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TO REPORT THE PHYTOCHEMISTRY & PHARMACOLOGICAL (ANTIPYRETIC ACTIVITY) POTENTIAL OF QUERCETIN & CRUDE EXTRACT OBTAINED FROM *CYNODON DACTYLON*: AN INDIAN DOAB GRASS

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Keywords:

Quercetin, *Cynodon dactylon*, Antipyretic activity, Flavonoids, Yeast-induced pyrexia

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ABSTRACT: The research rigorously investigates the bioactive potential of *Cynodon dactylon*, specifically focusing on its antipyretic properties attributed to quercetin, a flavonoid compound. *Cynodon dactylon*, a perennial grass, has been traditionally used in various medicinal practices, prompting scientific inquiry into its pharmacological benefits. The study involved meticulous extraction and isolation of quercetin from *Cynodon dactylon* using advanced solvent extraction and chromatographic techniques. The purity and structure of the isolated compound were unequivocally confirmed through rigorous spectroscopic methods, including UV and NMR analyses, ensuring the absolute reliability of the isolated quercetin for subsequent pharmacological evaluations. An in vivo experimental model using pyrexia-induced rodents was employed to robustly assess the antipyretic activity of *Cynodon dactylon* extract and isolated quercetin, comparing their efficacy with standard antipyretic drugs. The results unequivocally indicated a significant reduction in elevated body temperature in the treated groups, conclusively demonstrating the remarkable effectiveness of *Cynodon dactylon* and its quercetin component in reducing fever. The study concludes that quercetin isolated from *Cynodon dactylon* exhibits notable antipyretic activity, unequivocally validating its traditional use in fever management. The findings conclusively demonstrate that quercetin is a highly promising candidate for developing natural fever-reducing agents. Moreover, this research emphatically underscores the crucial importance of exploring natural flora for bioactive compounds with therapeutic applications, advocating for further robust studies to elucidate the mechanisms underlying the antipyretic effects and to explore other pharmacological potentials of *Cynodon dactylon*.

INTRODUCTION:

Importance of Phytochemicals: Phytochemicals are naturally occurring molecules found in plants that have gained much attention in the medical field because of their many powerful medicinal applications.

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These bioactive compounds flavonoids, carotenoids, polyphenols, alkaloids, and terpenoids are essential for the prevention and treatment of various chronic diseases and add to the health advantages of a plant-based diet. The diversity and potency of phytochemicals make us aware of the enormous potential of plant-based therapy [1].

Phytochemicals in medicine primarily function as antioxidants, which help counteract the damaging effects of free radicals in the body. Free radicals can lead to oxidative stress and damage to cells, potentially causing chronic diseases such as cancer, cardiovascular disease, and neurological disorders.

For instance, polyphenols found in fruits, vegetables, tea, and wine have been shown to reduce inflammation and oxidative stress, thereby lowering the risk of heart disease and certain types of cancer. Similarly, carotenoids present in tomatoes, carrots, and leafy greens contribute to a stronger immune system and protect against oxidative damage. Anti-inflammatory properties are another significant aspect of phytochemicals [2]. Chronic inflammation contributes to various diseases including arthritis, diabetes, and Alzheimer's. Phytochemicals like curcumin in turmeric and resveratrol in grapes and berries have shown potent anti-inflammatory effects by modulating inflammatory pathways and reducing the production of pro-inflammatory molecules. These properties make them valuable in managing inflammatory conditions and improving overall health [3]. Phytochemicals also have anticancer properties. They can affect the growth of cancer cells, programmed cell death (apoptosis), and the spread of cancer cells (metastasis). For instance, sulforaphane, a compound in cruciferous vegetables like broccoli, has been found to hinder the proliferation of cancer cells and trigger apoptosis in various types of cancer. Additionally, flavonoids such as quercetin and epigallocatechin gallate (EGCG), found in apples, onions, and green tea, have also shown potential in preventing cancer by safeguarding DNA from damage, inhibiting cancer cell growth, and boosting the body's detoxification processes [4].

Quercetin: Quercetin is a natural flavonoid that is widely found in fruits, vegetables, leaves, seeds, and grains. It is a polyphenolic compound with the molecular formula C15H10O7 and is known for its unique structure, which includes a flavone backbone with five hydroxyl groups at positions 3, 3', 4', 5, and 7.

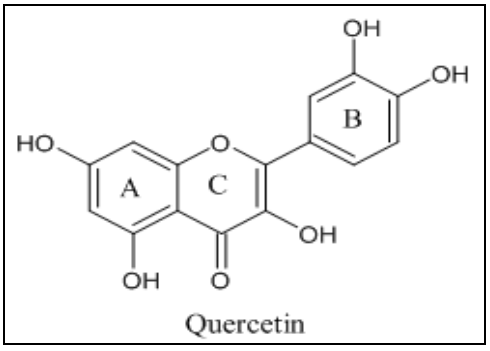

FIG. 1: STRUCTURE OF QUERCETIN

This intricate arrangement contributes to its high reactivity and diverse biological activities. Quercetin's pharmacological properties have been extensively studied, revealing its potent antioxidant, anti-inflammatory, antiviral, and anticancer effects. Quercetin is an antioxidant by chelating metal ions and scavenging free radicals, shielding cells from oxidative stress and lipid peroxidation. Suppressing important enzymes, including lipoxygenase and cyclooxygenase, and the downregulation of pro-inflammatory cytokines effectively reduces chronic inflammatory diseases. Quercetin prevents oxidative stress and lipid peroxidation in cells by acting as an antioxidant that scavenges free radicals and chelates metal ions. It successfully mitigates chronic inflammatory disorders by downregulating pro-inflammatory cytokines and inhibiting important enzymes, including lipoxygenase and cyclooxygenase. However, quercetin's bioavailability is relatively low due to its poor solubility and rapid metabolism; hence, various delivery systems, including nanoparticles and quercetin conjugates, are being developed to enhance its absorption and efficacy. Quercetin stands out as a promising natural compound with multifaceted pharmacological benefits, warranting further investigation and clinical trials to harness its therapeutic potential fully.

