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Original Article

Phytochemical Profiling and Therapeutic Potential of Ficus benjamina L.: Insights into Anticancer and Anti-inflammatory Activities

Farzana Khan[1,2], Samina Afzal[1]*., Anjum Khursheed[3], Benish Saeed[1,6], Sunder Zahra[1], Huma Majeed[2], Sumreen Sarwar[4], Muhammad Sheraz Qureshi[5], Muhammad Saleem Qureshi[7], Muhammad Imran Qureshi[8], Hafiz Muhammad Usman Abid[9]*

[1]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Bahauddin Zakariya University, Multan, Punjab, Pakistan.
[2]Bakhtawar Amin College of Pharmaceutical Sciences, Multan, Punjab, Pakistan.
[3]Faculty of Pharmacy, Grand Asian University Sialkot, Pakistan.
[4]University of Agriculture Faisalabad.
[5]Department of Pharmacognosy, Multan University of Science and Technology, Multan, Punjab, Pakistan.
[6]Southern Punjab Institute of Health Sciences, Department of Pharmacy, Multan, Punjab, Pakistan.
[7]Akbar Institute of Medical Sciences Bahawalpur, Punjab, Pakistan.
[8]Institute of Research and Advanced Studies, Multan, Punjab, Pakistan.
[9]Department of Pharmaceutics, Bahauddin Zakariya University, Multan, Punjab, Pakistan.
Corresponding Author: Samina Afzal, Hafiz Muhammad Usman Abid; Email: samina.afzal@bzu.edu.pk, usmanabid394@gmail.com; https://orcid.org/0000-0001-8881-9181

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ABSTRACT

Background: Ficus benjamina L., commonly known as the weeping fig, is a plant traditionally used for various medicinal purposes, particularly in Southeast Asia. Despite its widespread use, there is a scarcity of scientific data validating its therapeutic properties, especially its anti-inflammatory and anticancer potentials.

Objective: This study aimed to investigate the phytochemical profile of Ficus benjamina L. and to evaluate its anticancer and anti-inflammatory activities to provide a scientific basis for its traditional uses.

Methods: The plant materials were collected and subjected to extraction using methanol and dichloromethane. The extracts were analyzed for primary and secondary metabolites. The anticancer activity was tested using the MTT assay on HeLa cells, and anti-inflammatory activity was assessed using a luminol-enhanced chemiluminescence method. Data analysis was conducted using IBM SPSS Statistics Version 25, focusing on inferential statistics to compare the bioactivity of the extracts against standard drugs.

Results: Phytochemical analysis confirmed the presence of proteins, lipids, carbohydrates, alkaloids, flavonoids, and tannins. The dichloromethane extract showed significant anti-inflammatory activity with 58.6% inhibition at a concentration of 50 µg/ml and an IC50 of 8.966±1.03. The methanol extract exhibited minimal anticancer activity with an 8.3% inhibition rate at 30 µg/ml, suggesting low efficacy against HeLa cells compared to the standard drug, Doxorubicin, which showed a 101.2% inhibition.

Conclusion: The study confirms that Ficus benjamina L. contains bioactive compounds with potential anti-inflammatory benefits and limited anticancer activity. These findings support the traditional use of the plant in treating inflammatory conditions and highlight the need for further research to optimize extraction techniques and expand biological testing.

Keywords: Ficus benjamina L., phytochemical analysis, anticancer activity, anti-inflammatory activity, traditional medicine, bioactive compounds.

INTRODUCTION

Medicinal plants have long been a cornerstone of healthcare throughout human history, providing a rich source of therapeutic agents across various cultures. Ficus benjamina L., commonly known as the weeping fig or benjamin fig, is a prime example of such botanical resources, revered not only for its ornamental value but also for its extensive use in traditional medicine. Native to Southeast Asia, this evergreen tree is a member of the Moraceae family, distinguished by its graceful drooping branches and glossy leaves. Its petite fruits further contribute to its aesthetic appeal, making it a popular choice for decoration in gardens, parks, and indoor spaces across

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the globe. Historically, different parts of the Ficus benjamina L. tree, including its leaves, bark, roots, and latex, have been utilized by indigenous communities to treat a variety of ailments ranging from gastrointestinal disorders to inflammatory diseases (1).

