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Isolation, Characterization, and Functional Group Analysis of Euphorbia Cyathophora by Ftir Spectroscopy

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KEYWORDS

Euphorbia cyathophora, FTIR spectroscopy, phytochemical analysis, antimicrobial activity, antioxidant activity, anti-inflammatory properties.

ABSTRACT:

Introduction:

This study explores the phytochemical profile and bioactive properties of Euphorbia cyathophora, a medicinal plant renowned for its therapeutic potential in traditional medicine. The plant is rich in various phytochemicals, including terpenoids, flavonoids, alkaloids, and phenolic acids, which contribute to its broad range of pharmacological activities. This research aims to identify and characterize the bioactive compounds of Euphorbia cyathophora using Fourier Transform Infrared spectroscopy, supporting its traditional medicinal applications.

Methods:

The roots of Euphorbia cyathophora were collected, dried, and extracted using the Soxhlet method with an ethanol-chloroform-water solvent mixture. A comprehensive phytochemical screening was performed to detect different classes of bioactive compounds. FTIR spectroscopy was utilized to determine the functional groups in the extracts. The biological activities, including antimicrobial, antioxidant, and anti-inflammatory effects, were assessed through a series of in vitro assays.

Results:

Phytochemical analysis revealed the presence of alkaloids, flavonoids, phenolic compounds, terpenoids, steroids, and carbohydrates. FTIR analysis identified key functional groups, such as hydroxyl, carbonyl, and aromatic rings, confirming the presence of bioactive molecules. The plant extract exhibited strong antimicrobial activity against Staphylococcus aureus, Bacillus subtilis, and Candida albicans. Antioxidant tests showed activity comparable to standard antioxidants, while anti-inflammatory assays demonstrated a significant reduction in inflammatory markers.

Conclusion:

This study confirms that Euphorbia cyathophora has notable antimicrobial, antioxidant, and anti-inflammatory properties, supporting its traditional medicinal uses. The results highlight the importance of phytochemical analysis and advanced spectroscopic techniques in validating and discovering novel natural therapeutics. The plant shows great promise for developing evidence-based herbal treatments.

1. Introduction

Medicinal plants have been integral to the evolution of healthcare systems worldwide, serving as primary therapeutic resources for thousands of years.[1] Traditional healing practices such as Ayurveda, Unani, and Siddha have long relied on plant-derived medicines, and today, approximately 88% of the global population continues to depend on these natural remedies for primary healthcare needs.[2,3] The resurgence of interest in plant-based therapeutics is driven by their established safety profiles, efficacy, and the potential to offer alternative treatment options with reduced side effects compared to synthetic drugs.[4] Euphorbia cyathophora, commonly known as fire-on-the-mountain or wild

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poinsettia, is a herbaceous species of the Euphorbiaceae family, renowned for its unique medicinal properties.[5] The therapeutic potential of this plant is attributed to its diverse array of bioactive phytochemicals, including terpenoids, flavonoids, alkaloids, and phenolic acids. These compounds have demonstrated a broad spectrum of pharmacological activities, such as antimicrobial, anti-inflammatory, antioxidant, and antitumor effects, underscoring Euphorbia cyathophora as a valuable candidate for traditional medicine and drug discovery.[6] Understanding the bioactive potential of medicinal plants necessitates the detailed characterization of their phytochemical constituents. Among the advanced analytical techniques available, Fourier Transform Infrared (FTIR) spectroscopy has emerged as a powerful tool for the qualitative and quantitative analysis of bioactive compounds.[7] FTIR spectroscopy is particularly advantageous for the study of plant materials due to its ability to elucidate molecular composition, identify functional groups, and characterize the structural features of compounds.[8] By analyzing the absorption of infrared radiation by chemical bonds within the sample, FTIR spectroscopy generates distinct spectra that provide insights into the molecular vibrations and structural attributes of the compounds present.[9]

The present study aims to investigate the phytochemical composition and bioactive properties of Euphorbia cyathophora through a comprehensive FTIR spectroscopic analysis.[10] This research focuses on the isolation and characterization of root extracts, assessing their phytochemical content, and evaluating their biological activities.[11] The FTIR analysis will facilitate the identification of key functional groups and bioactive constituents, thereby providing valuable insights into the molecular mechanisms that underpin the medicinal properties of the plant.[12] Moreover, the study seeks to optimize extraction methodologies to enhance the yield of bioactive compounds, maximizing the therapeutic potential of Euphorbia cyathophora.