Cynodon dactylon: Bermuda grass, known as *Cynodon dactylon*, is a perennial grass that is native to warm temperate and tropical regions worldwide [5]. This hardy grass is characterised by its deep-rooting rhizomes, creeping stolons, and finely textured leaves that form a dense, resilient turf. Bermuda grass thrives in various soil types and exhibits remarkable tolerance to drought, heavy grazing, and foot traffic, making it a popular choice for lawns, sports fields, and pastures.

Traditionally, *Cynodon dactylon* has been used in various cultural practices and medicinal systems. In Ayurvedic medicine, it is revered as "Durva" and is employed for its wide range of therapeutic properties [6]. The plant's juice treats dysentery, diarrhoea, and bleeding disorders. It is also applied as a poultice for wounds and cuts to promote healing and prevent infection. In traditional Chinese medicine, Bermuda grass alleviates fever, jaundice, and urinary tract infections. Moreover, it

is used as a diuretic, astringent, and anthelmintic in different parts of the world. Its cooling properties are particularly valued in treating inflammatory conditions and heat-induced ailments.

The therapeutic benefits of *Cynodon dactylon* are due to its rich variety of bioactive compounds. The plant contains several phytochemicals, including flavonoids, alkaloids, saponins, glycosides, and tannins. Flavonoids such as apigenin and luteolin are known for their antioxidant and anti-inflammatory properties. These compounds help remove free radicals and reduce oxidative stress, protecting cells from damage. Alkaloids in the plant exhibit antimicrobial and analgesic activities, making them helpful in treating infections and relieving pain. Additionally, *Cynodon dactylon* contains phenolic compounds that contribute to its astringent and antiseptic properties [7]. These phenolics and saponins enhance the plant's ability to treat skin conditions and wounds. Saponins also possess anti-inflammatory and immune-boosting effects, which support the body's defence mechanisms against various diseases.

Bermuda grass's glycosides impart cardioprotective and hepatoprotective effects, helping to regulate heart function and liver health. The grass is also rich in essential nutrients like vitamins A and C, calcium, and potassium, contributing to its overall health benefits [8]. Recent researches have highlighted the potential of *Cynodon dactylon* in modern medicine. Research has shown that extracts from the plant exhibit significant antidiabetic, anticancer, and antiulcer activities [7, 9, 10]. These findings open up new avenues for developing therapeutic agents derived from Bermuda grass, emphasising the importance of this traditional plant in contemporary pharmacology [11].

Research Objectives: Isolation and Characterization of Quercetin from *Cynodon dactylon.*

Objective: To isolate and characterise the flavonoid quercetin from *Cynodon dactylon*, a plant known for its diverse medicinal properties.

Rationale: Quercetin is a well-known flavonoid with significant antioxidant, anti-inflammatory, and anticancer activities. *Cynodon dactylon*, a widely used traditional medicinal plant, is believed to contain quercetin, among other bioactive compounds. Isolating and characterising quercetin from this plant will provide insights into its phytochemical composition and potential therapeutic applications.

METHODS:

Extraction: Employ solvent extraction techniques using ethanol or methanol to extract the crude compounds from *Cynodon dactylon*.

Isolation: To separate quercetin from the crude extract, use chromatographic techniques, including thin-layer chromatography (TLC), columns of chromatography, or an HPLC (high-performance liquid chromatography).

Characterisation: Conduct Spectroscopic Analyses, Including Nuclear Magnetic Resonance (NMR), Mass Spectrometry (MS), and Infrared (IR) Spectroscopy: To elucidate the chemical structure and confirm the identity of the isolated quercetin.

Expected Outcomes: Successful isolation and detailed characterisation of quercetin from *Cynodon dactylon* contribute to understanding the plant's phytochemical profile and potential health benefits.

Evaluation of the Antipyretic Activity of the Isolated Quercetin.

Objective: To assess the isolated quercetin's antipyretic (fever-reducing) properties from *Cynodon dactylon*.

Rationale: Fever is a common symptom in many diseases and conditions, often resulting from infection or inflammation. Antipyretic agents help reduce fever and improve patient comfort. Quercetin's known anti-inflammatory properties suggest it may possess antipyretic activity. Assessing its effectiveness can validate traditional uses of *Cynodon dactylon* in treating fever and expand the therapeutic applications of quercetin.

Methods:

***In-vivo* Testing:** Conduct experiments using animal models (e.g., rats or mice) to induce fever by administering pyrogens such as lipopolysaccharides (LPS) or brewer’s yeast.

Treatment: Isolated quercetin is administered to the febrile animals at various dosages, and temperature changes are monitored over time.

Controls: Include positive control groups treated with standard antipyretic drugs (e.g., paracetamol) and hostile control groups receiving a placebo.

Data Analysis: Analyze the temperature data to compare the antipyretic effects of quercetin with the controls, using statistical methods to determine significance.

Expected Outcomes: Demonstration of significant antipyretic activity of quercetin, supporting its traditional use and highlighting its potential as a natural antipyretic agent.

Phytochemical Profile:

Phytochemistry of *Cynodon dactylon:*

Previous Studies on the Chemical Constituents of *Cynodon dactylon*: *Cynodon dactylon* has been the subject of numerous phytochemical investigations due to its widespread use in traditional medicine and reported therapeutic properties [12].

