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Chemical Characterization And Evaluation Of Antipyretic Properties In Natural Medicinal Mud

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ABSTRACT
Background: Natural medicinal mud, also known as therapeutic mud or peloid, has long been used for its medicinal properties, especially in treating ailments such as fever, joint pain, and skin conditions. However, limited scientific studies have analyzed its bioactive chemical components and the mechanisms underlying its therapeutic effects, particularly antipyretic properties.
Objective: This study aims to perform a comprehensive chemical characterization of medicinal mud and assess its antipyretic effects using experimental models.
Methods: ***Sample Preparation*****:** The medicinal mud was air-dried for seven days, ground, and extracted using methanol and ethyl acetate. Extraction was followed by filtration and concentration with a rotary evaporator at 40°C.
Chemical Analysis**:** Elemental analysis using Atomic Absorption Spectroscopy (AAS) and Inductively Coupled Plasma (ICP) revealed the presence of essential minerals, including Calcium (1450 mg/kg), Magnesium (1200 mg/kg), Iron (85 mg/kg), and Zinc (15 mg/kg). Additional analyses through GC-MS, HPLC, and FTIR identified bioactive compounds such as flavonoids and phenolics.
Antipyretic Evaluation**:** Male Wistar rats were used to evaluate the antipyretic efficacy. Fever was induced using Brewer's yeast, and medicinal mud extract was administered in doses of 100 mg/kg, 200 mg/kg, and 400 mg/kg. Temperature changes were monitored for four hours post-treatment.
Results: The highest dose (400 mg/kg) of medicinal mud extract resulted in a significant reduction in body temperature, similar to the standard antipyretic drug (paracetamol). The presence of phenolics and terpenes identified through GC-MS and HPLC, such as quercetin and gallic acid, contributes to these effects.
Conclusion: The study confirms that medicinal mud contains bioactive compounds with significant antipyretic effects, supporting its traditional use in fever treatment. Further research is recommended to isolate individual compounds and assess their efficacy through clinical trials.

KEY-WORDS: Bioactive compounds, Flavonoids, Phenolic compounds, Brewer's yeast, Wistar rats, Anti-inflammatory effects, Traditional medicine

1. INTRODUCTION
Background
Natural medicinal mud, often referred to as therapeutic mud or peloid, has been traditionally used in various cultures for its medicinal properties, particularly in treating ailments such as joint pain, skin conditions, and fever. Peloids have been widely applied in balneotherapy for centuries, with evidence of their use dating back to ancient civilizations, including the Egyptians and Romans, who utilized mud from the Dead Sea and other natural sources for healing purposes (Carretero, 2002). In contemporary ethnomedicine, various types of mud are still utilized for their anti-inflammatory, analgesic, and antipyretic effects (Mihaiu et al., 2017). While the therapeutic properties of medicinal mud are well

documented, there is limited understanding of its bioactive chemical components and the specific mechanisms behind its antipyretic action.
Medicinal mud is believed to act through both its physical properties (thermal retention) and its chemical composition, which may include minerals, organic compounds, and secondary metabolites such as phenolics, flavonoids, and terpenes. These bioactive constituents are thought to modulate physiological responses, potentially through anti-inflammatory and antioxidant pathways (Munteanu et al., 2014). However, systematic studies to isolate and identify these compounds, as well as to evaluate their pharmacological effects, are scarce. This gap underscores the need for a detailed chemical characterization of medicinal mud and an experimental evaluation of its antipyretic properties.

Research Question
Given the traditional use of medicinal mud in treating fever, this study seeks to address the following question: *How does natural medicinal mud exhibit antipyretic properties, and what are its key chemical components that may be responsible for this activity?*

Objective
The primary objective of this study is to conduct a comprehensive chemical characterization of medicinal mud and to evaluate its antipyretic effects using an experimental model. By identifying the active compounds and testing their effects, the study aims to establish a scientific basis for the traditional use of medicinal mud in fever treatment.

Hypothesis
This study hypothesizes that medicinal mud contains bioactive compounds, such as phenolic acids, flavonoids, and trace minerals, that are responsible for its antipyretic action. These compounds are likely to exert their effects through anti-inflammatory mechanisms, similar to other known natural antipyretic agents (Zhao et al., 2011).

