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Evaluation of Anti-Inflammatory, Antipyretic & Analgesic Activity of Aqueous Extract of Flower from Kydia Calycina on Experimental Animal Models

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ABSTRACT

This study set out to test the efficacy of an extract from the flowers of the Kydia calycina plant in reducing inflammation, fever, and pain using animal models. After being collected, dried in the shade, ground, and rinsed with water, the flowers were subjected to a battery of tests, including physicochemical evaluation, preliminary phytochemical screening, thin-layer chromatography (TLC), and Fourier transform infrared (FTIR) analysis. Using appropriate benchmark medications for comparison, the pharmacological activities were assessed in Wistar rats in models of formalin-induced pain, pyrexia caused by Baker's yeast, and carrageenan-induced paw oedema. According to the physicochemical examination, there were 4.31% water-soluble extractives, 2.18% alcohol-soluble extractives, 7.345% drying loss, and 1.97% total ash. Flavonoids, alkaloids, saponins, triterpenoids, carbohydrates, phenolic chemicals, and tannins were found during the initial phytochemical screening. TLC research revealed three spots with Rf values of 0.72, 0.73, and 0.78, whereas FTIR analysis was used to identify functional groups in the extract. The three pharmacological activities were assessed at dosages of 30, 100, and 300 mg/kg; however, measurements in the several animal trials varied, making it challenging to determine efficacy and statistical significance. The results provide initial physicochemical and phytochemical profile of the flowers of K. calycina and justify its continued study for pharmaceutical applications with confirmed experimental results and systematic analysis.

Keywords: *Kydia calycina, aqueous flower extract, anti-inflammatory activity, antipyretic activity, analgesic activity, phytochemical screening, Wistar rats.*

1. INTRODUCTION

There are traditional medical systems in India such as the Ayurvedic, Siddha, Unani and other indigenous medical systems, where medicinal plants have been important in the preventive and therapeutic measures against diseases [1]. The use of herbal medicines (or phytotherapy) remains an important topic as it continues to be used and is still believed to have therapeutic applications, with a variety of bioactive compounds still being identified. The medicinal plants are rich in flavonoids, alkaloids, terpenoids, tannins, steroids and glycosides which might be responsible for their pharmacological action [2]. The traditional knowledge of these plants offers a rich source of knowledge for identifying potential therapeutic agents and the evidence-based herbal medicines. In order to determine their efficacy, safety and mechanisms of action, however, there is a need for scientific investigation. With the increasing trend of plant-based therapeutics, research has been conducted on medicinal plants for their phytotherapeutic properties for different ailments such as pain, inflammation and fever [3].

Kydia calycina is a part of family Malvaceae and it is a medicinal plant found in various geographical areas of South and Southeast Asia such as India. Traditionally, the plant is considered as a valuable medicinal, ecological and economical valued plant. All parts of K. calycina such as its leaves, roots and bark have been traditionally utilized for the treatment of body pain, arthritis, lumbago, skin diseases, ulcers, wounds, swellings and other conditions [4]. According to reports, its leaves have hepatoprotective, analgesic, and anti-inflammatory qualities that may have therapeutic use. Additionally, it has historically been used to treat a variety of painful and inflammatory illnesses, suggesting that it may contain biologically active ingredients with potential pharmacological effect related to these conditions [5]. Although these traditional uses and early results have been found, there is need to scientifically investigate the therapeutic potential of individual plant parts. Especially, K. calycina flower extract has not been thoroughly explored for its antipyretic, anti-inflammatory, and analgesic properties.

Physiological response to tissue injury, infection and various pathological conditions include pain, inflammation and fever [6]. While pain occurs when nociceptors are activated and these signals are sent to the peripheral and central nervous system, inflammation is an organized response of the cells and molecules to harmful stimuli. Fever (pyrexia) occurs when endogenous pyrogens such as interleukin-1, interleukin-6 and tumour necrosis factor-alpha affect the function of the hypothalamic thermoregulatory system, in part through the action of prostaglandin E2 [7]. These responses are important in host defence and tissue protection but extended or intensified manifestations may NSAIDs are commonly used to treat these conditions. But gastrointestinal irritation, ulceration, bleeding and renal complications are potential side effects of their use. These restrictions have spurred search for medicinal plants as a source

of pharmacologically active compounds. However, the safety and efficacy of use due to their herbal origin is not guaranteed, and systematic experiments should be performed to assess their therapeutic effect and possible restrictions [8].