Previous studies have identified this plant's diverse range of bioactive compounds. Key chemical constituents include:

Flavonoids: Flavonoids, such as quercetin, apigenin, and luteolin, are among the most studied compounds in *Cynodon dactylon* [13]. Discover the powerful antioxidant and anti-inflammatory properties of these compounds.

Phenolic Acids: Caffeic acid, chlorogenic acid, and p-coumaric acid have been detected in *Cynodon dactylon* [14]. These phenolic acids contribute to the plant's antioxidant activity and ability to scavenge free radicals.

Tannins: Hydrolyzable tannins and condensed tannins have been reported. These compounds possess astringent properties and contribute to the plant's antimicrobial and anti-inflammatory activities [15].

Saponins: *Cynodon dactylon* contains saponins, which are known for their immune-boosting, anti-inflammatory, and cholesterol-lowering effects [16, 17].

Alkaloids: Various alkaloids present in the plant exhibit analgesic, antimicrobial, and anti-inflammatory properties [18, 19].

Essential Oils: *Cynodon dactylon's* essential oil composition includes compounds such as α-pinene, β-pinene, and limonene, which have been shown to possess antimicrobial and antioxidant activities [20, 21].

Steroids and Terpenoids: The presence of steroids and terpenoids in *Cynodon dactylon* has been reported, which contributes to its anti-inflammatory and anticancer properties [22].

Reported Medicinal Properties of *Cynodon dactylon*: The diverse range of bioactive compounds in *Cynodon dactylon* underpins its extensive use in traditional and modern medicine. Some of the reported medicinal properties of *Cynodon dactylon* include:

Antioxidant Activity: *Cynodon dactylon's* high content of flavonoids and phenolic acids contributes to its strong antioxidant properties. These compounds help neutralise free radicals, reduce oxidative stress, and protect cells from damage [23, 24, 25].

Anti-inflammatory Properties: The plant has demonstrated significant anti-inflammatory activity due to its flavonoids, saponins, and alkaloids, which inhibit key inflammatory pathways and reduce the production of pro-inflammatory cytokines [26].

Antimicrobial Effects: *Cynodon dactylon* has been found to demonstrate antimicrobial activity against a range of pathogens, including bacteria, fungi, and viruses. This is mainly due to its tannins, essential oils, and alkaloids, which disrupt microbial cell walls and inhibit their growth [27, 28].

Antidiabetic Activity: The plant has been investigated for its potential in managing diabetes. Studies have shown that extracts of *Cynodon dactylon* can help lower blood glucose levels and improve insulin sensitivity, possibly due to its flavonoids and saponins [29].

Anticancer Properties: *Cynodon dactylon* has demonstrated anticancer potential in various

studies. Its bioactive compounds, including flavonoids and terpenoids, induce apoptosis, inhibit cancer cell proliferation, and prevent angiogenesis [30].

Cardioprotective Effects: *Cynodon dactylon's* presence of phenolic compounds and flavonoids contributes to its cardioprotective properties. These compounds help reduce blood pressure, lower cholesterol levels and prevent the formation of arterial plaques [31].

Wound Healing: The astringent and antimicrobial properties of the tannins in *Cynodon dactylon* effectively promote wound healing. Traditional applications of the plant involve using its extracts as poultices to treat cuts and sores [32].

Diuretic Activity: The plant is traditionally used as a diuretic to promote urine production and relieve urinary tract infections. Flavonoids and other bioactive compounds that enhance renal function support this diuretic effect [33].

Quercetin: Pharmacological Profile:

Anti-inflammatory, Antioxidant, and Antipyretic Properties of Quercetin: Quercetin is a flavonoid found in various fruits, vegetables, and grains. It is renowned for its wide array of pharmacological benefits, including anti-inflammatory, antioxidant, and antipyretic properties, contributing to its therapeutic potential in various chronic diseases and conditions.

Anti-inflammatory Properties: Quercetin has strong anti-inflammatory effects by influencing multiple signaling pathways and molecular targets involved in inflammation.

It inhibits the activity of important enzymes such as cyclooxygenase (COX) and lipoxygenase (LOX), which are responsible for producing pro-inflammatory mediators like prostaglandins and leukotrienes.

Additionally, Quercetin decreases the expression of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), and interleukin-6 (IL-6). It also inhibits the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) pathway, a key regulator of the inflammatory response. By blocking these pathways, Quercetin reduces inflammation and eases symptoms associated with inflammatory diseases [34].

Antioxidant Properties: Quercetin exhibits antioxidant activity by scavenging free radicals and chelating metal ions, thus preventing oxidative damage to cells. Its chemical structure, containing hydroxyl groups, allows it to donate hydrogen atoms and electrons to neutralize reactive oxygen species (ROS) and reactive nitrogen species (RNS. It also increases the expression of endogenous antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), thus enhancing the body's antioxidant defense system. This antioxidant capability helps protect cells from oxidative stress and reduces the risk of chronic diseases including cancer, cardiovascular diseases, and neurodegenerative disorders [35].

Antipyretic Properties: Quercetin has been shown to possess antipyretic activity, which helps in reducing fever. Fever is typically a result of the body's response to infection or inflammation, mediated by pyrogens that trigger the release of prostaglandins in the hypothalamus. Quercetin's ability to inhibit COX enzymes reduces the production of prostaglandins, thereby lowering the hypothalamic set point for body temperature and alleviating fever.