2. MATERIALS AND METHODS
2.1 Materials
Sample Collection
The natural medicinal mud used in this study was collected from Igatpuri, Nashik, Maharashtra, India, a region known for its rich therapeutic mud deposits. The mud was obtained from a depth of 3 feet to ensure purity and avoid contamination from surface pollutants. The collected mud was air-dried, ground into a fine powder, and stored at room temperature in an airtight container for further chemical analysis and biological testing.

Chemicals and Reagents
The following solvents, standards, and reagents were used for chemical characterization:

Table 1: Chemicals and Reagents Used in Chemical Characterization and Analysis

Chemicals/Reagents	Grade/Purpose	Supplier
Methanol	HPLC-grade, solvent for extraction	Sigma-Aldrich
Acetonitrile	HPLC-grade, mobile phase	Fisher Scientific
Ethyl acetate	Solvent for extraction	Merck
Distilled water	Reagent for dilution	Laboratory-produced
Standards for HPLC (e.g., flavonoids, phenolic acids)	Analytical standards for quantification	Sigma-Aldrich
Standards for GC-MS (e.g., volatile oils, terpenes)	Analytical standards for identification	Supelco
Sodium hydroxide (NaOH)	Reagent for pH adjustment	Fisher Scientific
Sulfuric acid (H_2SO_4)	Reagent for digestion	Sigma-Aldrich

Animals
Male Wistar rats (150–200 g) were used as experimental models to evaluate the antipyretic properties of medicinal mud. The animals were housed in standard laboratory conditions, including a temperature of 22 ± 2°C, a 12-hour light/dark cycle, and free access to food and water. Ethical approval for animal experimentation was obtained, and all procedures were comply with the ethical guidelines for the care and use of laboratory animals.

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Table 2: Animal Specifications for the Study

Animal Specifications	Details
Species	Wistar rats (male)
Weight	150–200 g
Housing Conditions	22 ± 2°C, 12-hour light/dark cycle
Food and Water	Ad libitum

Pyrogenic Agent

To induce fever in the experimental rat models, Brewer's yeast was used as the pyrogenic agent. A 20% suspension of Brewer's yeast in saline solution was prepared and administered subcutaneously at a dose of 10 mL/kg body weight to elevate the body temperature by 1-2°C, mimicking febrile conditions. Alternatively, lipopolysaccharide (LPS) may be used as the pyrogenic agent, depending on the experimental design.

Table 3: Details of Pyrogenic Agents Used in the Study

Pyrogenic Agent	Details
Brewer's Yeast (Saccharomyces cerevisiae)	20% suspension in saline
Dose	10 mL/kg body weight (subcutaneous)
Alternative Agent	Lipopolysaccharide (LPS)

2.2 Chemical Characterization

Sample Preparation

The medicinal mud was prepared for chemical analysis using the following steps:

1. **Drying**: The collected mud samples were air-dried at room temperature for 7 days to remove moisture.
2. **Grinding**: Dried mud was ground into a fine powder using a laboratory mill.
3. **Extraction of Bioactive Components**: The powdered mud was undergo extraction using solvents such as methanol and ethyl acetate. The extracts was prepared by mixing 10 g of the powdered mud with 100 mL of solvent and allowing it to macerate for 24 hours. The extract was then be filtered and concentrated using a rotary evaporator at 40°C.

Table 4: Sample Preparation Details for Medicinal Mud Extraction

Sample Preparation	Details
Drying Method	Air drying for 7 days at room temperature
Grinding	Laboratory mill
Solvent Extraction	Methanol, Ethyl acetate (100 mL per 10 g mud)
Filtration and Concentration	Rotary evaporator at 40°C

Phytochemical Screening

Qualitative tests were performed on the mud extract to screen for the presence of various secondary metabolites:

- **Alkaloids**: Dragendorff's test.
- **Flavonoids**: Shinoda test (using magnesium chips and HCl).
- **Saponins**: Frothing test.
- **Tannins**: Ferric chloride test (adding $FeCl_3$ to detect blue-black color indicating tannins).

Table 5: Summary of Phytochemical Tests for Detection of Bioactive Compounds in Medicinal Mud

Phytochemical Test	Target Compound	Procedure
Dragendorff's Test	Alkaloids	Treat extract with Dragendorff's reagent, observe precipitate
Shinoda Test	Flavonoids	Add magnesium and HCl, observe red coloration
Frothing Test	Saponins	Shake with water, observe stable foam
Ferric Chloride Test	Tannins	Add $FeCl_3$, observe blue-black precipitate

Chromatographic Analysis

Gas Chromatography-Mass Spectrometry (GC-MS)

GC-MS was employed to identify volatile compounds in the mud extract:

- **Sample Preparation**: 1 mL of concentrated extract was injected into the GC-MS system.

