Inhibition of the enzyme cyclo-oxygenase and reducing the levels of PGE2 in the hypothalamus of rats by using smilax china., leaves extract

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ABSTRACT

Smilax china, a climbing plant belonging to the family Smilacaceae, naturally thrives and exhibits resistance to temperatures as low as -15 °C. It can be cultivated in well-drained soils, preferably in a sunny or partially shaded location. Its habitat spans mossy forests at elevations ranging from 1,600 to 2,400 meters in provinces such as Bontoc, Lepanto, Benguet, and Zambales in Luzon, as well as Mindanao and Negros. Indigenous to Japan and China, it has a pantropical distribution and extends into adjacent temperate zones in the north and south. In Central America and the Caribbean, there are recognized 29 species of this plant. The leaves of Smilax china have been traditionally used for their antipyretic properties. However, limited information is available in the literature regarding the leaves of this plant. Therefore, a study was conducted to investigate the inhibitory effects on the cyclo-oxygenase enzyme and the reduction of PGE2 levels in the hypothalamus of rats. Preliminary pharmacognostical studies, including physicochemical parameters, were also conducted. The results revealed that the leaves of Smilax china exhibited a total ash content of 1.69%, acid insoluble ash content of 0.5%, and water-soluble ash content of 2.2%.Pharmacological studies were carried out to evaluate the antipyretic activity of the leaves using a rat model with yeast-induced pyrexia. The aqueous extract of the leaves demonstrated a significant antipyretic effect in a dose-dependent manner. Furthermore, at a dose of 500 mg/kg, the extract showed inhibitory effects on the cyclo-oxygenase enzyme and a reduction in PGE2 levels in the hypothalamus. The leaves were extracted with water and concentrated to obtain a residue for these experiments. This study provides evidence supporting the pharmacognostical and antipyretic properties of Smilax china leaves, thereby corroborating the claims made by traditional medicine practitioners.

Key words: - Cyclo-oxygenase, pyrexia, smilax china and yeast.

1.Introduction

In developing countries like India and China, traditional medicinal systems are widely used as alternative healthcare services for treating various ailments. This preference is driven by the safety and cost-effectiveness of herbal medications. In different regions of India, the use of medicinal plants in folk medicine to treat numerous diseases is quite common.In India, practitioners of traditional medicine, such as Ayurveda and homeopathy, rely on their experience rather than scientific knowledge to prescribe approximately 1000–3000 medicinal plants for treating a wide range of disorders. The demand for effective and alternative medicines derived from natural sources is also growing in the developed world.[1] Herbal plants have gained significant attention globally and are extensively utilized in the form of herbal formulations and agents for combating various infectious diseases. It is estimated that around one quarter of approved modern medicines have been derived from botanical sources. This highlights the significant contribution of herbal medicine to the pharmaceutical industry. Efforts are being made to explore the therapeutic potential of botanicals and harness their healing properties to develop new medications.By incorporating traditional medicinal practices and exploring the potential of herbal remedies, countries worldwide are embracing a holistic approach to healthcare.[2] The utilization of medicinal plants not only offers a wide range of treatment options but also emphasizes the importance of natural sources in the pursuit of improved health and well-being.

Smilax china, commonly known as China root, is a glabrous evergreen climber found in various regions of China and India. It is referred to by different names in different languages and regions, including Chopchini or Chobchini in Hindi, Kaitha in Mizoram, Ayadi in Tamil, Kaltamara in Malayalam, Ghotvel in Marathi, and Kondadantena in Telugu.[3]This plant has a wide range of applications in traditional medicine. The flowers are used for wound healing and repairing the skin barrier in HaCaT human skin keratinocytes. The rhizomes are applied for the treatment of conditions such as rheumatism, gout, epilepsy, skin diseases, chronic nervous diseases, syphilis, flatulence, dyspepsia, colic, neuralgia, constipation, helminthiasis, psoriasis, and seminal weakness. It has been found to inhibit the migration and invasion of cancer cells by suppressing the TGF-β1 pathway.[4]The leaves of Smilax china exhibit antioxidant, antidiabetic, antiobesity, and antimicrobial activities. The roots are known for their antiscrophulatic, carminative, depurative, diaphoretic, diuretic, and tonic properties. The tubers are used as a diuretic and for the treatment of syphilis, rheumatic arthritis, detoxication, lumbago, gout, tumors, and inflammatory diseases.[5]Although there is limited information available in the existing literature regarding the leaves of this plant, the present study focuses on pharmacognostical and pharmacological investigations, specifically exploring the antipyretic effects of Smilax china leaves. These leaves are commonly used in traditional medicine due to the presence of bioactive compounds such as rutin, bismilachinone, and smilachinin.