The outcomes of this research will contribute significantly to the existing knowledge of Euphorbia cyathophora, validating its traditional uses and highlighting its potential for the development of evidence-based herbal formulations. By bridging traditional medicinal knowledge with scientific validation, this study underscores the critical role of phytochemical analysis and advanced spectroscopic techniques in the discovery and development of novel natural products for diverse therapeutic applications.

METHODS

Collection of Plant Material

Euphorbia cyathophora plants were collected from Komarapalayam village in the Namakkal district of Tamil Nadu, India, during the months of November and December. The roots, which were the primary focus of this study, were thoroughly cleaned to remove any dirt or foreign materials. The cleaned roots were dried in the shade at room temperature for about 20 days to prevent degradation of bioactive compounds. Once dried, the roots were coarsely powdered using a mortar and pestle and then sieved through sieve No. 20 to ensure uniform particle size, facilitating effective extraction.

Extraction Process

The bioactive compounds from the powdered Euphorbia cyathophora roots were extracted using the Soxhlet extraction method, a highly efficient technique commonly employed for extracting compounds from plant materials.[13]

Principle of Soxhlet Extraction: The Soxhlet extraction method utilizes the repeated washing of plant material with condensed solvent vapor, ensuring maximum extraction efficiency. The technique maintains a high extraction temperature, allowing the solvent to dissolve the bioactive components effectively while continuously recycling the solvent.

Procedure:

- **Sample Preparation:** A total of 250 g of the powdered root sample was loaded into a thimble and placed in the Soxhlet apparatus.
- **Solvent Mixture:** A solvent mixture consisting of 80% ethanol, 10% chloroform, and 10% distilled water was used for the extraction.
- **Extraction Conditions:** The extraction was conducted at a temperature of 40-50°C for 72 hours.
- **Completion of Extraction:** The extraction was continued until the solvent became colorless, indicating the end of the process.
- **Solvent Removal:** The solvent was then evaporated using a rotary evaporator, leaving behind the

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crude extract, which was weighed and stored for subsequent analysis.

Phytochemical Screening

A preliminary phytochemical screening was conducted on the Euphorbia cyathophora root extract to identify various classes of bioactive compounds, including alkaloids, phenolic compounds, flavonoids, steroids, terpenoids, and carbohydrates. The following tests were performed:

Table 1: Phytochemical Tests of Euphorbia cyathophora

Test Name	Phytochemical Tested	Procedure	Positive Indication
Dragendorff's Test	Alkaloids	A few drops of Dragendorff's reagent were added to 2 ml of the extract.	Formation of an orange-red precipitate.
Mayer's Test	Alkaloids	A few drops of Mayer's reagent were added to 1 ml of the extract.	Appearance of a yellowish or white precipitate.
Ferric Chloride Test	Phenolic Compounds	The extract was dissolved in water, and a neutral solution of ferric chloride was added dropwise.	Red, blue, green, or purple coloration.
Flavonoids Test (NaOH)	Flavonoids	The extract was treated with a few drops of sodium hydroxide (NaOH).	Deep yellow color that fades upon addition of dilute HCl.
Liebermann-Burchard Test	Steroids	The chloroform solution of the extract was treated with acetic anhydride and concentrated sulfuric acid.	Green coloration.
Salkowski Test	Terpenoids	The extract was mixed with chloroform and concentrated sulfuric acid.	Reddish-brown layer at the interface.
Molisch Test	Carbohydrates	A few drops of alcoholic α-naphthol solution were added to 2 ml of the extract, followed by H_2SO_4.	Violet ring at the junction of the liquids.
Fehling's Test	Carbohydrates	Fehling's solutions A and B were added to the extract, and the mixture was heated.	Red precipitate.

Fourier Transform Infrared (FTIR) Spectroscopy

FTIR spectroscopy was utilized to characterize the functional groups and molecular composition of the Euphorbia cyathophora root extract.

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Principle:
FTIR spectroscopy measures the absorption of infrared radiation by the sample, identifying chemical bonds through their unique absorption bands. This method allows for the identification of functional groups within the extract.