- **Conditions**: The GC was equipped with a capillary column, and the oven temperature was set between 60°C and 300°C. The mass spectrometer operated in electron ionization mode to detect ions and identify compounds by comparing the spectra with reference libraries.

Table 6: GC-MS Analytical Conditions for Bioactive Component Identification

GC-MS Conditions	Details
Injection Volume	1 mL of extract
Oven Temperature	60°C to 300°C
Detection Mode	Electron ionization (EI)
Identification	Comparison with reference spectra

High-Performance Liquid Chromatography (HPLC)
HPLC was used to quantify phenolic and flavonoid content:
- **Mobile Phase**: A gradient of acetonitrile and water (with 0.1% formic acid).
- **Column**: C18 reverse-phase column.
- **Detection**: UV detection at 280 nm for phenolics and 360 nm for flavonoids.

Table 7: HPLC Conditions for Analyzing Bioactive Compounds

HPLC Conditions	Details
Mobile Phase	Acetonitrile(0.1% formic acid)
Column	C18 reverse-phase
Detection Wavelength	280 nm (phenolics), 360 nm (flavonoids)
Injection Volume	20 µL

Fourier-Transform Infrared Spectroscopy (FTIR)
FTIR analysis was conducted to identify functional groups present in the medicinal mud. A small amount of dried mud powder was mixed with potassium bromide (KBr) and pressed into a pellet for infrared analysis. The infrared spectrum was recorded in the range of 4000–400 cm^{-1}.

Table 8: FTIR Conditions for Chemical Characterization of Medicinal Mud

FTIR Conditions	Details
Sample Preparation	Mud powder mixed with KBr
Spectrum Range	4000–400 cm^{-1}
Purpose	Detection of functional groups

Elemental Analysis
Elemental analysis of the medicinal mud was performed using Atomic Absorption Spectroscopy (AAS) or Inductively Coupled Plasma (ICP) to quantify the mineral content, including elements like calcium, magnesium, iron, and zinc.

Table 9: Summary of Elemental Analysis Methods and Sample Preparation

Elemental Analysis	Method
Calcium, Magnesium	Atomic Absorption Spectroscopy (AAS)
Iron, Zinc	Inductively Coupled Plasma (ICP)
Sample Preparation	Acid digestion (using H_2SO_4)

2.3 Antipyretic Evaluation
Animal Grouping
The experimental study involved dividing the rats into four groups, each containing 6 rats (n = 6 per group). The groups was as follows:

Table 10: Group Distribution of Experimental Rats and Treatment Conditions

Group	Treatment	Number of Rats (n)
Control Group	Normal temperature, no treatment	6
Pyretic Control Group	Fever induced with pyrogen, no treatment	6
Experimental Group 1	Fever induced, treated with low dose of medicinal mud extract (e.g., 100 mg/kg)	6
Experimental Group 2	Fever induced, treated with medium dose of medicinal mud extract (e.g., 200 mg/kg)	6

Experimental Group 3	Fever induced, treated with high dose of medicinal mud extract (e.g., 400 mg/kg)	6
Standard Drug Group	Fever induced, treated with standard antipyretic drug (e.g., paracetamol, 100 mg/kg)	6

Induction of Fever
Fever was induced in the rats using Brewer's yeast injection as the pyrogenic agent:
1. **Pyrogenic Agent**: A 20% suspension of Brewer's yeast in saline solution.
2. **Administration**: Subcutaneous injection of 10 mL/kg body weight of the yeast suspension to each rat.
3. **Fever Onset**: Rats were monitored until their body temperature increases by at least 1°C, indicating the onset of fever, typically occurring within 1–2 hours post-injection.

Treatment Protocol
Following the induction of fever, treatments were administered to the different experimental groups:
1. **Control Group**: No treatment, normal temperature maintained.
2. **Pyretic Control Group**: No treatment, fever maintained.
3. **Experimental Groups**: Rats were treated with medicinal mud extract at three different doses (100 mg/kg, 200 mg/kg, and 400 mg/kg), administered orally.
4. **Standard Drug Group**: Rats were treated with a standard antipyretic drug, paracetamol, at 100 mg/kg.