2. Experimental

2.1. Materials and methods

The leaves of *smilax china*., were collected, washed thoroughly with water, dried under shade at room temperature and powdered using hand crushing to make a coarse powder and stored in well-closed light resistant container until further used.

2.2. Preparation of the extracts

The shade-dried leaves of Smilax china weighing 250 grams were carefully packed in a Soxhlet apparatus. Continuous hot extraction with distilled water was performed until the extraction process was complete. The hot extract was then filtered, and the resulting filtrate was subjected to vacuum distillation under reduced pressure to completely remove the distilled water. The extract was subsequently dried and stored in a desiccator until further experimentation. The weight of the obtained extract was measured, and the percentage yield was calculated based on the air-dried powdered crude material for future use. The extract was further subjected to phytochemical investigation following the prescribed protocol.[10].

2.3. Animals:

Healthy male albino Wistar rats were chosen for this study. They were housed under standard conditions, including a temperature of 25±2 °C, a 12-hour light-dark cycle, and a relative humidity of 45-55% in the animal house. The rats were provided with a pellet diet and water ad libitum, ensuring they had unrestricted access to food and water. Prior to the experiments, the rats were deprived of food for 24 hours, although they were allowed to drink tap water. For the antipyretic activity and anti convulsant activity experiments, the tests were conducted between 10:00 AM and 1:00 PM with free access to food and water. Throughout the study, a mortality rate of 8% was observed among the rats. The utmost care was taken to ensure the well-being of the animals, and all procedures involving the rats were carried out in aseptic conditions to prevent any infections or contamination. Only chemicals, solvents, and reagents of analytical grade were utilized in this experiment to ensure accuracy and reliability of the results. The use of high-quality materials maintained the integrity and consistency of the study, minimizing any potential sources of error. Animals were kept six in one group and all operation on animals was done in aseptic condition[11].

2.4. Pharmacognostical studies

The macroscopical characters of the leaves of *smilax china*., are described (Fig.1).

Colours **:** green.

Taste **:** bitter

Odour **:** Characteristic[12]

Size **:** The leaves are simple, alternate, elliptically oblong to subrounded, 5 to 8 centimeters long, 2.5 to 4 centimeters wide[13]; those toward the end of the branches are much smaller and veined. Petioles are about 7 millimeters long, with adnate spiculate stipules which frequently are extended into tendrils. Inflorescence arises from the upper leaf axils, 3 to 5 centimeters long[14].

2.4.1. Physicochemical parameters

Ash Values

The determination of various physicochemical parameters such total ash, water-soluble ash, alkalinity of water soluble ash and acid- insoluble ash values of the fruit powder was determined as per the Indian Pharmacopoeia[15].

Extractive Values

Extract of the powdered fruit were prepared with water solvents for the study of extractive value[16] .

Fluorescence Analysis

A small quantity of dried and finely powdered leaf and fruit was placed on grease free clean microscopic slide and added 1-2 drops of the freshly prepared reagent solution, mixed gently by tilting the slide and waited for 1-2 minutes. Then the slide was viewed in day light and (365 nm) ultraviolet radiations. The colors observed by application of different reagents in different radiations were recorded[17,18]

2.4.2. Screening for antipyretic activity

Brewer's yeast induced Pyrexia in Rats:

Antipyretic activity on albino rats was studied with fever induced by 20% Brewer's yeast. Albino rats (200-250g) were fed uniformly till 24 hours, and food was withdrawn before giving drugs. After measuring rectal temperature of the rats by introducing 1.5 cm of digital thermometer in rectum, pyrexia was induced by injecting subcutaneously, 20% suspension of dried yeast in 2% gum Acacia at a dose of 20 ml/kg of body weight. After 18 hour of yeast injection, rats which showed a rise in temperature of at least 1°C were taken for the study[19].