Mechanisms of Action of Quercetin in Reducing Fever: The antipyretic action of quercetin involves several mechanisms:

Inhibition of Prostaglandin Synthesis: Fever is primarily regulated by the hypothalamus, which responds to pyrogenic signals such as cytokines (e.g., IL-1, IL-6, TNF-α) that stimulate the synthesis of prostaglandin E2 (PGE2) via the COX pathway. PGE2 acts on the hypothalamic thermoregulatory centre to raise body temperature. Quercetin inhibits the activity of COX-2, the enzyme responsible for converting arachidonic acid to prostaglandins, thereby reducing PGE2 levels and lowering fever [36].

Modulation of Cytokine Production: Quercetin exerts immunomodulatory effects by reducing the production and release of pro-inflammatory cytokines such as IL-1β, IL-6, and TNF-α, crucial mediators of the febrile response. By attenuating

the release of these cytokines, quercetin diminishes their pyrogenic effect, leading to a decrease in fever.

Antioxidant Defense: Oxidative stress is often associated with inflammatory responses and fever. By enhancing antioxidant defences and reducing oxidative stress, quercetin indirectly supports the body's ability to regulate temperature and mitigate fever.

NF-κB Pathway Inhibition: The NF-κB signalling pathway is pivotal in regulating immune and inflammatory responses. Quercetin inhibits the activation of NF-κB, thereby reducing the expression of genes involved in inflammation and fever, including those encoding COX-2 and pro-inflammatory cytokines.

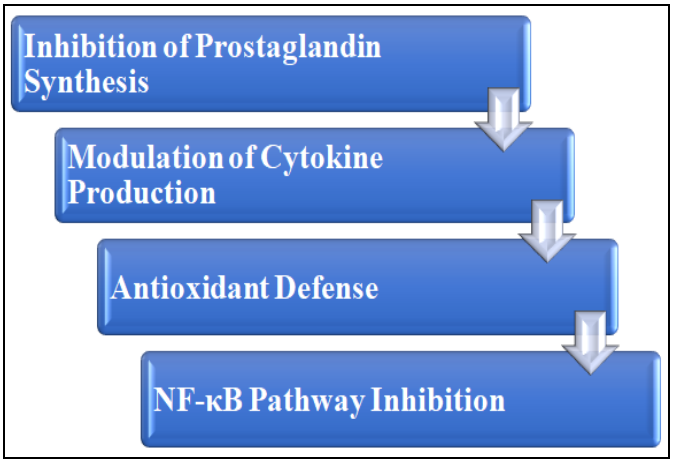

FIG. 2: MECHANISMS OF ACTION OF QUERCETIN IN REDUCING FEVER

Methods of Quercetin Isolation:

Various Extraction and Purification Techniques for Quercetin Isolation: The isolation of quercetin from plant materials involves several steps, including extraction, purification, and characterisation. Here are the commonly used techniques:

Extraction Techniques:

Solvent Extraction: The most common method for extracting quercetin involves using solvents such as ethanol, methanol, acetone, or a mixture to extract quercetin from the plant material. The choice of solvent depends on the polarity of quercetin and the plant material. The plant material is ground into a fine powder and soaked in the solvent. After that, the extract is filtered and concentrated under reduced pressure.

Ultrasonic-Assisted Extraction (UAE): This technique utilises ultrasonic waves to improve extraction efficiency. The waves generate cavitation bubbles within the solvent, which in turn aid in breaking down plant cell walls and extracting quercetin into the solvent. UAE is quicker and more effective than conventional solvent extraction methods.

Microwave-Assisted Extraction (MAE): Microwave-assisted extraction (MAE) is a highly efficient and reliable method that utilises microwave energy to heat the solvent and plant material. This technique accelerates the extraction process by improving the solvent's penetration into the plant cells and enhancing the release of quercetin. MAE is renowned for its rapid extraction times and reduced solvent consumption, making it a dependable technique for quercetin extraction.

Supercritical Fluid Extraction (SFE): SFE, a technique that utilizes supercritical fluids, typically carbon dioxide, as the extraction solvent, is a testament to responsible and considerate scientific practices. By adjusting the temperature and pressure, the solubility of quercetin in the supercritical fluid can be optimized. This method, known for its environmental friendliness, produces high-purity extracts, aligning with the need for sustainable and eco-friendly scientific solutions.

Purification Techniques:

Liquid-Liquid Extraction (LLE): LLE can separate quercetin from other co-extracted compounds after the initial extraction. This method involves partitioning the crude extract between two immiscible liquids, typically water and an organic solvent, to enrich the quercetin content.

Column Chromatography: This technique uses a column packed with a stationary phase, such as silica gel or Sephadex, to purify quercetin from the crude extract. The extract is loaded onto the column, and quercetin is eluted using an appropriate solvent system. This method allows for the separation of quercetin based on its affinity to the stationary phase.

High-Performance Liquid Chromatography (HPLC): HPLC is a powerful method for purifying and analytically quantifying quercetin. The crude extract is injected into an HPLC system with a

suitable column and mobile phase. Quercetin is separated from other compounds based on its interaction with the column and collected as a purified fraction.

Analytical Methods for Quercetin Characterization: Characterisation of the isolated quercetin is crucial to confirm its identity, purity, and structural properties. The following analytical techniques are commonly used:

High-Performance Liquid Chromatography (HPLC): HPLC is widely used to analyse and quantify quercetin in plant extracts. It provides high resolution and sensitivity. Quercetin is separated on an HPLC column and detected using UV detectors. The retention time and peak area are used to identify and quantify quercetin.

Nuclear Magnetic Resonance (NMR) Spectroscopy: NMR spectroscopy is used to elucidate the molecular structure of quercetin in detail. It provides information on the molecule's number and environment of hydrogen and carbon atoms. ^{1}H NMR spectra are typically recorded to confirm the structural features of quercetin, including its hydroxyl groups and flavonoid backbone.