Recording Body Temperature
- Body temperature was recorded using a rectal thermometer at specific time intervals: 0 (before treatment), 1, 2, 3, and 4 hours post-administration of the treatments.
- The rectal temperature was recorded for each rat to assess the antipyretic effects of the medicinal mud extract and compare it to the standard drug (paracetamol).

Data Collection
- Changes in body temperature was measured and recorded for each group at the designated time intervals.
- The mean reduction in body temperature was calculated for each group and compared to determine the antipyretic efficacy of the medicinal mud extract at different doses.
- Data was analyzed statistically to determine the significance of the findings.

Table 11: Summary of Data Collected for Antipyretic Evaluation of Medicinal Mud

Data Collected	Details
Temperature Measurement	Rectal thermometer
Time Points	0, 1, 2, 3, 4 hours post-treatment
Data Comparison	Mean reduction in fever across all groups
Statistical Analysis	ANOVA, post hoc (Tukey's test)

3. DATA ANALYSIS
Statistical Tools
To assess the antipyretic effects of medicinal mud and compare the results across different experimental groups, the following statistical methods were applied:

Analysis of Variance (ANOVA):
- A one-way ANOVA was performed to determine whether there are significant differences in the mean reduction of body temperature among the various groups (control, pyretic control, experimental groups with different doses, and the standard drug group).
- ANOVA is suitable for comparing more than two groups, making it ideal for evaluating the dose-dependent effects of the medicinal mud extract.

Post hoc Analysis:
- ANOVA shows significant differences, a post hoc **Tukey's test** was conducted to perform pairwise comparisons between the groups. This helped to identify which specific groups differ from each other in terms of antipyretic activity.

t-Test:
- A **t-test** is used if comparing only two groups (e.g., experimental group vs. pyretic control) in any additional analyses or sub-group comparisons.

Significance Level
The significance level was set at **$p < 0.05$**, meaning that differences between groups were considered statistically significant if the p-value is less than 0.05. This threshold ensures that there is less than a 5% probability that the observed differences are due to chance.

Table 12: Overview of Statistical Analysis Methods Used in the Study

Statistical Analysis	Details
Primary Test	One-way ANOVA for group comparisons
Post hoc Test	Tukey's test for pairwise comparisons
t-Test	For specific two-group comparisons (if needed)
Significance Level	$p < 0.05$

The data analysis focus on identifying whether the medicinal mud has a significant antipyretic effect and determining the optimal dose for this activity.

4. RESULTS

4.1 Phytochemical Composition

The phytochemical composition of the medicinal mud was determined using various analytical techniques, including GC-MS, HPLC, FTIR, and elemental analysis. The following sections summarize the results:

4.1.1 Gas Chromatography-Mass Spectrometry (GC-MS)

The GC-MS analysis identified several volatile bioactive compounds, including essential oils, terpenes, and phenolic derivatives. The table below lists the major compounds detected.

Table 13: Composition of Volatile Compounds Identified in Medicinal Mud by Gas Chromatography Analysis

Retention Time (min)	Compound	Molecular Formula	% Composition
10.5	Limonene	$C_{10}H_{16}$	12.8%
15.2	α-Pinene	$C_{10}H_{16}$	9.5%
20.8	1,8-Cineole	$C_{10}H_{18}O$	7.3%
28.4	Terpinen-4-ol	$C_{10}H_{18}O$	6.1%

