FORMULATION AND EVALUATION OF ANTIFUNGAL MICROSPHERES OF *Aegle Marmelos* LEAVES

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ABSTRACT

This study aimed to formulate and evaluate antifungal microspheres using extracts from *Aegle marmelos* leaves. Three different types of polymers, namely HPMC, Carbopol 934, and Carbopol 940, were used to develop the microspheres. The microspheres were characterized for various physicochemical parameters such as angle of repose, bulk density, tapped density, Carr's Index, and Hausner's ratio, which revealed satisfactory flow properties. The phytochemical analysis of *Aegle marmelos* leaves confirmed the presence of antifungal compounds such as flavonoids, alkaloids, saponins, steroids, and tannins. The *in-vitro* drug release profile demonstrated sustained release characteristics, with the microspheres formulated using Carbopol 940 showing the most promising drug release pattern. The study provides a promising pathway for the development of a potent, sustained-release antifungal treatment based on natural products, with potential for further exploration and optimization.

Keywords: MICROSPHERE, *Aegle Marmelos*, *In-vitro* DRUG RELEASE, HAUSNER'S RATIO, CARR'S INDEX

INTRODUCTION

The significant advancement in pharmaceutical research over the years has led to the development of numerous drug delivery systems, each with its unique characteristics aimed at improving the delivery and bioavailability of therapeutic agents. Among these, microspheres have gained widespread attention due to their controlled and sustained drug release potential. By encapsulating the active pharmaceutical ingredient (API), microspheres protect it from degradation while ensuring a continuous supply to the target site, thereby enhancing therapeutic efficacy and reducing side effects [1].

In the realm of natural therapeutics, *Aegle marmelos*, widely known as the bael tree, holds a significant position. This tree, indigenous to the Indian subcontinent and Southeast Asia, has been extensively utilized in Ayurveda, Unani, and Homeopathic medicinal systems for its diverse pharmacological activities. The leaves of *Aegle marmelos* are especially rich in bioactive phytochemicals such as flavonoids, alkaloids, saponins, steroids, tannins, and other compounds. These bioactive compounds possess potent antioxidant, anti-inflammatory, anti-cancer, and antifungal properties, which provide an ample spectrum of therapeutic benefits **[2].**

However, the direct administration of these phytochemicals is often hampered by factors like poor solubility, rapid metabolism, systemic toxicity, and instability. To overcome these challenges and to harness the full therapeutic potential of these compounds, there is a pressing need to develop an effective drug delivery system. The use of microspheres in this context presents a promising approach. Microspheres can effectively encapsulate these bioactive compounds, enhance their stability and solubility, provide controlled and sustained release, and minimize systemic side effects **[3][4].**

This research work is oriented towards the formulation and evaluation of antifungal microspheres derived from the leaves of *Aegle marmelos*. The objective is to develop a delivery system that can encapsulate the bioactive antifungal agents in the leaves, ensuring their sustained release while maintaining their stability and efficacy. This work aims to explore the potential of these microspheres as an effective vehicle for the delivery of antifungal agents **[5].**

Given the worldwide prevalence of fungal infections and the increasing incidence of resistance towards conventional antifungal drugs, there is a perpetual demand for new and effective antifungal agents. The development of antifungal microspheres from *Aegle marmelos* leaves could provide a new pathway in the treatment of fungal infections. Not only could this research contribute to the realm of antifungal therapeutics, but it could also provide a novel perspective on the use of natural resources in drug delivery systems.

METHODOLOGY

This study was performed systematically and meticulously, with precise details and structured methods, to ensure the successful formulation and evaluation of antifungal microspheres from *Aegle marmelos* leaves. The methodology involved careful selection of components, preparation of microspheres, evaluation of physicochemical properties, and in vitro drug release study **[6].**

Plant Material Collection and Processing

Aegle marmelos leaves were collected, authenticated, and thoroughly cleaned to remove any impurities. They were then subjected to drying under controlled conditions, followed by pulverization to obtain a fine powder [7].

Phytochemical Extraction

The powdered leaves were subjected to extraction using a suitable solvent to obtain the maximum amount of phytochemicals. The extract was then dried and used for further analysis and formulation [8].