Despite its widespread traditional use, scientific studies exploring the phytochemical composition and pharmacological potential of Ficus benjamina L. are surprisingly sparse (2). While anecdotal evidence and ethnobotanical surveys provide some validation of its medicinal properties, a comprehensive understanding of its bioactive components and their therapeutic mechanisms remains elusive. This gap between traditional knowledge and scientific evidence presents a significant research challenge, emphasizing the need for rigorous scientific investigation to validate and potentially expand upon the traditional uses of Ficus benjamina L.

Our research focuses on bridging this gap by conducting a detailed phytochemical profiling of Ficus benjamina L. and exploring its therapeutic potential, particularly in the areas of cancer therapy and inflammation management. By employing suitable solvent systems, we aim to extract bioactive compounds from various parts of the tree and perform a thorough phytochemical analysis to identify both primary and secondary metabolites. The anticancer properties of these extracts will be evaluated through in-vitro assays against cancer cell lines, focusing on their cytotoxic effects and the mechanisms behind these actions. Similarly, the anti-inflammatory properties of the extracts will be assessed using established in-vitro models to understand their ability to modulate inflammatory pathways and alleviate symptoms associated with inflammation (3). Through these investigations, we aspire to provide a scientific foundation for the medicinal use of Ficus benjamina L., potentially leading to novel therapeutic applications for this historically important plant.

MATERIAL AND METHODS

In this study, all chemicals and reagents were sourced from reputable institutions to ensure consistency and reliability in experimental outcomes. The Department of Pharmaceutical Chemistry at the Faculty of Pharmacy in Multan, Punjab, Pakistan supplied most of the chemicals used, including 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), penicillin, and streptomycin. These were procured in collaboration with the HEJ Research Institute of Chemistry at the University of Karachi, Sindh, Pakistan, which also provided the solutions required for the in-vitro anti-inflammatory activity assays. Additional materials, including Doxorubicin and Ibuprofen used as reference standards, were obtained from the same department in Multan (1).

The plant material, Ficus benjamina L. from the Moraceae family, was collected from Allah Ditta Nursery Farm on the Northern Bypass, Boson Road, Multan, Pakistan. Post-collection, the plant was cleansed with water to remove any adherent dust and subsequently separated into its constituent parts—stem, roots, and leaves. These were then shade-dried on a clean paper sheet for 30 days at a room temperature of 25°C (2).

For the extraction process, 400 grams of the dried plant powder underwent a simple maceration with 1.5 liters each of methanol and dichloromethane. This procedure was repeated three times for each solvent, with the mixtures maintained at 25°C for three days with intermittent shaking. The resulting extracts were then concentrated using a rotary evaporator, weighed, and duly labeled according to the specified plant codes, as detailed in Table 1 (3).

Phytochemical assays were conducted on the finely ground powder to confirm the presence of primary and secondary metabolites such as proteins, lipids, carbohydrates, alkaloids, anthraquinones, glycosides, and flavonoids among others (6). For the cytotoxic assay, the dichloromethane extract was tested using the MTT colorimetric assay on HeLa cells cultured in Minimum Essential Medium Eagle supplemented with fetal bovine serum, penicillin, and streptomycin. Post-incubation, the cytotoxic effects were quantified by measuring the absorbance at 570 nm, and the percentage inhibition was calculated to evaluate the anticancer potential against the standard drug, Doxorubicin (7).

The in-vitro anti-inflammatory activity was assessed using a Luminol-enhanced chemiluminescence method, with each assay conducted in triplicate. The assessments involved treating freshly dissolved blood samples with solutions containing Hanks Balanced Salt with added $CaCl_2$ and $MgCl_2$, followed by zymosan and a solution for reactive oxygen species detection (4).

Data collection was conducted in strict adherence to ethical standards outlined in the Declaration of Helsinki, ensuring the welfare of all biological samples used during the study. Data analysis was performed using the SPSS software version 25, applying appropriate statistical tests to determine the significance of the findings (5). This robust methodological framework not only underscores the scientific rigor of the study but also enhances the reliability and validity of the results obtained, paving the way for further exploration into the pharmacological potentials of Ficus benjamina L.

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RESULTS

In our study, the extraction efficiency of Ficus benjamina L. varied significantly depending on the solvent used. As shown in Table 1, the methanol extraction (FBWM) yielded 12 grams of extract from 400 grams of dried plant material, with 4.77 grams remaining after processing. In contrast, the extraction with dichloromethane (FBWD) was slightly more efficient, yielding 12.81 grams of extract and leaving 9.68 grams remaining. This indicates a notable difference in the solubility of bioactive compounds in methanol versus dichloromethane, which could influence their pharmacological potential.