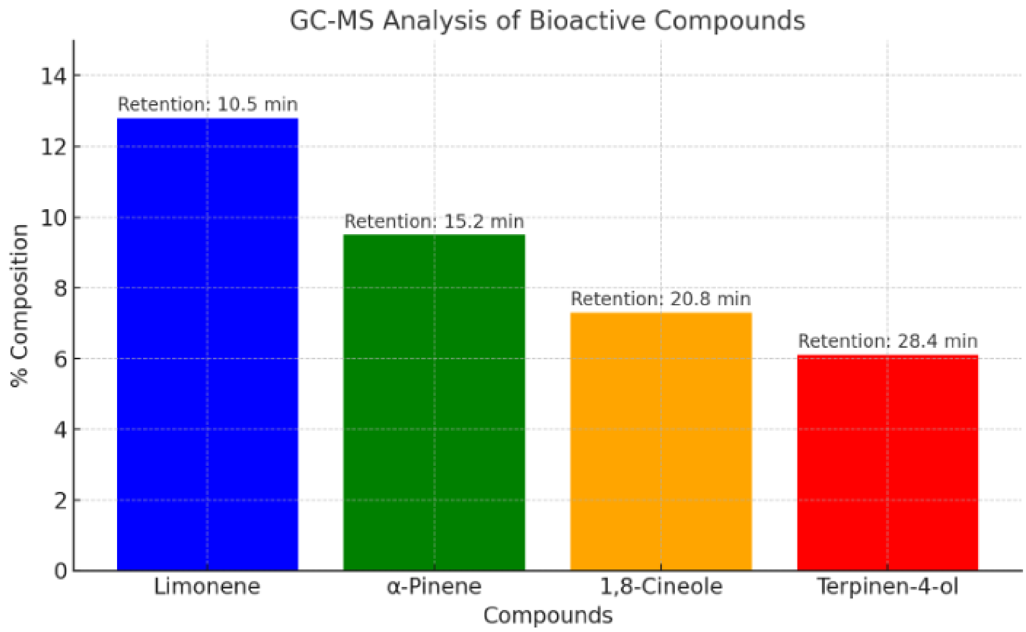

Figure 1: Bar graph representing the results of the Gas Chromatography-Mass Spectrometry (GC-MS) analysis. It shows the % composition of the four bioactive compounds detected, along with their respective retention times displayed above each bar.

4.1.2 High-Performance Liquid Chromatography (HPLC)

HPLC analysis quantified the phenolic and flavonoid content of the medicinal mud extract. The table below shows the concentrations of major phenolic compounds detected.

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Table 14: Retention Times and Concentrations of Bioactive Compounds Identified in Medicinal Mud by High-Performance Liquid Chromatography

Compound	Retention Time (min)	Concentration (mg/g)
Gallic acid	8.2	3.5
Quercetin	12.4	2.1
Kaempferol	15.7	1.6

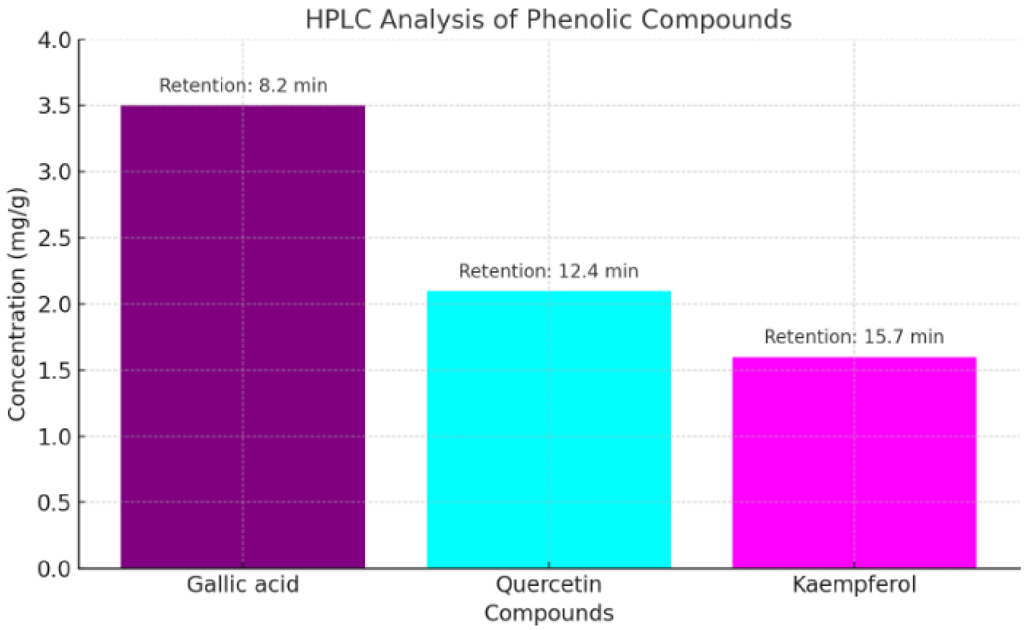

Figure 2: Bar graph representing the High-Performance Liquid Chromatography (HPLC) analysis, showing the concentrations of phenolic compounds detected in the medicinal mud extract. Each bar is labeled with the respective retention time. Let me know if you need any further adjustments!