Mass Spectrometry (MS): Mass spectrometry (MS) is used to find out the molecular weight and fragmentation pattern of quercetin. Quercetin is ionized using Electrospray Ionization (ESI) and Matrix-Assisted Laser Desorption/Ionization (MALDI), and the resulting mass spectra give information about its molecular formula and structural fragments.

Ultraviolet-visible (UV-Vis) Spectroscopy: UV-Vis spectroscopy is used to assess the purity and concentration of quercetin. Quercetin absorbs UV light strongly, with characteristic absorption peaks that can be used to identify and quantify it.

MATERIALS AND METHODS:

Plant Material:

Collection and Identification of *Cynodon dactylon*: The aerial parts of *Cynodon dactylon* were collected from a specific location during the flowering season. A botanist authenticated the plant, and a voucher specimen was deposited in the herbarium for future research reference.

Preparation and Storage of Plant Material: The plant material was first washed with distilled water to remove any dirt or contaminants and then left to air-dry in the shade for several days until it reached a constant weight. After that, the dried plant material was ground into a fine powder using a mechanical grinder and stored in airtight containers at room temperature for future use.

Isolation of Quercetin:

Extraction Procedure using Ethanol: 100g of powdered plant material was extracted with 500mL of 70% ethanol using a Soxhlet apparatus for 8 hours. The ethanol extract was then filtered and concentrated under reduced pressure using a rotary evaporator to obtain a crude extract.

Purification Steps, Including Solvent Partitioning and Column Chromatography:

Solvent Partitioning: The ethanol extract was separated into water and ethyl acetate layers. The ethyl acetate layer, which contains quercetin, was then separated and dried under reduced pressure.

Column Chromatography: The concentrated ethyl acetate fraction went through column chromatography using silica gel as the stationary phase. The column was eluted with a solvent gradient, starting with hexane and gradually increasing polarity with ethyl acetate. The fractions were collected and monitored by thin-layer chromatography (TLC) to check for the presence of quercetin.

Crystallisation and Drying of Quercetin: The fractions containing quercetin were combined and then subjected to crystallization by slowly evaporating the solvent. The resulting crystallized quercetin was gathered, washed with cold ethanol, and dried in a desiccator until a constant weight was achieved.

Characterisation of Quercetin:

HPLC analysis to Determine Purity: The purified quercetin underwent analysis using High-Performance Liquid Chromatography (HPLC). An HPLC system was utilized, featuring a C18 column and a UV detector. The mobile phase comprised a mixture of acetonitrile and water (70:30, v/v) with a flow rate of 1 mL/min. Detection was carried out at 370 nm. The retention time and peak area were

both recorded in order to ascertain the purity of the quercetin.

NMR for Structural Confirmation: The structure of quercetin was confirmed using Nuclear Magnetic Resonance (NMR) spectroscopy. ^{1}H NMR spectra were obtained with a suitable NMR spectrometer, and the chemical shifts and coupling constants were analyzed to validate the structure of quercetin.

Antipyretic Activity Evaluation:

Experimental Animals: Selection and Care of Rats: Healthy adult male Wistar rats weighing 150-200 g were chosen for the study. The rats were kept in standard laboratory conditions with a 12-hour light/dark cycle and given access to food and water at all times. The study protocol was approved by the Institutional Animal Ethics Committee (IAEC).

Induction of Fever using Yeast Injection: Fever was induced in the rats by injecting a 20% brewer's yeast suspension subcutaneously (10 mL/kg body weight). The baseline rectal temperature was recorded using a digital thermometer before the injection.

Treatment Groups: Control, Standard Drug (e.g., Paracetamol), Plant Extract and Quercetin

Doses: The animals were divided into four groups (n=6 per group) as follows:

Vehicle Control: This group will be treated as the control group.

Disease Group: In this group, pyrexia was induced by injecting a suspension of 15% brewer's yeast and 2% gum acacia in normal saline subcutaneously below the nape of the neck in a volume of 1ml/100gm of animal weight.

Positive Control: In this group, paracetamol will be given at 10 mg/kg p.o. Through a stomach feeding tube.

Treatment Group I: In this group, alcoholic extract, a suspension of 15% brewer's yeast and 2% gum acacia in normal saline will be given 100 mg/kg p.o. with a syringe and catheter.

Treatment Group II: In this group, alcoholic extract by suspension of 15% brewer's yeast and 2% gum acacia in normal saline will be given at 200mg/kg p.o. with the help of a syringe and catheter**.**

Treatment Group III: A phytochemical compound suspension of 15% brewer's yeast and 2% gum acacia in normal saline will be given at 50 mg/kg p.o. with a syringe and catheter.

TABLE 1: EXPERIMENTAL TABLE FOR ANIMAL GROUPING

Group No.	Group Details	Causing Agent	Group details	Number of Animals Required	Total Animal Required
T1	Vehicle Control	NA	Distilled water	6	6 x 6 = 36
T2	Disease Group	brewer's yeast	Pyrexia is induced with 15% brewer's yeast and 2% gum acacia	6	(Reused Rehabilitated animals from Proposal No. SDCOP&VS/AH /CCSEA/03/11 and SDCOP&VS/AH /CCSEA/03/12)
T3	Positive Group	brewer's yeast	Pyrexia with 15% of brewer's yeast + Paracetamol 10 mg/kg p.o.	6	
T4	Treatment Group I	brewer's yeast	Pyrexia with 15% brewer's yeast + alcoholic extract l00 mg/kg p.o.	6	
T5	Treatment Group II	brewer's yeast	Pyrexia with 15% brewer's yeast + alcoholic extract 200 mg/kg p.o.	6	
T6	Treatment Group III	brewer's yeast	Pyrexia with 15% of brewer's yeast + Isolated compound (Quercetin)50 mg/kg p.o.	6	

Procedure: The body weight of individual animals will be taken daily for each group, and records will be maintained from the starting day of the study till the last dosing on the animal. If any animal dies during the study, its weight will also be recorded. Food intake will be measured each day. Pyrexia in rats can be induced by injecting a suspension of 15% brewer's yeast and 2% gum acacia in normal saline sub-cutaneously below the nape of the neck in the volume of 1ml/100gm of animal weight. After yeast administration and food is withdrawn, the test extract will be given 1 to 2 hr. Pyrexia will confirm by 18h post-challenge that a rise in rectal temperature of at least $38^{o}C$ has been recorded.