Since K. calycina has historically been used medicinally, this study aims to assess the antipyretic, analgesic, and anti-inflammatory properties of the plant's flowering part using appropriate experimental animal models as a substitute source of pharmacologically active compounds [9]. The goal of the study is to offer experimental support for the potential anti-febrile, anti-inflammatory, and anti-pain properties of K. calycina's flowering portion. The findings may shed light on the pharmacological profile of the plant and serve as a foundation for identifying the bioactive substances that underlie its traditional therapeutic applications. Additionally, the study may serve as a scientific basis for further investigations into the phytochemical constituents, mode of action, safety, and medicinal applications of this plant [10]. When taken as a whole, these three pharmacological activities will aid in assessing K. calycina's potential as a source of medicinal plant-derived products from an evidence-based standpoint.

2. REVIEW OF LITERATURE

Bhukya et al. (2009) [11] investigated for a variety of pharmacological effects, including as analgesic and anti-inflammatory effectiveness, in experimental animal models. Wistar rats' carrageenan-induced paw oedema was used to measure the anti-inflammatory activity, while albino mice were used to measure the analgesic effectiveness using the hot-plate test and the acetic acid-induced writhing test. The analgesic effects of the extract were most noticeable between 200 and 400 mg/kg, which led to faster reactions and less stomach writhing. In addition, the extract showed anti-inflammatory capabilities by considerably reducing paw oedema. The discovered activities provided scientific support for additional research into the pharmacological properties of K. calycina and were linked to its traditional applications as a medicinal plant for pain and inflammatory illnesses.

Pavithra et al. (2013) [12] tested the extracts of the Kydia calycina flower in chloroform, methanol, and petroleum ether for their antioxidant and antibacterial properties. Scientists used the agar well diffusion technique to check how well the compound fights both Gram-positive and Gram-negative bacteria. To check how well the antioxidant works, they used tests called DPPH free-radical scavenging and ferric reducing assays. The studied microorganisms were shown to be susceptible to the antibacterial activity of the floral extracts; Pseudomonas aeruginosa was more susceptible to the activity of the methanol and chloroform extracts. In terms of free-radical scavenging activity, antioxidant research also demonstrated antioxidant capacity; reported inhibition values ranged from roughly 59% to 91%. The findings

demonstrated the existence of potentially bioactive components in K. calycina flowers and the necessity of additional research into the pharmacological characteristics of these flowers.

Bhukya et al. (2014) [13] investigated if a methanolic extract from Kydia calycina leaves may prevent liver damage in female albino Wistar rats brought on by carbon tetrachloride (CCl_4). The extract was given by mouth at doses of 250 and 500 mg per kilogram, and it was compared to silymarin, which is a commonly used drug that helps protect the liver. The medicine caused a drop in aspartate aminotransferase, total bilirubin, alanine aminotransferase, and alkaline phosphatase levels, which suggests the liver was damaged by the drug. The results demonstrated that the leaf extract can protect the liver from damage caused by chemicals. The pharmacological potential of K. calycina was established, supporting the need for systematic studies on the various portions of the plant, even if the investigation was focused on hepatoprotection rather than analgesic, antipyretic, or anti-inflammatory activities.

Kumari and Padmaja (2012) [14] The antioxidant activity of the hydroalcoholic extract and its different parts—hexane, ethyl acetate, and methanol—was checked using sections from the aerial parts of Kydia calycina. The FRAP assay was used to measure the ability of the extracts and their fractions to reduce ferric ions. The findings showed that the tested preparations had antioxidant activity, albeit their claimed reducing capacity differed from that of the ascorbic acid standard. These findings suggested that the K. calycina extract contains elements that may be involved in antioxidant processes. Given that oxidative stress and inflammatory responses have been linked in a number of clinical disorders, it makes sense to investigate the plant's potential anti-inflammatory properties given its documented antioxidant activity. However, based on the aforementioned antioxidant activity, there are no claims for analgesic or antipyretic action; instead, it must be assessed in experimental settings.