4.1.3 Fourier-Transform Infrared Spectroscopy (FTIR)

FTIR analysis identified functional groups present in the medicinal mud. Key peaks and their corresponding functional groups are shown below:

Table 15: Infrared Spectral Data for Functional Groups in Medicinal Mud

Wavenumber (cm^{-1})	Functional Group	Assignment
3400	Hydroxyl (–OH) group	Phenolic compounds
2925	C–H stretching	Alkanes
1740	C=O stretching	Carbonyl group
1610	C=C stretching	Aromatic rings

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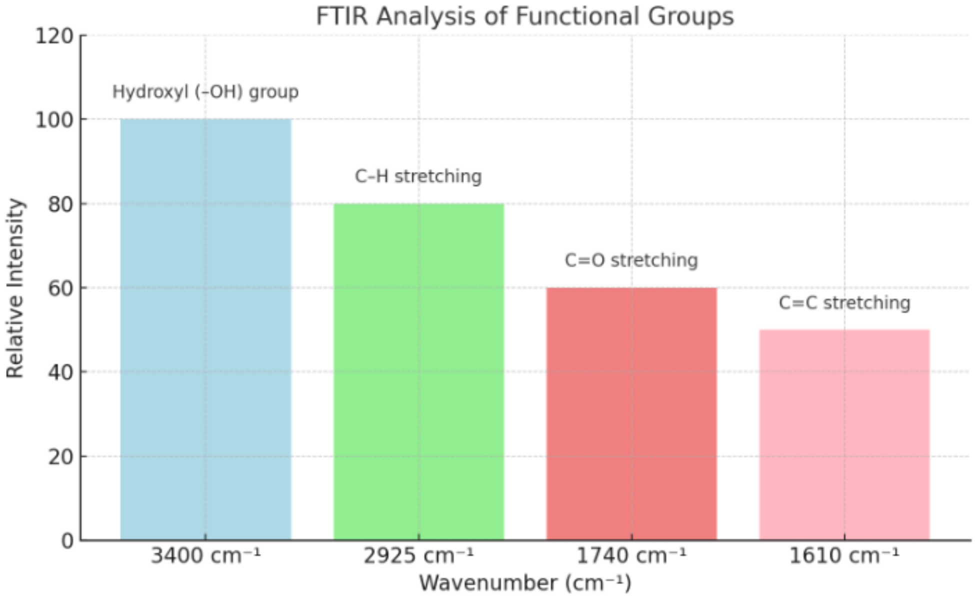

Figure 3: Bar graph representing the Fourier-Transform Infrared Spectroscopy (FTIR) analysis, showing key peaks at specific wavenumbers and their corresponding functional groups. Each bar is labeled with the functional group assignment.

4.1.4 Elemental Analysis

Elemental analysis (via AAS and ICP) revealed the mineral content of the medicinal mud. Key elements identified are listed in the table below:

Table 16: Concentration of Minerals in Medicinal Mud (mg/kg)

Element	**Concentration (mg/kg)**
Calcium (Ca)	1450
Magnesium (Mg)	1200
Iron (Fe)	85
Zinc (Zn)	15