3. Results and Discussion

The current study's objective is to examine *Smilax china* leaves for pharmacognostical research, including an initial evaluation of physicochemical parameters and an antipyretic screening.

3.1. Pharmacognostical studies

The pharmacognostical studies conducted on the leaves of Smilax china revealed important physiochemical parameters. The total ash value was determined to be 1.69%, indicating the inorganic residue content of the leaves. The water soluble ash was found to be 2.2%, representing the amount of ash that dissolved in water. Additionally, the acid insoluble ash was measured to be 0.5%, indicating the portion of ash that remained undissolved in acid. Furthermore, the water soluble extractive value of the leaves was calculated to be 36.56%, providing insight into the solubility of active constituents in water. The loss on drying was determined to be 15%, which indicates the amount of moisture lost during the drying process. The foaming index, a measure of the ability of the extract to produce foam, was observed to be 166.97.

To further analyze the characteristics of the aqueous extract of Smilax china leaves, fluorescence analysis was conducted. The results of fluorescence analysis are presented in Tables 3 and 4, providing valuable information regarding the presence of specific compounds or substances in the extract. These pharmacognostical findings contribute to our understanding

of the chemical composition and quality of the aqueous extract of *Smilax china* leaves, offering insights into its potential therapeutic properties and supporting its traditional medicinal use.

3.2. Screening for antipyretic activity

The effect of aqueous leaves extract of *smilax china*.,. plant on yeast induced pyrexia has been shown in (Table no:5). Treatment with control and extracts at dose of 100 mg/kg, 300 mg/kg and 500 mg/kg body weight decreased body temperature of yeast induced rats. The results obtained from both control and extracts treated groups were compared. A significant reduction in the yeast elevated rectal temperature was observed in the test drug.The effect of the aqueous extract of Smilax china leaves on rectal temperature in rats is presented in Table 5. After 18 hours of subcutaneous injection of yeast suspension, a significant increase in rectal temperature was observed. However, treatment with the aqueous extract at doses of 100 mg/kg, 300 mg/kg, and 500 mg/kg resulted in a dose-dependent decrease in the rats' rectal temperature. Notably, the extract at a dose of 500 mg/kg exhibited a significant reduction in body temperature as early as 1 hour after administration (34.20 ± 0.10). The maximal antipyretic effect was observed at a dose of 300 mg/kg, causing a significant reduction in body temperature ($P<0.01$) for up to 2 hours after administration (34.50 ± 0.11). The antipyretic effect persisted for a duration of 4 hours following administration, with the initial effect observed at 1 hour.When comparing the three doses of the tested Smilax china leaf extract, the dose of 500 mg/kg demonstrated significant reductions in yeast-elevated rectal temperature at the 2nd, 3rd, and 4th hour compared to the control group.

These findings highlight the potential antipyretic activity of the aqueous extract of Smilax china leaves, with a dose-dependent effect observed in reducing body temperature in rats

TABLE NO. 1

Physico-chemical parameters of the powdered leaves extract of *smilax china*.,

S. NO	Parameters	% w/w
1.	Ash values	
	(a) Total Ash	1.69
	(b) Acid Insoluble Ash	0.5
	(c) Water Soluble Ash	2.2
2.	Extractive Values	
	Water soluble Extractive	34.89
3.	Loss on Drying	15

TABLE NO. 2

Foaming index of the powdered leaves extract of *smilax china*.,

S. No.	Test of volumetric flask no. (10 ml)	Height of foam (cm.)
1.	1	0.3
2.	2	0.5
3.	3	0.7
4.	4	1.0
5.	5	1.2
6.	6	1.4
7.	7	1.5
8.	8	1.5
9.	9	1.6
10.	10	1.6

TABLE NO. 3

Data for fluorescence study on powder of leaves extract of *smilax china*.,

S. No	Extracts	Day Light	UV Light
1.	Aqueous Extract	Dark Brown	Greenish Brown

TABLE NO. 4

Data for fluorescence analysis of the powder of leaves extract of *smilax china*.,

