inflammatory properties and phytochemical components. Standard procedures were used to extract the bioactive components, and then phytochemical screening was carried out. Flavonoids, tannins, phenols, and glycosides were found to be present. Utilizing in vivo designs, anti-inflammatory efficacy was evaluated, and significant suppression of inflammation was noted. The results imply that *R. baccifera* has potential as a naturally anti-inflammatory medication source. It is necessary to do more research to extract and pinpoint the precise active ingredients causing its anti-inflammatory effects.

KEY-WORDS: Inflammation, Carrageenan, *Rhipsalis baccifera,* Glycosides

INTRODUCTION

From the Latin word "Inflammare," that means "burn," we get the term "inflammatory". Many chemical changes can occur in the afflicted area as a result of any kind of physical harm. Inflammation was once believed to be a single disease caused by anomalies in body fluids. According to the prevailing idea, inflammation is a typical response to a disease or other disruption. The traditional signs of inflammation are heat, redness, swelling, pain, and loss of function. Inflammation is frequently caused by a number of mechanisms that may be divided into three groups: the acute transitory phase, the delayed subacute phase, and the chronic proliferating phase. In the early stage, inflammatory exudates occur as a result of increased vascular permeability, leading to local edema. Leukocytes and phagocytes migrate through blood to vascular tissues during the second phase, while tissue fibrosis and degradation define the third phase. In reaction to inflammation, endogenous mediators such as prostaglandins, histamine, serotonin, and bradykinin are produced.[1]

Inflammation is a difficult process. The identification of a disease or injury is the initial step in the inflammatory cascade. One typical method for doing this is to identify pathogen-associated molecular patterns (PAMPs), which are specifically focused at general themes of pathogen-expressed molecules required for pathogen survival. Alarmins, also known as damage-associated molecular patterns (DAMPs), are naturally occurring molecules that the innate immune system identifies as signs of injury or necrosis. One advantage of identifying these signals is that it lessens unintentional targeting of host cells and organs. Unlike adaptive immunity, the innate immune system is unable to distinguish between various pathogen strains and evaluate their pathogenicity [2,3].

Nonsteroidal anti-inflammatory medicines are the most widely prescribed medications globally for the treatment of both acute and chronic inflammation-related pain (NSAIDs). The suppression of COX activity in the formation of prostaglandins and thromboxanes is a common feature of the actions of the NSAID medication class.NSAIDs work primarily by inhibiting central and peripheral COX, which stops arachidonic acid from being transformed into prostacyclins, thromboxanes, and prostaglandins E2. The functions of the COX-1 and COX-2 enzymes differ significantly in the way that NSAIDs function. A large number of cells, particularly those in the amniotic fluid and fetus, have COX-1, which is involved in several physiological functions like defense and control. Conversely, proinflammatory cytokines and inflammation stimulate COX-2. Despite the medications' early effectiveness, significant negative effects on the heart, kidneys, and gastrointestinal system have been reported since the introduction of selective COX-2 inhibitors [4,5].

Herbal medicine has been utilized for medicinal reasons since ancient times. Their quantity of medicinal ingredients, which may help prevent diseases and ailments, has made them highly

appreciated internationally. China and India are considered the "Botanical Garden of the World" with good reason, since they are the world's largest producers of medicinal plants. India holds a unique position in the world since it is home to various recognized indigenous medicinal systems that are used to cure ailments, such as Ayurveda, Siddha, Unani, homeopathy, yoga, and naturopathy. [6]

The Cactaceae family includes the widely dispersed epiphytic cactus Rhipsalis baccifera, which is prized for its aesthetic qualities. Traditional medical use of R. baccifera have aroused interest in investigating its pharmacological potential in addition to its aesthetic appeal. To determine which plant components are bioactive and have therapeutic effects, phytochemical study is necessary. Furthermore, because inflammatory illnesses are common and better substitutes for synthetic medications are needed, it is imperative to look into the anti-inflammatory action of plant extracts.[7]

The phytochemical screening, and assessment of R. baccifera's anti-inflammatory potential are the main objectives of this investigation. While phytochemical screening looks for the presence of different secondary metabolites, such as glycosides, alkaloids, flavonoids, tannins, and phenols, which are known to have a variety of biological activities, the extraction process aims to separate the bioactive compounds from the plant material. [8] Using in vivo models, the plant extract's anti-inflammatory activity is evaluated to ascertain how well it inhibits inflammation, a major contributing cause to a number of long-term disorders.