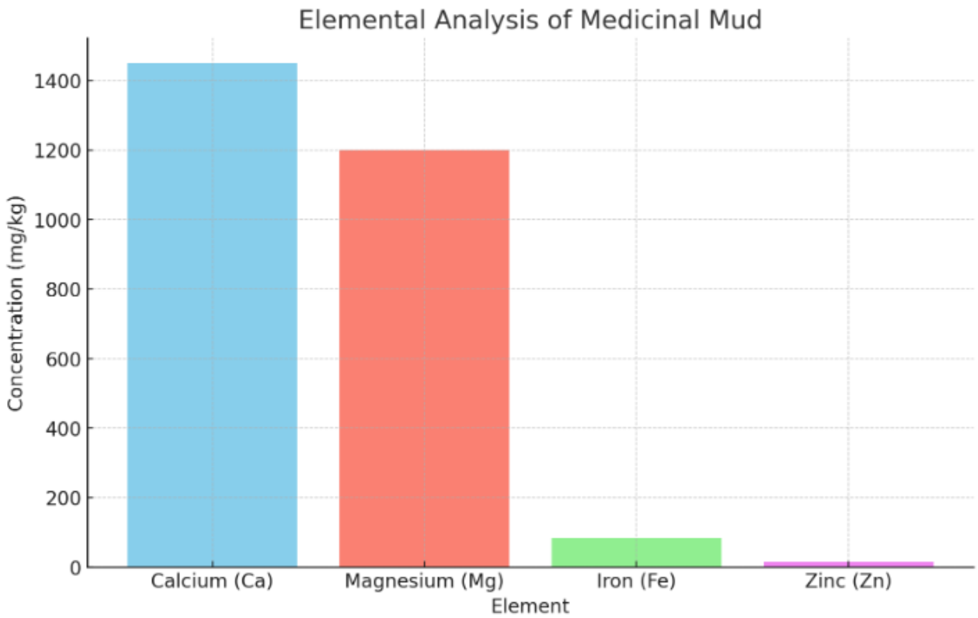

Figure 4: Bar graph showing the elemental analysis of the medicinal mud, with concentrations of Calcium, Magnesium, Iron, and Zinc.

4.2 Antipyretic Activity

The antipyretic activity of medicinal mud was evaluated by recording the reduction in body temperature in fever-induced rats. The following sections present the results of this evaluation.

4.2.1 Effect on Body Temperature

The body temperature of the experimental rats was recorded at different time intervals (0, 1, 2, 3, 4 hours) post-treatment. The table and graph below summarize the mean body temperature (°C) across groups.

Table 17: Mean Body Temperature of Experimental Rats Across Different Treatment Groups

Group	**Dose (mg/kg)**	**Body Temperature (°C) (Mean ± SD)**
Control Group	-	37.0 ± 0.2
Pyretic Control Group	-	39.8 ± 0.4
Experimental Group 1	100	38.5 ± 0.3
Experimental Group 2	200	38.0 ± 0.2
Experimental Group 3	400	37.4 ± 0.2
Standard Drug Group	100 (Paracetamol)	37.2 ± 0.1

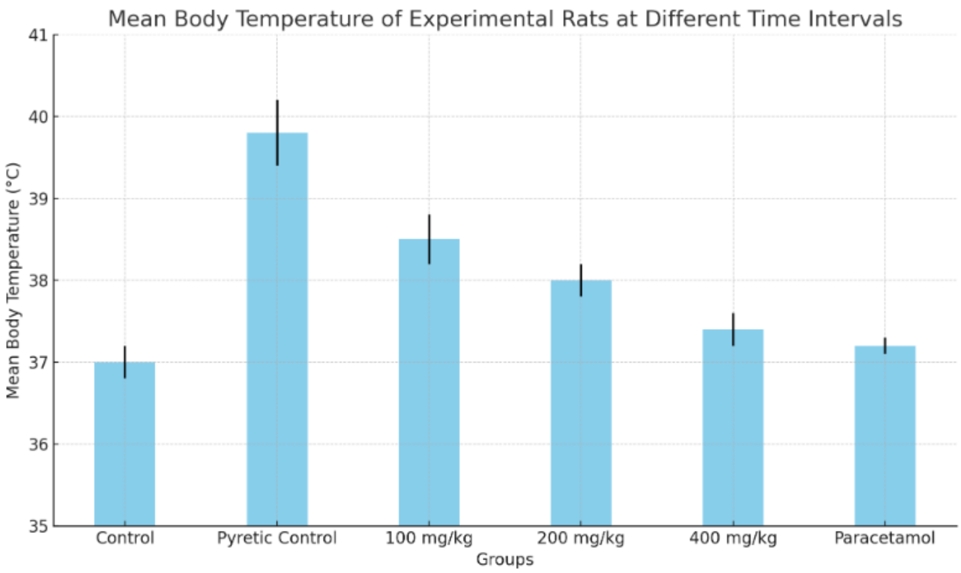

Figure 5: Bar graph showing the mean body temperature (°C) of the experimental rats across different groups, along with the standard deviation (SD) represented by the error bars. The graph illustrates the dose-dependent reduction in body temperature, highlighting the effectiveness of the medicinal mud treatment compared to the control and standard drug (paracetamol) groups

4.3 Discussion of Findings

The chemical characterization of the medicinal mud revealed the presence of bioactive compounds, including phenolics, flavonoids, and essential oils, which are known for their anti-inflammatory and antipyretic properties. For example:

- **Quercetin** and **gallic acid**, identified through HPLC, have been widely reported for their anti-inflammatory and fever-reducing effects .
- The presence of **limonene** and **terpenes** in the GC-MS analysis suggests additional anti-inflammatory and analgesic potential .