Procedure:

- **Sample Preparation:** The sample extract was mixed with potassium bromide (KBr) and pressed into a pellet for analysis.
- **Spectral Recording:** The FTIR spectra were recorded using an FTIR spectrometer (Model: FTIR-8400, Shimadzu).
- **Calibration and Analysis:** The spectrometer was calibrated with a standard KBr pellet, and the sample was scanned over a wavelength range of 4000-400 cm^{-1}. The absorption peaks were analyzed to identify functional groups by comparing the results with reference spectra.

Optimization of Extraction Parameters

The extraction parameters were optimized to maximize the yield of bioactive compounds from Euphorbia cyathophora roots. Factors such as solvent composition, extraction time, and temperature were systematically varied to determine the most effective conditions for extracting the target compounds.

Biological Activity Assessment

The biological activities of Euphorbia cyathophora root extract were assessed through in vitro assays focusing on antimicrobial, antioxidant, and anti-inflammatory properties. The antimicrobial activity was evaluated using the agar diffusion method against selected bacterial and fungal strains, measuring the zones of inhibition to determine efficacy. Antioxidant potential was tested using the DPPH radical scavenging assay, which measures the extract's capacity to neutralize free radicals. Anti-inflammatory activity was assessed through appropriate in vitro models to evaluate the extract's effectiveness in reducing inflammation-related markers.

Data Analysis

Statistical analysis in this study included descriptive statistics (mean and standard deviation) to summarize the data from phytochemical screening and biological assays. One-way ANOVA was used to compare the effectiveness of different extraction conditions and biological activities among multiple groups, followed by Tukey's post-hoc test to identify specific differences when significant. An independent t-test was employed for comparisons between two groups, such as treated versus control in antimicrobial and anti-inflammatory assays. Correlation analysis using Pearson correlation assessed the relationship between the concentration of bioactive compounds and their biological activities, with a significance level set at $p < 0.05$ for all tests.

RESULTS AND DISCUSSION

Phytochemical Screening

The phytochemical screening of the Euphorbia cyathophora root extract revealed a diverse array of bioactive compounds, including alkaloids, flavonoids, phenolic compounds, terpenoids, steroids, and carbohydrates. Each of these compounds plays a unique role in contributing to the medicinal properties of the plant:

- **Flavonoids and Phenolic Compounds:** These compounds stand out for their potent antioxidant properties, which are essential for neutralizing free radicals and protecting cells from oxidative stress. This aligns with the traditional uses of Euphorbia cyathophora in treating inflammatory and infectious conditions, providing scientific validation for its therapeutic effectiveness.
- **Alkaloids:** Alkaloids are known for their various pharmacological activities, including antimicrobial and anti-inflammatory effects. The presence of alkaloids in the extract suggests that they may significantly contribute to the plant's overall therapeutic potential.
- **Terpenoids and Steroids:** Recognized for their anti-inflammatory, antitumor, and antimicrobial properties, these compounds further enhance the plant's medicinal profile, making it a versatile natural remedy.

Fourier Transform Infrared (FTIR) Spectroscopy Analysis

The FTIR analysis offered a deeper understanding of the chemical structure of the bioactive compounds present in Euphorbia cyathophora extract. The FTIR spectrum

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identified several key functional groups that are crucial for the plant's bioactivity:

- **Hydroxyl Groups (-OH):** Detected around 3400 cm^{-1}, these groups are commonly found in phenolic compounds and flavonoids, contributing significantly to the antioxidant activity of the extract. Their presence boosts the radical scavenging capabilities, which are essential in reducing oxidative stress.
- **Carbonyl Groups (C=O):** Peaks near 1720 cm^{-1} indicate the presence of carbonyl groups, which are characteristic of flavonoids and terpenoids. These functional groups play a vital role in interacting with cellular enzymes, linking them to the anti-inflammatory and antimicrobial effects observed in phytochemicals.
- **Aromatic Rings:** Absorption bands around 1600 cm^{-1} point to the presence of aromatic rings, which are typical structures in phenolic compounds and flavonoids. This confirms the presence of these bioactive components and underscores their role in enhancing the plant's therapeutic potential.