Dangi et al. (2023) [15] tested the ability of ethanol, methanol, and water extracts from Kydia calycina flowers to kill different types of bacteria, both Gram-positive and Gram-negative, such as Pseudomonas aeruginosa, Bacillus cereus, Escherichia coli, and Staphylococcus aureus.” The antibacterial activity of the different extracts was tested using the serial broth dilution method. The findings demonstrated that every extract tested had antibacterial action, with the methanolic extract being more successful against the tested bacterial strains. The current study also aims to emphasise the biological activity of K. calycina's flowering component and, thus, the basis for future research on its therapeutic potential. Nevertheless, this antimicrobial activity alone is insufficient to demonstrate its impact on inflammation, discomfort, or fever. To close this gap, the current work is to experimentally examine the anti-inflammatory, antipyretic, and antinociceptive properties of K. calycina's flowering component.

3. MATERIAL & METHODOLOGY

Thin layer chromatography (TLC), FTIR analysis, physicochemical analysis, and preliminary phytochemical screening were performed on the Kydia calycina flowers after they were gathered and extracted using an aqueous technique. The extract's antipyretic, anti-inflammatory, and anti-analgesic properties were assessed in healthy Wistar rats at dosages of 30, 100, and 300 mg/kg, respectively, in models of formalin-induced pain, carrageenan-induced paw oedema, and Baker's yeast-induced pyrexia. Other haematological parameters were also evaluated and the results of the experiment were planned to be analyzed statistically to investigate the pharmacological potential of the plant extract.

3.1. Plant Material and Extraction

Flower of *Kydia calycina* were collected, cleaned, shade dried and coarsely powdered in Alwar Pharmacy College, Alwar, Rajasthan. The extracted powdered material was obtained by macerating it for 7 days with water at room temperature with periodic agitation. Concentrated under reduced pressure at approximately 40 °C with the rotary evaporator, and kept in a desiccator until further use. The suspension used for the administration of the extracts was prepared in 2% gum acacia.

3.2. Phytochemical and Physicochemical Analysis

Standard pharmacognostic methods have been used to determine the extractive values, loss on drying and ash content of the extract. Preliminary qualitative phytochemical screening was done to identify major phytoconstituents like alkaloids, flavonoids, tannins, saponins and carbohydrates. TLC was performed to separate chemical components using spot development and to determine the Rf values. Dried extract samples were made using KBr, and FTIR was used to collect spectra with a resolution of 4 cm-1 across the 400 to 4000 cm-1 range.

3.3. Experimental Animals and Grouping

Wistar rats of both sexes, 8-12 weeks old and weighing 150-175 g, were purchased from the animal house of Alwar Pharmacy College. The animals lived in standard laboratory conditions, with a 12-hour light/dark cycle and unrestricted access to food and water, with the exception of fasting intervals during the experiments. Their acclimation period was at least five days prior to the experiment. Each experimental model had four groups, each consisting of six animals. IAEC has approved the study, and it was conducted in accordance with the relevant CPCSEA criteria.

3.4. Evaluation of Antipyretic Activity

Wistar rats with pyrexia caused by Baker's yeast were used to examine the antipyretic properties of the floral aqueous extract. Animals were administered the extract orally at dosages of 30, 100, and 300 mg/kg, with paracetamol serving as the reference medication. Rectal temperature was monitored at baseline and at predetermined intervals for up to five hours following the development of pyrexia using Baker's yeast. By comparing the temperatures of the treatment and control groups, antipyretic activity was ascertained.

3.5. Evaluation of Anti-inflammatory Activity

We tested its anti-inflammatory properties in wistar rats using a model of paw oedema caused by carrageenan. Oral administration of diclofenac sodium (10 mg/kg) and aqueous floral extract (30, 100, and 300 mg/kg) were carried out in the same manner. To make the paw swell, a small amount of a 1% carrageenan solution, which is 0.1 mL, was injected under the skin in the area underneath the right hind paw. Using a plethysmometer, the paws' volume was measured at the conclusion of the induction and one, two, and four hours later. Results from the treatment groups were compared to the control group in order to calculate the percentage inhibition of oedema.

3.6. Evaluation of Analgesic Activity

The paw licking test using formalin was done to check how well the analgesic works. According to the approved study plan, the animals received the aqueous flower extract by mouth at doses of 30, 100, and 300 mg per kilogram of body weight. They also received a standard pain relief medication and a control solution as part of the study. Over the course of the observation period, we measured how long it took for the hind paw to lick after receiving a subcutaneous injection of formalin. To find the analgesic activity, we compared the reduction in paw licking time to the control group.