Table 1: Extraction Results of Ficus benjamina L.

Sr. No.	Plant Code	Plant Weight Used in Extraction	Solvent Used	Solvent Volume Used in Extraction	Extract Weight Obtained	Remaining Weight of Extract
1	FBWM	400g	Methanol	500 ml × 3	12g	4.77g
2	FBWD	400g	Dichloromethane	500 ml × 3	12.81g	9.68g

Table 2: Primary Metabolites of Ficus benjamina L.

Sr. No.	Metabolite Class	Test Name	Observation	Results
1	Proteins	Nitroprusside Test	Reddish and purple color observed	Proteins present
		Biuret Test	Violet color observed	Proteins present
		Ninhydrin Test	Purple color observed	Glycine present
2	Lipids	Solubility Test	Clear solutions observed	Lipids present
3	Carbohydrates	Fehling's Test	Red precipitates observed	Carbohydrates present
		Benedict Test	Greenish precipitates observed	Reducing sugars present
		Molisch Test	Deep violet color at junction observed	Carbohydrates present
		Iodine Test	Blue color observed	Polysaccharides present

Table 3: Secondary Metabolites of Ficus benjamina L.

Sr. No.	Metabolite Class	Test Name	Observation	Results
1	Alkaloids	Dragendorff's Reagent	Reddish brown precipitates observed	Positive
		Hager's Reagent	Yellow color precipitates observed	Positive
		Mayer's Reagent	Cream color precipitates observed	Positive
		Wagner's Reagent	Reddish brown precipitates observed	Positive
2	Anthraquinones	Borntrager's Test	Cherry red color observed	Positive
	glycosides	Modified Borntrager's Test	Intense pink color observed	Positive
3	Cardiac glycosides	Keller Kiliani Test	Pale green color at upper layer observed	Positive
4	Saponin glycosides	Froth Test	No frothing observed	Negative
5	Flavonoids	Alkaline Test	Yellow color observed	Positive
		Lead Acetate Test	Yellow color observed	Positive
6	Tannins	Gelatin Test	Tannins precipitates observed	Positive
		Catechin Test	Pink color observed	Positive
		Ferric Chloride Test	Blue or black precipitated observed	Positive

Table 4: Anticancer Activity on HeLa Cell Line of Ficus benjamina L.

Sample Code	Concentration (µg/ml)	% Inhibition/Stimulation	IC50 ± SD
FBWM	30 µg/ml	8.3%	Inactive
Standard Drug	Doxorubicin	30 µg/ml	101.2%

Table 5: Anti-inflammatory Activity of Ficus benjamina L.

Sample Code	Concentration (µg/ml)	% Inhibition/Stimulation	IC50 ± SD
FBWD	50 µg/ml	58.6%	8.966 ± 1.03
Standard Drug	Ibuprofen	25 µg/ml	73.2 ± 1.4%

Phytochemical screening revealed a diverse array of primary metabolites present in the plant, detailed in Table 2. The presence of proteins was confirmed through a variety of tests, including the Nitroprusside test, which produced reddish and purple colors, and

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the Biuret test, which showed a violet coloration, indicative of peptide bonds. Additionally, the Ninhydrin test confirmed the presence of amino acids such as glycine. Lipids were identified through a clear result in the solubility test, while multiple carbohydrate forms were detected. Fehling's and Benedict tests pointed towards the presence of reducing sugars and other carbohydrate forms, confirmed by Molisch and Iodine tests, which indicated the presence of polysaccharides.

Secondary metabolites, as summarized in Table 3, included several classes such as alkaloids, anthraquinones glycosides, and tannins, all of which tested positive in specific chemical assays. Notably, alkaloids were universally positive across several reagents, including Dragendorff's and Wagner's, indicating a strong presence. Anthraquinones also showed a significant reaction in both the standard and modified Borntrager's tests. However, the saponin glycosides were the exception, where the froth test did not produce frothing, indicating a negative result.

The anticancer activity of the extracts was quantified through cytotoxic assays on HeLa cell lines, with results presented in Table 4. The methanol extract (FBWM) demonstrated minimal activity, with only an 8.3% inhibition rate at a concentration of 30 µg/ml, rendering it inactive in cancer inhibition. In stark contrast, the standard drug Doxorubicin exhibited a dramatic 101.2% inhibition under the same conditions, underscoring the potent efficacy of this chemotherapeutic agent.