Color reaction	Day light	UV light 365nm
Powder + NaOH	Blakish green color	Dark red fluorescence
Powder + nitrocellulose	Reddish brown	Strong yellow fluorescence
Powder + Hcl	Greenish grey	Dark brown fluorescence
Powder + H2SO4	Brown	Black
Powder + HNO3	Maroon	Brown

TABLE - 5

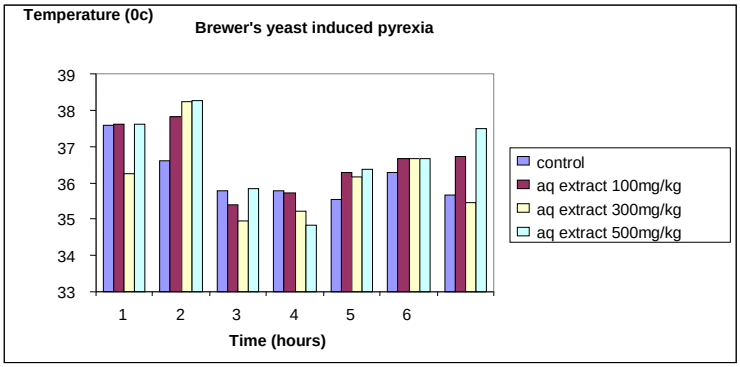
Antipyretic effect of leaves extract of *smilax china*., aqueous extract on adult albino rats

Sr.No.	Group	Treatment	Dose	Initial Rectal Temp. in 0C before Yeast Injection	Rectal Temperature in ^{0}C after 18hrs of Yeast Injection (Mean± SEM)						
					0hr	1hr	2hrs	3hrs	4hrs	5hrs	6hrs
1.	I	Control	-	37.63 ± 0.1	36.31 ± 0.11	36.89 ± 0.13	36.45 ± 0.15	36.59 ± 0.17	36.87 ± 0.18	35.90 ± 0.19	36 ± 0.17
2.	II	Aqueous extract	100 mg/kg	37.78 ± 0.2	37.93 ± 0.12	35.22 ± 0.16	35.50 ± 0.17	36.78 ± 0.14	36.89 ± 0.18	36.80 ± 0.12	37.40 ± 0.-09
3.	III	Aqueous extract	300 mg/kg	36.55 ± 0.4	38.22 ± 0.10	35.94 ± 0.13	34.50 ± 0.11*	36.76 ± 0.15	36.96 ± 0.08	35.64 ± 0.17	37.99 ± 0.11
4.	IV	Aqueous extract	500 mg/kg	37.80 ± 0.5	38.67 ± 0.18	34.20 ± 0.10*	35.99 ± 0.14	36.58 ± 0.12	36.68 ± 0.15	37.70 ± 0.13	37.80 ± 0.17

n = 6 in each group, * indicate P < 0.01 compared to control

GRAPH NO-1

Antipyretic effect of leaves extract of *smilax china*., **aqueous extract on adult albino rats, by Brewer's yeast induced Pyrexia**



4. Conclusion

The pharmacognostic characters documented in this study serve as valuable tools for the standardization of Smilax china, a medicinal plant. These parameters can be utilized for the identification of any potential adulterants, ensuring the authenticity of the plant material. The macroscopic features described in the study can also contribute to the establishment of morphological standards in line with the guidelines set by the World Health Organization (WHO) for the authentication of the drug.Based on the findings of the present investigation, it

can be concluded that the aqueous extract of Smilax china leaves exhibits antipyretic activity. This activity is attributed to the inhibition of the enzyme cyclo-oxygenase and the reduction of PGE2 levels in the hypothalamus of rats. However, further comprehensive studies are required to identify and evaluate the active principles responsible for the observed antipyretic effects. Additionally, a thorough assessment of the safety profile of this plant as a medicinal remedy for pyretic disorders is necessary.Overall, the results of this research highlight the significance of the pharmacognostic characteristics in standardizing the medicinal use of Smilax china. Furthermore, the antipyretic activity demonstrated by the plant extract holds promise for its potential therapeutic applications, but further investigations are essential for a better understanding of its medicinal properties.

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