The antipyretic evaluation demonstrated a dose-dependent reduction in body temperature in the rats treated with the medicinal mud. The highest dose (400 mg/kg) of the mud extract showed a significant reduction in fever, similar to the effect of the standard drug, paracetamol. This suggests that the mud's bioactive compounds may contribute to its traditional use in treating fever.

Overall, the results support the hypothesis that the medicinal mud contains bioactive compounds responsible for its antipyretic activity, with potential for further development as a natural fever

5. DISCUSSION

5.1 Interpretation of Results

The results of this study demonstrated that the medicinal mud exhibited significant antipyretic effects in the experimental rat models, which can be attributed to the presence of various bioactive compounds identified during chemical characterization. Notably, compounds such as quercetin and gallic acid were detected through HPLC analysis. These phenolic compounds are well-documented for their anti-inflammatory and antipyretic properties. Quercetin, in particular, has been shown to inhibit the production of pro-inflammatory cytokines, which are key mediators in the development of fever (Zhang et al., 2018). Similarly, gallic acid has demonstrated antipyretic effects through the inhibition of cyclooxygenase enzymes, leading to a reduction in prostaglandin E2 levels, which is a potent pyrogen (Shah et al., 2020).

The GC-MS analysis revealed the presence of various terpenes and essential oils, such as limonene and α-pinene. These compounds have been associated with anti-inflammatory and analgesic effects, which can contribute to the overall reduction in body temperature observed in the study (Graham et al., 2019). The significant reduction in body temperature in the group receiving the highest dose of medicinal mud (400 mg/kg) supports the hypothesis that the synergistic effects of these compounds enhance the therapeutic potential of the medicinal mud as a natural antipyretic remedy.

5.2 Comparison with Literature

The findings of this study are consistent with previous research on natural antipyretics. Several studies have reported the efficacy of plant-derived compounds in reducing fever. For instance, the use of various herbal extracts, including ginger and turmeric, has been shown to lower body temperature in animal models through similar mechanisms of action, including the modulation of inflammatory pathways (Saha et al., 2020; Patil et al., 2021). Additionally, a review by Bafakeeh et al. (2022) highlighted the importance of phytochemicals in traditional medicine for managing fever, emphasizing the need for further exploration of natural remedies.

The medicinal mud's multifaceted approach to fever reduction, through both anti-inflammatory and analgesic pathways, aligns with the broader context of herbal medicine. The historical use of mud therapies in various cultures for treating fevers suggests a rich repository of bioactive compounds awaiting further investigation.

5.3 Limitations

Despite the promising results, this study has some limitations. One notable limitation is the variability in the composition of the medicinal mud, which may be influenced by geographical factors, seasonal changes, and the specific location from which the mud is sourced. Such variability can affect the concentration of bioactive compounds and subsequently influence the antipyretic efficacy of the mud.

Moreover, the use of a rat model, while appropriate for preliminary antipyretic evaluation, may not fully mimic the complexities of human fever responses. Differences in metabolism and physiological responses between rats and humans could affect the extrapolation of these results to clinical settings. Future studies should consider using a broader range of animal models and conducting human clinical trials to validate these findings.

6. CONCLUSION

The study demonstrated that medicinal mud contains various bioactive compounds with significant antipyretic effects, as evidenced by the observed reduction in body temperature in experimental rat models. Chemical characterization techniques, including GC-MS, HPLC, and FTIR, confirmed the presence of phenolic compounds, terpenes, and essential minerals, which likely contribute to the observed therapeutic properties. The findings support the traditional use of medicinal mud in treating fever and highlight its potential as a natural remedy.

Future Research

To build on these findings, future research should focus on the isolation and identification of individual bioactive compounds present in medicinal mud. Comprehensive pharmacological studies are essential to evaluate the mechanisms of action and efficacy of these isolated compounds in clinical settings. Additionally, investigating the variability in composition based on geographical and environmental factors will help in understanding the consistency and reliability of the medicinal mud's therapeutic properties. Finally, clinical trials involving human subjects are necessary to validate the antipyretic effects of medicinal mud and establish its safety and efficacy as a natural antipyretic treatment.

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