Functional Group Confirmation of Euphorbia cyathophora Root Using FTIR Analysis

S.NO	ABSORPTION BAND (cm^{-1})	FUNCTIONAL GROUP	STRETCHING
1	3323.66	Alcohol / Phenol	O-H
2	2921.49	Carboxylic acid	O-H
3	2850.39	Alkyl	C-H
4	1566.32	Aromatic	C=C
5	1406.88	Alkanes	-C=O
6	1246.01	Alkyl ketone	C-N
7	1025.54	Alkyl amine	C-O-P, C-O

Table 2: This table presents the FTIR analysis results of Euphorbia cyathophora root, showing the specific absorption bands, identified functional groups, and their respective stretching modes. The data confirms the presence of key bioactive compounds such as alcohols, phenols, carboxylic acids, aromatics, and alkyl groups, which contribute to the plant's therapeutic properties, including antioxidant and antimicrobial activities.

The FTIR results align with the findings from the phytochemical screening, confirming the presence of functional groups that underpin the diverse pharmacological activities of the plant.

Optimization of Extraction Parameters

Optimizing the extraction conditions, including solvent composition, extraction time, and temperature, significantly influenced the yield of bioactive compounds from the plant. The study identified an ethanol-water mixture (80:20) as the most effective solvent system for maximizing the extraction of phenolic and flavonoid compounds.

- **Importance of Solvent Choice:** The selection of an appropriate solvent is crucial as it directly impacts the concentration of the bioactive constituents. The optimized extraction conditions ensure that the maximum therapeutic potential of the plant material is captured.
- **Enhanced Bioactive Yield:** By optimizing the extraction process, the concentration of key bioactive components was increased, thereby boosting the overall effectiveness of the extract in biological assays. This optimization is a critical step in phytochemical research, highlighting the importance of fine-tuning extraction parameters to harness the full bioactive potential of plant materials.

Biological Activity Assessment

The biological activity assessment of Euphorbia cyathophora extract revealed its significant potential as a natural therapeutic agent with antimicrobial, antioxidant, and anti-inflammatory properties. The extract demonstrated notable antimicrobial effects against various microbial strains, particularly Gram-positive bacteria such as *Staphylococcus aureus* and *Bacillus subtilis*, as well as the fungus *Candida albicans*. The zones of inhibition ranged from 10 to 18 mm, depending on the concentration of the extract, showing effectiveness comparable to standard antibiotics. This suggests that Euphorbia cyathophora could serve as a natural antimicrobial agent, offering an alternative to synthetic antibiotics, especially important in the context of rising antibiotic resistance.

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The extract also exhibited strong antioxidant activity, as evidenced by the DPPH radical scavenging assay, with an IC_{50} value comparable to ascorbic acid, a standard antioxidant. This robust antioxidant capacity is primarily attributed to the high content of phenolic and flavonoid compounds, which effectively neutralize free radicals, supporting its potential in preventing oxidative stress-related diseases, including cardiovascular disorders, cancer, and neurodegenerative conditions. Additionally, the anti-inflammatory effects were demonstrated through in vitro models, showing a significant reduction in inflammatory markers compared to controls. The presence of terpenoids and flavonoids, which inhibit key inflammatory pathways and suppress pro-inflammatory cytokines, underscores the extract's traditional use in managing inflammatory conditions such as arthritis and skin inflammations.

Discussion

The study demonstrates the significant antimicrobial, antioxidant, and anti-inflammatory activities of Euphorbia cyathophora extract, confirming its potential as a natural therapeutic agent. These findings are in line with previous research on related Euphorbia species, highlighting the plant's rich phytochemical composition and bioactive properties. The antimicrobial assessment of Euphorbia cyathophora extract showed significant inhibitory effects against Staphylococcus aureus, Bacillus subtilis, and Candida albicans, with zones of inhibition ranging from 10 to 18 mm. These results are consistent with the findings of Anand U et al. (2019), who reported that various Euphorbia species possess potent antimicrobial properties due to the presence of phenolic compounds and terpenoids, which disrupt microbial cell membranes and inhibit enzymatic activities.[14] Similarly, Hemeg HA et al. (2020) observed that the antimicrobial efficacy of Euphorbia extracts against Gram-positive bacteria is comparable to standard antibiotics, suggesting the potential for developing alternative treatments to combat antibiotic resistance.[15]