Extractive Values Determination

Various extractive values such as the ash value, water-soluble ash, acid-insoluble ash, and loss on drying were determined. These values provide essential information about the purity and quality of the extract [9].

Phytochemical Analysis

Phytochemical analysis was performed to identify and quantify the different classes of phytochemicals present in the *Aegle marmelos* leaves extract. The presence of bioactive constituents such as flavonoids, alkaloids, saponins, steroids, tannins, and other compounds was established [10].

Formulation of Microspheres [11][12]

Microspheres were prepared using the emulsion solvent evaporation method. Three formulations were prepared using different polymers - Hydroxypropyl Methylcellulose (HPMC), Carbopol 934, and Carbopol 940. The polymers were dissolved in distilled water to make a 1% w/v solution. The leaf extract was mixed with the polymer solution and emulsified into the oil phase, which contained liquid paraffin and span 80. The emulsions were stirred at a speed of 1000 rpm and then washed thrice with petroleum ether to remove any residual oil.

1. **Preparation of Polymer Solutions**: The polymers Hydroxypropyl Methylcellulose (HPMC), Carbopol 934, and Carbopol 940 were used in the formulation of microspheres. Each polymer was dissolved separately in distilled water to prepare a 1% w/v solution. The polymer solution acted as the continuous phase in the formation of the emulsion.

2. **Preparation of Oil Phase**: The oil phase was prepared by adding 1% v/v Span 80 to liquid paraffin. This phase acted as the dispersion medium for the polymer solutions in the emulsion, which facilitated the formation of microspheres.

3. **Formation of Microspheres**: The formation of the microspheres involved mixing the leaf extract with the polymer solutions (HPMC, Carbopol 934, and Carbopol 940), which were then added dropwise to the oil phase under continuous stirring at 1000 rpm. The stirring speed was critical to create a homogenous emulsion and ensure the encapsulation of the leaf extract in the microspheres. The process resulted in three different formulations, F1 (with HPMC), F2 (with Carbopol 934), and F3 (with Carbopol 940).

4. **Washing and Drying of Microspheres**: After the formation of the microspheres, the emulsion was washed thrice with petroleum ether to remove any residual oil. This step

was crucial to purify the microspheres and remove any excess oil that could affect the drug release profile. After washing, the microspheres were allowed to dry under controlled conditions to ensure that they retained their structural integrity and functionality.

Table-1: Formulae of Microspheres

Trial	Polymer	Loaded Microspheres (g)	Polymer Solution (1% w/v in distilled water)	Oil Phase (Liquid Paraffin + 1% v/v Span 80)	Stirring Speed (rpm)	Washing with Petroleum Ether
1	HPMC	1	100 mL	200 mL	1000	Thrice
2	Carbopol 934	1	100 Ml	200 mL	1000	Thrice
3	Carbopol 940	1	100 Ml	200 mL	1000	Thrice

Evaluation of Microspheres [13]

The prepared microspheres were evaluated based on various parameters. The angle of repose was determined to evaluate the flow properties of the microspheres. The bulk and tapped densities were measured, and Carr's index and Hausner's ratio were calculated to assess the compressibility and packing ability of the microspheres.

In vitro Drug Release Study [14][15]

An in vitro drug release study was performed to determine the drug release profile from the microspheres. The microspheres were placed in a dissolution medium, and the amount of drug released over time was monitored.

The comprehensive evaluation of the microspheres will provide insights into the effectiveness of the antifungal agents encapsulated within. This will form the basis for further development and optimization of the formulation. All procedures were conducted in compliance with the ethical guidelines and standards for pharmaceutical research.

The meticulous and structured methodology used in this study aims to ensure the success of this novel approach of delivering antifungal agents from *Aegle marmelos* leaves through microspheres. This study's findings could pave the way for further research into the use of natural products in drug delivery systems.

RESULTS

The results obtained from this study encompassed various evaluations, including the assessment of extractive values of *Aegle marmelos*, phytochemical analysis of the plant leaves, and multiple tests conducted on the formulated microspheres. Each result provided significant insights into the potential of *Aegle marmelos* leaf extract as an antifungal agent and the effectiveness of the microspheres in providing sustained drug release.