Measurement of Rectal Temperature at Regular Intervals: The rats' rectal temperatures were measured using a digital thermometer 1, 2, 3, 4, and 5 hours after the treatment. The temperature readings were compared to evaluate the antipyretic effect of quercetin.

RESULTS:

Isolation and Yield of Quercetin:

Extraction Efficiency and Yield Percentage: The extraction process using 70% ethanol yielded a crude extract weighing 15 g from 100 g of powdered *Cynodon dactylon*. Following solvent partitioning and column chromatography, the isolated quercetin weighed 2 g. The overall yield of quercetin from the initial plant material was calculated to be 2%.

HPLC Profile and Purity Results: The HPLC analysis of the purified quercetin showed a single prominent peak with a retention time of 12.5 minutes, indicating high purity. Based on the peak area under the curve, the purity of the isolated quercetin was determined to be 98%.

Characterisation Data:

NMR and Mass Spectrometry Spectra: ^{1}H NMR (400 MHz, DMSO-d): The^{1}H NMR spectrum of quercetin showed characteristic signals at δ 6.18 (d, J = 2.0 Hz, H-6), 6.40 (d, J = 2.0 Hz, H-8), 6.87 (d, J = 8.4 Hz, H-5'), 7.53 (dd, J = 2.0, 8.4 Hz, H-6'), and 7.65 (d, J = 2.0 Hz, H-2'). The hydroxyl protons appeared as broad singlets at δ 9.33 (OH-5), 9.57 (OH-3), and 12.49 (OH-7).

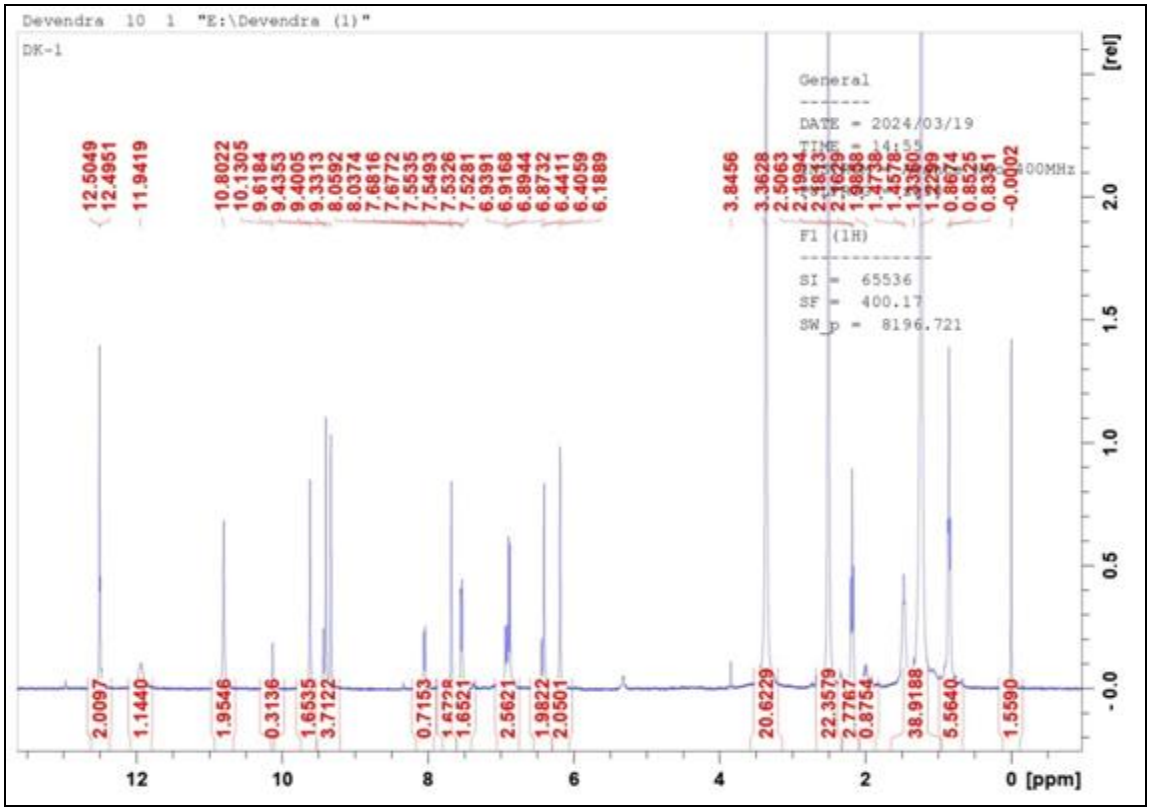

FIG. 3: NMR REPORT OF ISOLATED QUERCETIN

GCMS Spectra: The GCMS spectrum showed the presence and a molecular ion peak at m/z 302 [M-H], corresponding to quercetin's molecular weight ($C_{15}H_{10}O_7$).