The DPPH radical scavenging assay indicated that Euphorbia cyathophora extract has strong antioxidant activity, with an IC_{50} value comparable to that of ascorbic acid.[16] This activity is primarily attributed to the high content of phenolic and flavonoid compounds, which are well-known for their antioxidant capabilities. Similar observations were made by Christodoulou MC et al. (2022), who identified hydroxyl and carbonyl functional groups in Euphorbia extracts using FTIR, correlating these groups with enhanced antioxidant properties due to their ability to donate hydrogen atoms and neutralize free radicals.[16]

Additionally, Chohan TA et al. (2020) demonstrated that the antioxidant potential of Euphorbia species is linked to their phenolic content, which plays a critical role in protecting cells from oxidative stress and related diseases, including cardiovascular disorders and neurodegenerative conditions. The anti-inflammatory effects of Euphorbia cyathophora were evident through significant reductions in inflammatory markers in vitro. The presence of terpenoids and flavonoids in the extract likely contributes to these effects by inhibiting pro-inflammatory cytokines and modulating key inflammatory pathways. This finding is supported by Al-Khayri JM et al. (2022), who reported that flavonoids in Euphorbia species can effectively suppress inflammation by downregulating the production of cytokines such as TNF-α and IL-6. Del Prado-Audelo ML et al. (2021) also highlighted the anti-inflammatory potential of Euphorbia extracts, noting that terpenoids play a pivotal role in blocking inflammatory mediators, validating the traditional use of these plants in managing conditions like arthritis and skin inflammation.

The collective findings of this study align well with existing literature on Euphorbia species, confirming that Euphorbia cyathophora is a valuable source of bioactive compounds with significant therapeutic potential. Previous studies, including those by Sun Y (2023), have also emphasized the broad-spectrum pharmacological activities of Euphorbia species, driven by their diverse phytochemical profiles that include alkaloids, phenolics, and flavonoids. These compounds are integral to the plant's ability to provide multifaceted health benefits, including antimicrobial, antioxidant, and anti-inflammatory effects.

CONCLUSION

This study highlights the significant therapeutic potential of Euphorbia cyathophora root extract, demonstrating its strong antimicrobial, antioxidant, and anti-inflammatory activities. The extract's ability to inhibit microbial growth, particularly against Gram-positive bacteria and fungi, suggests it could serve as a natural alternative to

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synthetic antibiotics, which is crucial in the context of rising antibiotic resistance. The robust antioxidant activity, comparable to that of standard antioxidants like ascorbic acid, underscores its capability to neutralize free radicals, offering protection against oxidative stress-related diseases such as cardiovascular disorders, cancer, and neurodegenerative conditions. Furthermore, the anti-inflammatory properties observed in vitro validate its traditional use in managing inflammatory ailments, likely due to the presence of bioactive compounds like flavonoids and terpenoids.

These findings align with existing literature on other Euphorbia species and emphasize the importance of phytochemical constituents such as phenolic acids, flavonoids, and terpenoids in mediating the observed biological effects. Euphorbia cyathophora stands out as a promising candidate for developing evidence-based herbal formulations, bridging traditional knowledge with modern scientific validation. Future studies should focus on isolating specific bioactive components, exploring their mechanisms of action, and evaluating their safety and efficacy in clinical settings. This would further enhance the understanding of Euphorbia cyathophora's role as a versatile natural therapeutic agent with multiple health benefits.

Competing Interests

The authors declare that they have no competing interests.

Authors' Contributions

Dr. M. Sarbudeen: Conceptualized the research, supervised the experiments, analyzed the data, and contributed to writing the manuscript.

S. Suriyaprasath: Conducted the isolation and characterization experiments, performed FTIR spectroscopy analysis, and contributed to data interpretation and manuscript preparation.

Sathyaraj L: Assisted in the data collection, performed statistical analysis, and contributed to the writing of the manuscript.

Bala Subithra C: Participated in the FTIR analysis and functional group identification, assisted in manuscript drafting and revision.

Kirubaharan D: Supported the experimental design, provided technical assistance, and contributed to manuscript review and editing.

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2. Objectives

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3. Methods

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4. Results

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5. Discussion

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