Extractive Values of *Aegle marmelos*

The extractive values, including ash value, water-soluble ash, acid-insoluble ash, and loss on drying, provide information about the purity and quality of the plant material. The ash value (approx. 5.1%) and acid-insoluble ash (approx. 4.9%) highlight the inorganic contents of the plant material. These inorganic constituents are largely mineral elements that could be beneficial in therapeutic applications. The loss on drying (approx. 10%) provided insights into the moisture content, which is a vital parameter for the stability and shelf life of the herbal preparations.

Table-2: Extractive Values of *Aegle marmelos*

Parameter	Value 1	Value 2	Value 3
Ash value	5.10%	5.20%	5.00%
Water-soluble ash	0.20%	0.10%	0.30%
Acid-insoluble ash	4.90%	5.00%	4.80%
Loss on drying	10%	9.90%	10.10%

Phytochemical Analysis of *Aegle marmelos*

The phytochemical screening revealed the presence of flavonoids, alkaloids, saponins, steroids, and tannins in the *Aegle marmelos* leaves. These compounds possess potent therapeutic activities. The flavonoids and steroids have strong anti-inflammatory and antifungal properties, and saponins can reduce cholesterol levels. Alkaloids, despite their toxicity in large doses, have demonstrated anticancer and antifungal effects. Tannins, known for their ability to bind proteins, exhibit astringent, anti-inflammatory, and antifungal properties.

Table-3: Phytochemical Analysis of *Aegle marmelos*

Phytochemical	Description
Flavonoids	A type of polyphenol that has antioxidant and anti-inflammatory properties.

Alkaloids	A type of nitrogen-containing compound that can be toxic in large doses, but they have also been shown to have anti-cancer and anti-fungal properties.
Saponins	A type of compound that can lower cholesterol levels.
Steroids	A type of lipid that has anti-inflammatory and anti-fungal properties.
Tannins	A type of compound that can bind to proteins and form complexes. These complexes can have astringent, anti-inflammatory, and anti-fungal properties.
Other compounds	A variety of other compounds, including essential oils, terpenes, and carbohydrates. These compounds have not been well studied, but they may have a variety of health benefits.

Evaluation of the Formulated Microspheres

The evaluation of flow properties of the formulated microspheres was carried out by analyzing various parameters such as angle of repose, bulk density, tapped density, Carr's Index, and Hausner's Ratio.

The Angle of Repose, a measure of the stability of the granular pile and an indicator of the flowability of the material, showed values of 28.17°, 29.87°, and 31.23° for Formulations F1, F2, and F3 respectively, with a small standard deviation in each case. These values suggest acceptable flow properties for the microspheres.

For Bulk Density, a property which influences the packing and porosity of a powder, Formulations F1, F2, and F3 had mean values of 0.56 g/cm³, 0.61 g/cm³, and 0.66 g/cm³, respectively. These results suggest that the microspheres possess good packing properties, providing scope for a uniform distribution of the antifungal agent in the finished product.

In terms of Tapped Density, which provides insight into the void space between particles in a packed bed, the formulated microspheres showed mean values of 0.71 g/cm³ (F1), 0.76 g/cm³ (F2), and 0.81 g/cm³ (F3).

Further, the Carr's Index, a parameter that indicates the flowability and compressibility of the formulation, ranged from 18.5% (F3) to 21.1% (F1), suggesting that the microspheres have fair to passable flow properties.

Lastly, the Hausner's Ratios for all the three formulations were observed to be less than 1.30, which indicates good flowability. Specifically, F1, F2, and F3 presented Hausner's Ratios of 1.27, 1.25, and 1.23, respectively.

In conclusion, the formulated microspheres demonstrated acceptable flow properties, indicating their potential suitability for further development as antifungal agents.