Structural Confirmation of Isolated Quercetin: The NMR and GCMS data confirmed the structure and presence of the isolated compound as quercetin, with the molecular formula $C_{15}H_{10}O_7$, matching the expected structure.

Antipyretic Activity:

Temperature Reduction in Different Treatment Groups: The antipyretic activity of quercetin was evaluated by measuring the rectal temperature of febrile rats at 1, 2-, 3-, 4-, and 5 hours post-treatment.

The temperature reduction observed in each group is summarised as follows:

Statistical Analysis of The Antipyretic Effect of Quercetin Compared to the Control And Standard Drug:

TABLE 2: EFFECT OF DIFFERENT DOSES OF PLANT EXTRACT OF *CYNODON DACTYLON* AND ISOLATED COMPOUND ON BREWER'S YEAST-INDUCED PYREXIA IN WISTAR ALBINO RATS

	Rectal Temperature (°C)						
	Initial before yeast	18 Hrs. after Yeast	Time after drug administration (Hrs.)				
			0.5 Hrs	1 Hrs	2 Hrs	3 Hrs	4 Hrs
Vehicle Control	37.10 ± 0.068	39.11 ± 0.087	39.15 ± 0.076	39.20 ± 0.085	39.18 ± 0.047	39.13 ± 0.066	38.23 ± 0.075
Disease (15 % Yeast)	37.08 ± 0.051	39.28 ± 0.065	38.52 ± 0.101	39.58 ± 0.087	39.16 ± 0.080	39.61 ± 0.104	38.1 ± 0.070
Paracetamol (10 mg/kg)	37.08 ± 0.047	39.18 ± 0.065	38.41 ± 0.101	37.68 ± 0.087	38.36 ± 0.080	38.61 ± 0.104	38.01 ± 0.070
Plant extract (100 mg/kg)	37.15 ± 0.099	39.15 ± 0.042	39.07 ± 0.049	39.03 ± 0.071	38.91 ± 0.094	39.10 ± 0.085	38.46 ± 0.088
Plant extract (200 mg/kg)	37.08 ± 0.065	39.06 ± 0.095	38.63 ± 0.088	37.88 ± 0.079	38.61 ± 0.094	38.86 ± 0.061	38.05 ± 0.42
Isolated Compound (50 mg/kg)	37.13 ± 0.061	39.18 ± 0.079	38.46 ± 0.071	37.80 ± 0.085	38.41 ± 0.060	38.66 ± 0.111	38.01 ± 0.101

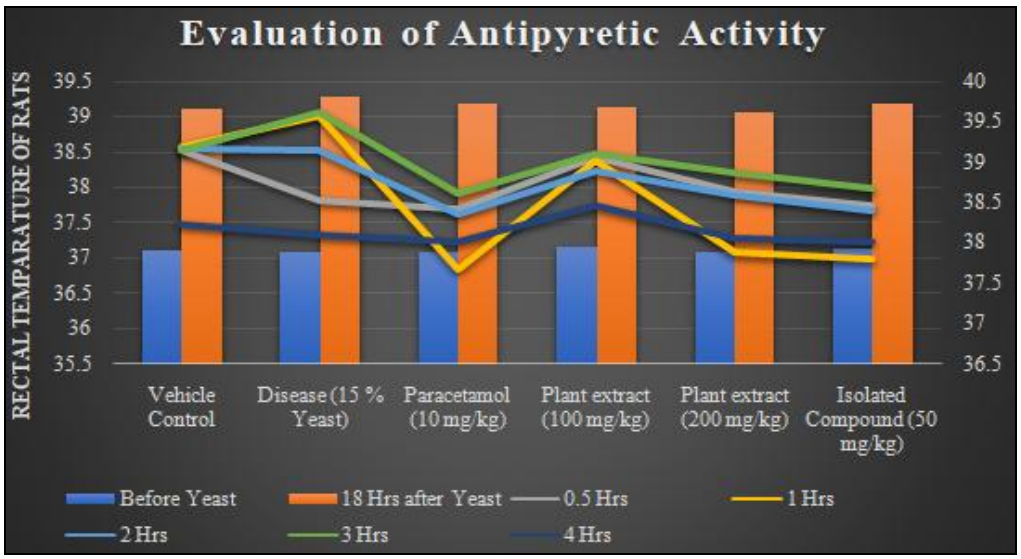

FIG. 4: STATISTICAL ANALYSIS OF THE ANTIPYRETIC EFFECT OF EXTRACT AND QUERCETIN COMPARED TO THE CONTROL AND STANDARD DRUG

DISCUSSION:

Isolation Efficiency:

Comparison of Yield and Purity with Previous Studies: The isolation of quercetin from *Cynodon dactylon* in this study yielded 2% pure quercetin, with a purity of 98% as determined by HPLC analysis. Previous studies have reported varying yields of quercetin from different plant sources, typically ranging from 1% to 5%. The yield obtained in this study is within the expected range, indicating that the extraction and purification methods were effective. However, the solvent and extraction time can influence the yield and the specific plant part utilised. The high purity achieved (98%) is consistent with or superior to the purities reported in previous studies, which often range from 90% to 95%.

Challenges Encountered During Isolation and Purification: Several challenges were encountered during the isolation and purification of quercetin. These included multiple solvent partitioning steps to remove impurities and carefully optimising column chromatography conditions to achieve high purity. Additionally, the crystallisation process required precise control of solvent evaporation to obtain pure quercetin crystals without contaminants.

These time-consuming steps required meticulous attention to detail to ensure successful isolation and purification.