MATERIALS & METHODS

Gathering of Botanical Specimens

A portion of the stems of *Rhipsalis baccifera* were dried at room temperature after being cleaned with tab water. The materials were crushed and put through a 20-mesh filter after they had dried. The powdered drugs were kept out of direct light and in sealed containers until they were needed.

Extraction Using the Maceration Method

Rhipsalis baccifera stem powder that has been dried and pulverized has been extracted with ethanol using a 48-hour maceration procedure, filtered, and dried at 40ºC in a vacuum evaporator [9].

Screening for Phytochemicals [10]

All of the extracts underwent phytochemical analyses using accepted techniques.

Table 1: Different Phytochemicals and its test procedures

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

Carrageenan induced *in vivo* antiinflammatory activity of *Rhipsalis baccifera* extract

Animals

Six 150–200 g wistar rats were housed in groups with controlled humidity and 12-hour light/dark cycles. There was always water and regular rat food accessible. The rats were given seven days to acclimate to the laboratory setting before the experiments began.

Acute toxicity study

It was carried out in accordance with Organization for Economic Co-operation and Development (OECD) standards 425 (up and down approach). All mice were administered an oral dose of 2000

mg/kg of *Rhipsalis baccifera* hydroalcoholic extract, and daily for 14 days as well as hourly for 24 hours, their behaviors, grooming, exploring, writing reaction, eye movements, convulsions, and other activities were observed [11]. Trial dosages of 100 and 200 mg/kg/p.o. for the extracts were selected.

Table 2: Experimental designs [9]

Group –1	Carrageenan control (0.1 ml of 1% w/v)
Group –2	Carrageenan (0.1 ml of 1% w/v) + Indomethacin
Group –3	Carrageenan control (0.1 ml of 1% w/v) + extract of *Rhipsalis baccifera* 100 mg/kg
Group –4	Carrageenan (0.1 ml of 1% w/v) + extract of *Rhipsalis baccifera*200 mg/kg

Carrageenan induced paw edema model

Before the investigation, the animals were separated into four groups of six each, and they fasted for twenty-four hours. Group 1 was given Carrageenan control (0.1 ml of 1% w/v) , whereas Group 2 got Carrageenan (0.1 ml of 1% w/v) + Indomethacin . *Rhipsalis baccifera* extract (10O mg/kg) combined with a carrageenan control (0.1 ml of 1% w/v) was administered to Group 3. Carrageenan (0.1 ml of 1% w/v) and 200 mg/kg of *Rhipsalis baccifera* extract were administered to Group 4. Periodically, the thickness was measured with a vernier caliper.

RESULTS AND DISCUSSION

Alkaloids and glycosides were not found in the ethanolic extract, but flavonoids, proteins, phenol, carbohydrates, tannins, and saponins were found by phytochemical screening. The positive findings of the Molisch's test for carbohydrates suggest that the extract may include these energy-producing molecules. Carbohydrates are essential for cellular metabolism and may enhance the overall nutritional value of the extract. The presence of flavonoids in the hydroalcoholic extract is verified by the positive alkaline reagent test result. Flavonoids are well known for their antioxidant and anti-inflammatory properties.

The extract's purported anti-inflammatory qualities may be influenced by them. The positive Xanthoproteic test result indicates the presence of proteins in the extract. Proteins may enhance the overall nutritional value of the extract and are necessary for several physiological processes. A positive ferric chloride test result means that phenolic compounds are present. It is generally known that phenols contain antioxidant and free radical-scavenging properties, which might increase the extract's total biological activity.

According to the research, the extract from *Rhipsalis baccifera* may have anti-inflammatory effects on carrageenan-induced paw edema in rats. The extract exhibited dosage-dependent effects, with the highest dose (200 mg/kg) demonstrating the greatest reduction of edema at later time periods in particular. These findings support the traditional use of *Rhipsalis baccifera* as an anti-inflammatory and urge further investigation to elucidate the underlying mechanisms responsible for its purported advantages.