3.7. Haematological Evaluation and Data Analysis

Blood was drawn in accordance with authorised animal protocol for haematological evaluation. An auto haematology analyser was used to estimate RBC, Hb, WCC, and platelets. The results were shown as the mean plus or minus the standard error of the mean (SEM) when it was suitable to do so. A p-value less than 0.05 was considered statistically significant after suitable statistical methods were used for the investigation.

4. RESULTS AND DISCUSSION

This section of the study examined the physicochemical characteristics, phytochemical components, and pharmacological potential of the flower aqueous extract of *Kydia calycina*. TLC showed three spots with

Rf values of 0.72, 0.73, and 0.78, which suggests there are multiple groups of plant chemicals present, such as flavonoids, alkaloids, saponins, tannins, and phenolic compounds. FTIR analysis was used to find out the different groups of chemicals in the extract. In the experimental animal models, they looked into the antipyretic, anti-inflammatory, and pain-relieving effects; but the results about how the extract affected body temperature, swelling in the paw, and pain response were not clear and need more research to prove if they really work and are statistically significant.

4.1. Morphological and Physicochemical Evaluation

The flowers of *Kydia calycina* were subjected to morphological and organoleptic examination, which showed that the flowers were characterized by coarse powder, green-to-brown colour, characteristic odor and astringent taste. The aqueous extract was found to be semi-solid and dark reddish-brown. The physicochemical analysis showed that the water-soluble extractive value was 4.31% and the alcohol-soluble extractive value was 2.18%. The total ash loss was 1.97%, water soluble ash was 0.71%, acid insoluble ash was 1.23%, and the drying loss was 7.345%. The results show the initial quality checks for the plant materials and the water-based extract.

Table 1: Organoleptic and physicochemical characteristics of *Kydia calycina*

Parameter	Observed value
Powder colour	Green to brown
Odour	Characteristic
Taste	Astringent
Aqueous extract appearance	Semisolid, dark reddish-brown
Alcohol-soluble extractive value	2.18% w/w
Water-soluble extractive value	4.31% w/w
Loss on drying	7.345% w/w
Total ash	1.97% w/w
Water-soluble ash	0.71% w/w
Acid-insoluble ash	1.23% w/w

4.2. Preliminary Phytochemical Screening

The plant's aqueous flower extract contained triterpenoids, saponins, alkaloids, carbohydrates, flavonoids, phenolic compounds, tannins, protein, glycoside, and fixed oils or fatty acids, according to the qualitative testing. Phytosterols were not detected by the Salkowski reaction. Comparatively strong reactions were observed with carbohydrates and saponins and the phenolic compounds, indicating the need for further studies to be conducted on these phytochemical groups. Qualitative screening, however, does not provide information on the concentrations of the possible classes of constituents nor the direction of contribution to the observed pharmacological activities.

Table 2: Preliminary phytochemical screening of the aqueous flower extract

Phytochemical group	Reported observation
Phytosterols	Absent
Triterpenoids	Present
Saponins	Present
Alkaloids	Present
Carbohydrates	Present
Flavonoids	Present
Phenolic compounds and tannins	Present
Proteins	Partially positive
Glycosides	Inconsistent test results
Fixed oils and fatty acids	Present

4.3. Thin-Layer Chromatography and FTIR Analysis

Three spots were seen with Rf values of 0.72, 0.73, and 0.78 after the aqueous flower extract was tested on TLC. The TLC used silica gel G as the stationary phase and a mixture of chloroform and methanol in a ratio of 50:10 as the mobile phase. The values are very close together indicating that there may be several chromatographically separable components in the extract, but this must be confirmed by further analytical characterization. An FTIR analysis was conducted to analyze the range of spectral frequencies

between 400 to 4000 cm -1 which was used as a basis for the determination of the functional groups present in the extract. The available results do not allow for the specific identification of chemical bonds or compounds, as individual absorption peaks and their assignment were not given.

Table 3: TLC and FTIR characterization

Parameter	Reported result
Stationary phase	Silica gel G
Mobile phase	Chloroform–methanol (50:10)
Number of TLC spots	3
Rf values	0.72, 0.73, 0.78
TLC detection	Iodine chamber
FTIR spectral range	400–4000 cm^{-1}
FTIR resolution	4 cm^{-1}, as specified in the methodology

4.4. Antipyretic Activity

Wistar rats with pyrexia caused by Baker's yeast were used to test the antipyretic effect of the flower's aqueous extract. The data presented demonstrate the variations in body temperature following the administration of the normal medication (paracetamol) and extract doses (30, 100, and 300 mg/kg). The temperature values in the original table are not consistent with the normal range of physiological temperatures, and the time point names are not clear. Therefore, the amount of temperature drop, dose dependency effects, and statistical significance cannot be determined from the data. Thus, the antipyretic activity of the extract needs to be read subject to the original temperature records and calculations, which must be reviewed.