The anti-inflammatory potential of the extracts was also assessed and is detailed in Table 5. The dichloromethane extract (FBWD) exhibited significant activity, achieving 58.6% inhibition at a concentration of 50 µg/ml, with an IC50 value of 8.966 ± 1.03. This compares favorably with the standard drug Ibuprofen, which had a slightly higher inhibition rate of 73.2% at a lower concentration of 25 µg/ml but a higher IC50 value of 11.2 ± 1.9, suggesting that the extract has a noteworthy anti-inflammatory effect.

These results not only highlight the pharmacological potential of Ficus benjamina L. but also emphasize the importance of solvent selection in the extraction of bioactive compounds for therapeutic uses. The findings suggest that while the plant possesses significant anti-inflammatory properties, its anticancer potential may require further enhancement or might be dependent on different extraction techniques or conditions.

DISCUSSION

The findings from our investigation into the phytochemical composition and bioactivity of Ficus benjamina L. provide an insightful addition to the existing body of knowledge concerning its medicinal value, especially in anti-inflammatory and anticancer applications. Previous studies have highlighted the plant's use in traditional medicine for treating various ailments, and our results align with and expand upon these traditional uses by scientifically validating the presence of significant bioactive compounds (11-14).

Our phytochemical analysis confirmed the presence of primary metabolites such as proteins, lipids, and carbohydrates, which are crucial for basic cellular functions and have been extensively reported in literature as common in many medicinal plants (15-16). The diversity in secondary metabolites, including alkaloids, flavonoids, and tannins, which our study identified, are compounds known for their antioxidant, anti-inflammatory, and anticancer properties (17-19). The presence of these metabolites in Ficus benjamina L. supports the traditional claims and provides a basis for its therapeutic potentials. However, the absence of saponin glycosides, which are often credited with anti-inflammatory properties, was a surprising find and may suggest variability in metabolite concentration due to environmental factors or genetic differences within species (20).

The anticancer activities of Ficus benjamina L., as revealed by our cytotoxic assays, showed minimal inhibition on HeLa cells, which contrasts with the higher efficacy reported by other studies (4). This disparity could be attributed to the differences in extraction methods or the solvent used, as our results notably highlighted methanol's lower extraction efficiency compared to dichloromethane. This suggests that the solvent type significantly affects the extraction of bioactive compounds that may be crucial for anticancer activity (16). The minimal activity could also stem from the possible degradation of sensitive compounds during extraction or the absence of synergistic effects that are present in whole plant formulations used traditionally. In terms of anti-inflammatory efficacy, the dichloromethane extract showed significant activity, supporting the use of Ficus benjamina L. for inflammatory conditions in folk medicine. The comparative effectiveness of the extract to ibuprofen, especially at higher concentrations, offers promising implications for developing plant-based anti-inflammatory therapies that could potentially offer fewer side effects or enhanced biocompatibility (5, 11, 17).

Despite these promising findings, our study had limitations. The variability in plant material due to environmental factors, the limited range of solvents and concentrations tested, and the focus on only two biological activities restrict the generalizability of our results.

Additionally, the absence of in vivo studies to confirm the efficacy and safety of the extracts limits the clinical applicability of our findings.

Moving forward, it would be advisable to conduct further studies involving a wider range of extraction solvents and methods to optimize the yield of bioactive compounds. Expanding the research to include in vivo models would also help in validating the therapeutic potential and safety profile of the extracts. Moreover, isolating specific bioactive compounds could lead to the development of novel therapeutic agents with targeted properties. Overall, our study reinforces the medicinal value of Ficus benjamina L., but also highlights the need for comprehensive scientific validation to fully integrate such traditional remedies into modern medicinal practice (6, 20).

CONCLUSION

The research on Ficus benjamina L. underscores its significant potential in the development of novel therapeutic agents, particularly for anti-inflammatory and, to a lesser extent, anticancer applications. Our findings confirm the presence of bioactive compounds that align with the plant's traditional uses, offering a scientific foundation that supports its integration into modern healthcare. This study not only enhances our understanding of Ficus benjamina L.'s pharmacological properties but also suggests a broader implication for the use of medicinal plants in developing safer, plant-based medical treatments that could complement or offer alternatives to conventional therapies. Further investigations into optimizing extraction methods and conducting clinical trials are necessary to fully harness the therapeutic potentials of this plant in human healthcare.

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