Table 3: Different Physicochemical tests and results

Phytochemical	Test	Result
Alkaloids	Dragendroff's Test	-ve
Glycosides	Legal's Test	-ve
Flavonoids	Alkaline Reagent Test	+ve
Saponins	Froth Test	+ve
Tannins	Gelatin Test	+ve
Phenols	Ferric Chloride Test	+ve
Proteins and Amino acids	Xanthoproteic Test	+ve
Carbohydrates	Molisch's Test	+ve

Table 4: Effect of extract of *Rhipsalis baccifera* on paw edema induced by carrageenan in rats by different timelines

Groups	Dose (mg/kg)	0 hr	30 min	1 hr	2 hr	4 hr
Group-I	Carrageenan control (0.1 ml of 1% w/v)	4.6±0.08	5.6±0.02	5.7±0.08	5.8 ±0.08	6.4 ±0.07
Group-II	Carrageenan (0.1 ml of 1% w/v) + Indomethacin	2.3 ±0.05	2.1 ±0.05	1.9±0.07	1.7±0.04	2.15±0.06
Group- III	Carrageenan control (0.1 ml of 1% w/v) + extract of *Rhipsalis baccifera* 100 mg/kg	3.6 ±0.06	3.7 ±0.05	3.8±0.12	3.9 ±0.15	3.9 ±0.05
Group - IV	Carrageenan (0.1 ml of 1% w/v) + extract of	2.8 ±0.12	2.7 ±0.05	2.5 ±0.1	2.3 ±0.25	2.1±0.05

	Rhipsalis baccifera 200 mg/kg					

SUMMARY & CONCLUSION

In conclusion, the goal of this study was to investigate the anti-inflammatory qualities, phytochemical testing, and extraction of the *Rhipsalis baccifera* extract. Numerous significant findings from the study contributed to our understanding of the potential health advantages of the plant extract. The comprehensive process of extraction, phytochemical screening, and anti-inflammatory activity assessment enhances our understanding of *Rhipsalis baccifera*'s potential as a medicine. The study highlights the importance of traditional medicinal herbs as sources of novel bioactive compounds and the need for more research to fully understand the health benefits of these plants. In summary, the *Rhipsalis baccifera* extract has promising anti-inflammatory properties, which may be attributed to the diverse range of phytochemicals it comprises. This study lays the groundwork for future research into the extract's potential to produce effective anti-inflammatory medications with a wider range of therapeutic applications.

REFERENCES

1. NB Sridharamurthy, Dhiman Vinay, R Yogananda. Extraction and Screening of Antioxidant, Anti-inflammatory and Analgesic Activity of Rhynchosia beddomel Leaves. Research J. Pharmacology and Pharmacodynamics. 2012; 4(5): 319-327.
2. Netea, M.G., Balkwill, F., Chonchol et.al, A guiding map for inflammation. Nature Immunology, 18, 826–831
3. Cronstein, B.N. (1992) Molecular mechanism of methotrexate action in inflammation. *Inflammation*, 16, 411– 423
4. Vane, J.R. & Botting, R.M. (1998) Anti-inflammatory drugs and their mechanism of action. *Inflammation Research*, 47 (Supplement 2), S78– S87
5. Moses, V.S. & Bertone, A.L. (2002) Nonsteroidal anti-inflammatory drugs. Veterinary clinics. *Equine Practice*, 18, 21–37.
6. Anonymous. Sectoral Study an Indian Meditional Plants- status, perspective and strategy for growth. Biotech Consortium India Ltd., New Delhi. 1996
7. Nascimento, V.T. et al. (2020) Ethnopharmacological and pharmacological aspects of Rhipsalis baccifera (Sol.) Stearn (Cactaceae). *Journal of Ethnopharmacology*, 251, 112484
8. Santos, J.P.A. et al. (2014) Chemical constituents and antioxidant activity of the stems of Rhipsalis baccifera (Sol.) Stearn (Cactaceae). *Journal of the Brazilian Chemical Society*, 25, 985– 992
9. Mukherjee, P.K. (2007). *Quality Control of Herbal Drugs*, 2nd edn, Business Horizons, pp. 2–14.

10. Kokate, C.K., editor Practical Pharmacognosy (1994), 4th edn, *Vallabh Prakashan*:, 112, 120.
11. OECD (2001). Guideline for Testing of Chemicals-Acute Oral ToxicityAcute Toxic Class Method. OECD: Paris.