Table 4: Experimental evaluation of antipyretic activity

Treatment	Dose (mg/kg, orally)	Reported interpretation
Paracetamol	150	Standard treatment
Aqueous flower extract	30	Antipyretic effect requires verification

Aqueous flower extract	100	Antipyretic effect requires verification
Aqueous flower extract	300	Antipyretic effect requires verification

4.5. Anti-inflammatory Activity

Carrageenan-induced paw oedema in Wistar rats was used to investigate the anti-inflammatory efficacy of oral diclofenac sodium 10 mg/kg. After the flower's aqueous extract was given at three distinct doses 30, 100, and 300 mg/kg the outcome demonstrated a change in paw volume. The values that are recorded, however, are not always given as paw volume or percentage inhibition and some entries have implausible magnitudes or unclear time points. Given this, the claim that the extract reduced inflammation cannot be statistically substantiated until the original paw-volume measurements are obtained and percentage inhibition is calculated once again.

Table 5: Experimental evaluation of anti-inflammatory activity

Treatment	Dose (mg/kg, orally)	Assessment
Diclofenac sodium	10	Standard treatment
Aqueous flower extract	30	Paw-volume data require verification
Aqueous flower extract	100	Paw-volume data require verification
Aqueous flower extract	300	Paw-volume data require verification

4.6. Analgesic Activity

The anti-analgesic activity of the flower extract (aqueous) was determined on formalin-induced paw licking test. Observations provided suggested differences in the biting and licking behaviors that occurred during the observation periods after extract treatment. However, the table fails to differentiate between raw response times and percentage inhibition and the nature and dose of the reference analgesia is unknown. These restrictions make it difficult to compare efficacy of the treatments on the analgetic effect. Results should be expressed as the percentage inhibition of paw licking or biting time in comparison with the appropriate vehicle control before conclusions are drawn about the effects of the drug on the paw-licking or biting behavior.

Table 6: Experimental evaluation of analgesic activity

Treatment	Dose (mg/kg, orally)	Reported assessment
Control/standard	Requires clarification	Treatment identity requires verification
Aqueous flower extract	30	Response recorded; inhibition requires recalculation
Aqueous flower extract	100	Response recorded; inhibition requires recalculation
Aqueous flower extract	300	Response recorded; inhibition requires recalculation

4.7. Overall Discussion

The initial results assess the physicochemical characteristics and suggest that the flower aqueous extract of *Kydia calycina* is a source of several phytochemical groups. The TLC further indicates that there was a mixture of extractable constituents and will allow for FTIR analysis to yield some framework for future functional-group characterization. The results indicate the plant's pharmacological potential and warrant further research. The antipyretic, anti-inflammatory and analgesic effects, however, cannot be proven definitively from the animal study tables provided due to inconsistencies in the measurements and calculations reported. The effectiveness and dose-response relationship of the extract must be determined after appropriate validation of the raw experimental data, appropriate comparison with control groups and statistical analysis.

5. CONCLUSION

The current study assessed the aqueous flower extract of Kydia calycina's anti-inflammatory, antipyretic, and anti-analgesic effects in experimental animal models, as well as its physicochemical and phytochemical characteristics. Flavonoids, saponins, triterpenoids, phenolic compounds, tannins, and alkaloids were among the initial phytochemical compounds found in the data, with extractive values of 4.31% and 2.18% for the water-soluble and alcohol-soluble extracts, respectively. An initial profile of the extract was obtained by TLC, which showed 3 spots with an Rf value of 0.72, 0.73 and 0.78. However, the lack of consistency in the measurements of temperature, paw volume and pain response did not allow reliable confirmation of its pharmacological efficacy and statistical significance. Hence, study provides preliminary physicochemical and phytochemical data of *Kydia calycina* flowers and

further study with validated experimental data, proper statistical analysis and systematic safety evaluation is required to identify its anti-inflammatory, antipyretic and the anti-analgesic properties.

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