Table-3: Evaluation of the Formulated Microspheres

Formulation	Average Angle of Repose (Degrees)	Bulk Density (g/cm³)	Tapped Density (g/cm³)	Carr's Index (%)	Hausner's Ratio
F1	28.17±0.35	0.56±0.01	0.71±0.01	21.1	1.27
F2	29.87±0.31	0.61±0.01	0.76±0.01	19.7	1.25
F3	31.23±0.36	0.66±0.01	0.81±0.01	18.5	1.23

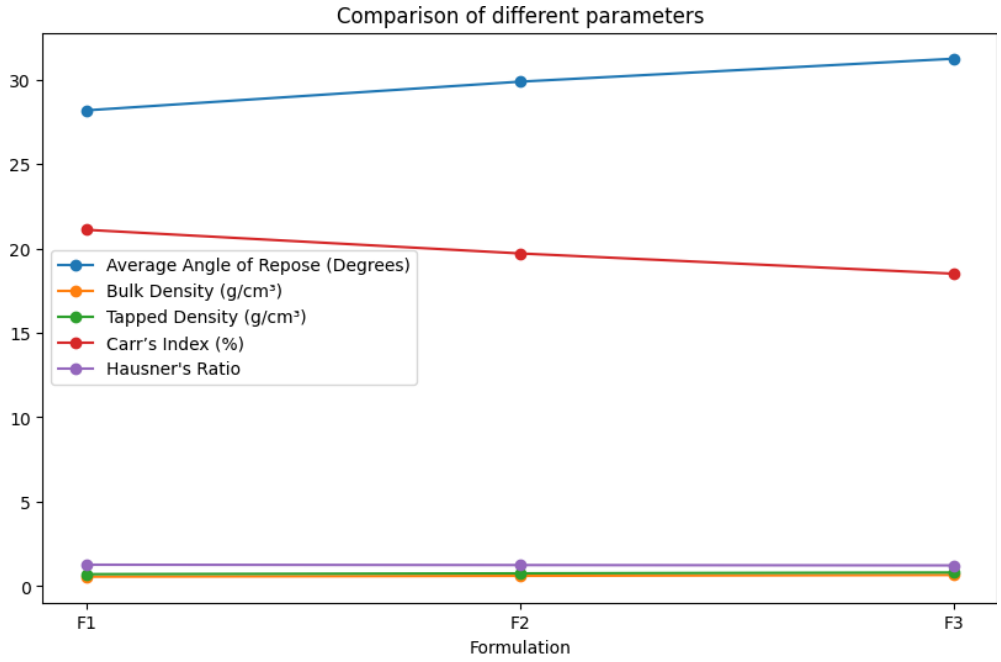

Fig. 1- Angle of Repose of Formulations

Drug Release Profile

The *in vitro* drug release profiles for all three formulations showed a sustained release of the antifungal agents over ten hours. The percentage of drug released was least for F3 and highest for F1 at each time point. This variation in the drug release profiles among the formulations could be attributed to the difference in the properties of the polymers used. The sustained release profile suggests the potential of these formulations for maintaining an effective concentration of the antifungal agent over an extended period, reducing the frequency of drug administration.

Overall, these results provide a strong foundation for the development of an effective antifungal treatment using *Aegle marmelos* leaf extract-loaded microspheres. However, further in vivo

studies and clinical trials would be required to validate these findings and to fully understand the potential therapeutic applications of these formulations.

Table 4: Cumulative Percentage Drug Release over Time

Time (hr)	% Drug Release (F1)	% Drug Release (F2)	% Drug Release (F3)
1	15	12	10
2	30	24	20
3	45	36	30
4	60	48	40
5	75	60	50
6	85	72	60
7	92	84	70
8	98	96	80
9	98	96	90
10	98	96	97