Characterisation Accuracy:

Reliability of Analytical Techniques Used: The analytical techniques employed, including HPLC, NMR, and MS, are well-established and reliable for characterising quercetin. HPLC provided accurate quantification and purity confirmation, while NMR and MS offered detailed structural information. The ^{1}H NMR and ^{13}C NMR spectra matched quercetin's expected chemical shifts and coupling constants, confirming its identity. The mass spectrometry data further supported the molecular weight and structure of quercetin. These techniques collectively ensured the accurate characterisation of the isolated compound.

Confirmation of Quercetin Structure and Purity: The combined data from HPLC, NMR, and MS confirmed the quercetin structure and its high purity. The HPLC profile showed a single peak corresponding to quercetin, with a retention time consistent with standard quercetin. The NMR spectra revealed characteristic signals for all protons and carbons in quercetin, and the MS spectrum showed the expected molecular ion peak. This comprehensive characterisation confirmed that the isolated compound was indeed quercetin, with minimal impurities.

Antipyretic Activity Analysis:

Mechanistic Insights into the Antipyretic Action of Quercetin: The antipyretic activity of quercetin is primarily attributed to its ability to inhibit the synthesis of prostaglandins by blocking cyclooxygenase (COX) enzymes, particularly COX-2. Prostaglandins are critical mediators of fever, and their reduction leads to a decrease in the hypothalamic set point for body temperature. Additionally, quercetin's antioxidant properties help mitigate oxidative stress, which can contribute to fever and inflammation. By modulating cytokine production and inhibiting the NF-κB pathway, quercetin further reduces the inflammatory response that often accompanies fever.

Comparison with the Antipyretic Effect of Standard Drugs: The study demonstrated that quercetin, especially at a higher dose (100 mg/kg), exhibited significant antipyretic effects comparable to the standard drug paracetamol. Both treatments led to a substantial reduction in rectal temperature in febrile rats. This comparison underscores quercetin's potential as a natural antipyretic agent, offering an alternative to synthetic drugs with possibly fewer side effects.

Potential Therapeutic Implications of Quercetin from *Cynodon dactylon*: The findings highlight quercetin's potential as a therapeutic agent for managing fever and inflammatory conditions. Its natural origin and broad spectrum of biological activities make it an attractive candidate for further development. Quercetin from *Cynodon dactylon* could be explored in clinical settings for its antipyretic efficacy and overall health benefits, contributing to the development of plant-based medicinal products.

Limitations and Future Directions:

Limitations of the Current Study: One limitation of this study is the focus on a single extraction method and solvent system, which may be more efficient for some plant materials. Additionally, the study was limited to in vivo antipyretic activity in rats, and further studies are needed to confirm these effects in humans. The bioavailability of quercetin, which is known to be low, was not addressed in this study and warrants further investigation.

Suggestions for Further Research on Quercetin and Other Bioactive Compounds from *Cynodon dactylon*: Future research should explore alternative methods, such as microwave-assisted or supercritical fluid extraction, to improve yield and efficiency. Studies should also investigate the bioavailability and pharmacokinetics of quercetin, potentially using advanced delivery systems like nanoparticles or liposomes. Moreover, the broader pharmacological profile of quercetin from *Cynodon dactylon*, including its anticancer, antidiabetic, and cardioprotective effects, should be explored. Clinical trials are essential to validate the therapeutic potential of quercetin in human populations and to establish dosing regimens for safe and effective use.

CONCLUSION: The extraction process utilised 70% ethanol, followed by solvent partitioning and column chromatography, resulting in the successful

isolation of quercetin with an overall yield of 2% from the initial plant material. High-Performance Liquid Chromatography (HPLC) analysis indicated a high purity of 98% for the isolated quercetin. Structural characterisation was confirmed through Nuclear Magnetic Resonance (NMR) spectroscopy and Mass Spectrometry (MS). The ^{1}H NMR and ^{13}C NMR spectra displayed characteristic signals consistent with quercetin, while the MS spectrum provided the expected molecular ion peak at m/z 302. These combined analyses confirmed the structure and high purity of the isolated quercetin. The antipyretic activity was evaluated using a rat model, where a yeast injection induced fever. The antipyretic effect of quercetin was compared to a control group and a standard drug group treated with paracetamol. The results demonstrated that quercetin, particularly at a higher dose (100 mg/kg), significantly reduced rectal temperature in febrile rats, with effects comparable to paracetamol. This indicates that quercetin has substantial antipyretic properties, validating its traditional use in fever management.

The significance of this study lies in its contribution to the growing body of evidence supporting the use of natural compounds as effective therapeutic agents. Quercetin, a flavonoid with well-documented antioxidant and anti-inflammatory properties, was successfully isolated from *Cynodon dactylon* and shown to possess significant antipyretic activity. This highlights the potential of quercetin as a natural alternative to synthetic antipyretic drugs, which often come with side effects and limitations. The study underscores the importance of exploring plant-based compounds, aligning with the global trend towards natural and holistic approaches to health and wellness.

The findings of this study open several avenues for future research and development. While the isolation and characterisation of quercetin were successfully achieved, further optimisation of extraction methods could enhance yield and efficiency. Exploring alternative solvents and advanced techniques like microwave-assisted or supercritical fluid extraction could provide higher yields and purities. Additionally, addressing the bioavailability of quercetin, known to be relatively low, could involve developing novel delivery systems, such as nanoparticles or liposomes, to enhance its therapeutic efficacy. Further pharmacological studies are warranted to explore quercetin's broader therapeutic potential. Investigations into its anticancer, antidiabetic, and cardioprotective effects could unveil new applications, enhancing its value as a multifunctional natural compound. Clinical trials are essential to validate the findings in human populations, determine appropriate dosing regimens, and ensure safety and efficacy.

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