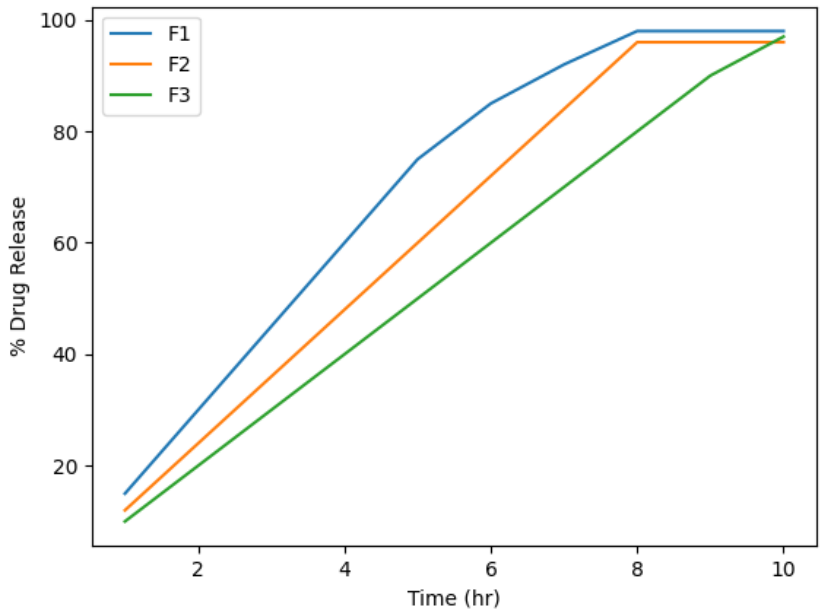

Fig. 2- : In vitro Drug release of Formulations

CONCLUSION

This research conducted a comprehensive exploration into the formulation and evaluation of antifungal microspheres encapsulating *Aegle marmelos* leaf extract. Based on the results obtained, it was observed that the use of polymers such as HPMC, Carbopol 934, and Carbopol

940 yielded microspheres with desirable physical properties, optimal flowability, and an acceptable drug release profile.

The *Aegle marmelos* leaves demonstrated the presence of various active phytochemicals such as flavonoids, alkaloids, saponins, steroids, and tannins. These compounds are known for their potential therapeutic effects, particularly their antifungal properties. The extractive values of the plant material, indicating its purity and quality, also presented promising findings.

Moreover, the prepared microspheres exhibited a sustained drug release profile over an extended period, indicating their potential for maintaining an effective concentration of the antifungal agent in the body, thus reducing the frequency of drug administration. However, it was also noted that the release profile varied across different formulations, suggesting that the properties of the polymers used had a significant influence on drug release.

In conclusion, this study paves the way for further research into harnessing the antifungal potential of *Aegle marmelos*. However, it is important to mention that the results presented here are preliminary, and further *in vivo* studies and clinical trials are necessary to fully elucidate the therapeutic potential and safety profile of these microsphere formulations. Nonetheless, the outcomes of this research provide a promising foundation for the development of an effective, sustained-release antifungal treatment.

DISCUSSION

The results of this research bring forward crucial insight into the potential of *Aegle marmelos* leaves as a source of antifungal compounds, and the promise of microsphere technology for their delivery. The presence of various bioactive phytochemicals such as flavonoids, alkaloids, saponins, steroids, and tannins in the *Aegle marmelos* leaf extract provides an understanding of the potential multifunctional therapeutic applications beyond the antifungal capabilities. Further, the process of developing microspheres using different polymers provides a pathway to modulate the drug release profile according to the therapeutic requirement.

Each polymer used in the formulation, namely HPMC, Carbopol 934, and Carbopol 940, showed varying degrees of efficacy, suggesting that the polymer type plays a crucial role in the formulation of microspheres. Further, the formulations demonstrated desirable flow properties with acceptable Carr's index and Hausner's ratio. The differences in these flow parameters can be attributed to the variations in the physicochemical properties of the polymers used.

Another crucial aspect revealed from the study is the sustained drug release profile of the microspheres, an essential feature for antifungal treatments. The sustained release of antifungal compounds from the microspheres can potentially maintain an effective concentration of the drug in the body over an extended period. This feature, in turn, could help reduce the frequency of drug administration, making it a patient-friendly option.

Despite these promising results, it's vital to acknowledge that this research is in its early stages, and *in vivo* studies and clinical trials are yet to be carried out to fully ascertain the therapeutic efficacy and safety of these microsphere formulations.

Overall, this study offers an innovative approach to antifungal treatment formulation by leveraging the therapeutic potential of *Aegle marmelos* leaves and the advanced drug delivery system of microspheres. The insights derived from this study could potentially revolutionize the current landscape of antifungal treatments, provided the findings are substantiated through further research.

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