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Research Article

Evaluation of Antifungal Potential of *Syzygium cumini (L.) Skeels* Ethosomal Gel

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Abstract:
The present study focuses on the development of a gel formulation incorporating leaf extract of Syzygium cumini L. and its evaluation for in-vitro anti-fungal activity. Syzygium cumini L. (Skeels), a widely cultivated medicinal plant, is known for its diverse pharmacological properties. In this investigation, ethosomal formulations of S. cumini extract were prepared and incorporated into a gel base. The anti-fungal efficacy of the formulation was assessed against Aspergillus flavus and Aspergillus niger using the agar well diffusion method. The results revealed a concentration-dependent increase in anti-fungal activity, with significant inhibition observed against A. niger. The performance of the gel formulation was compared with fluconazole, used as the standard reference at a concentration of 150 µg/mL. The S. cumini ethosomal gel demonstrated promising anti-fungal potential, suggesting its applicability as a natural therapeutic agent for fungal infections. Further studies are warranted to confirm its mechanism of action and efficacy in vivo.
Keywords: Syzygium cumini, ethosomal gel, anti-fungal activity, Aspergillus niger, Aspergillus flavus, agar well diffusion, fluconazole.

Introduction

Fungi constitute a separate kingdom of eukaryotic organisms, distinct in their cellular structure and ecological role. Unlike prokaryotic bacteria, fungal cells possess a true nucleus and organelles, and although they share some molecular features with plants and animals, they are unique in their physiology. A notable characteristic is their rigid cell wall made primarily of chitin, which differentiates them from mammalian cells that lack such a structure. Furthermore, fungi are non-photosynthetic and do not contain chlorophyll, thereby excluding them from the plant kingdom. Their structure varies from unicellular forms to multicellular filaments known as hyphae, allowing them to thrive in a wide range of environments. Species such as Aspergillus and Candida are ubiquitous and are frequently isolated from soil, air, water, plant surfaces, and even human skin and mucous membranes. These fungi have been implicated in opportunistic infections, particularly in immunocompromised patients [1].

Syzygium cumini Skeels, commonly referred to as Jamun or black plum, is a well-known plant that is extensively cultivated in the Indian subcontinent and several Southeast Asian countries including Bangladesh, Nepal, Sri Lanka, and Indonesia [2]. Traditionally, the leaves of S. cumini have been employed in folk medicine for their stimulant, diuretic, and expectorant properties [3]. Phytochemical investigations have confirmed the presence of various bioactive compounds such as flavonoids, glycosides, phenolic compounds, sterols, triterpenes, proteins, and carbohydrates [4-6]. Previous studies have reported multiple pharmacological activities of S. cumini leaf extract, including antioxidant [7], antidiabetic [8], anti-nociceptive, anti-hyperglycaemic [9], and even anticancer effects [10].

To enhance the delivery and efficacy of plant-based extracts, novel carriers such as ethosomes have emerged as effective drug delivery systems. Ethosomes are phospholipid-based vesicles containing high concentrations of ethanol, which enhances the permeability of bioactive compounds through the stratum corneum by disrupting its lipid organization [11-12]. These vesicles are characterized by their flexible and malleable membranes, allowing for deeper skin penetration and better drug retention compared to conventional liposomes. Their sizes typically range from nanometers to micrometres, making them suitable for topical and transdermal applications [13].

Given the therapeutic potential of S. cumini and the advantages of ethosomal drug delivery, the current study aims to develop an ethosomal gel formulation of S. cumini leaf extract and evaluate its in-vitro antifungal activity against Aspergillus flavus and Aspergillus niger. This research seeks to assess the feasibility of using a natural, plant-based formulation as an effective antifungal treatment [14].

Materials and Methods

Material: Around 1kg S. cumini leaves were Collected, washed in running tap water and then rinsed with distilled water was subjected to drying at room temperature for about two weeks. The dried leaves were powdered using mixer grinder and passed through sieve no 80. The coarse powder was extracted with Methanol in Soxhlet's apparatus at a temperature not exceeding 40°C for 96 hours. The extract was concentrated under reduced pressure in rotary evaporator to yield a crude semi-solid mass percentage of yield. After drying the extracts was used for Photochemical, chromatography and pharmacological screening.

Preparation of ethosomal gel: Ethosomal vesicles suspensions were incorporated by dispersion method using Carbopol 934 dispersing gelling agent. The specified amount of Carbopol 934 powder was allowed to swell overnight, tri ethanolamine was added to drop by drop neutralized mixture, optimized ethosomal dispersion was added and mixed properly. Mixing was continued until a transparent gel appeared and added preservative; The prepared gels were filled in glass container stored at 4-8 °C.

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

GC-MS information

- Make: Perkin Elmer Gc
- Model: Clarus 680
- Mass Spectrometer: Clarus 600 (Ei)
- Software: Turbomass Ver 5.4.2
- Library Year: Nist-2008

Acquisition Parameters

- Oven: Initial Temp 60°C For 2 Min, Ramp 10°C/Min To 300°C, Hold 6 Min,
- Total Run Time: 32.00 Mint
- Inject auto = 260°C,
- Volume =1 Ml, Split=10:1,
- Flow Rate: 1 Ml/Mint
- Carrier Gas = He,
- Column = Elite-5MS (30.0m, 0.25mmid, 250µm Df)

Gc-Ms Analysis: The spectrums of the components were compared with the database of

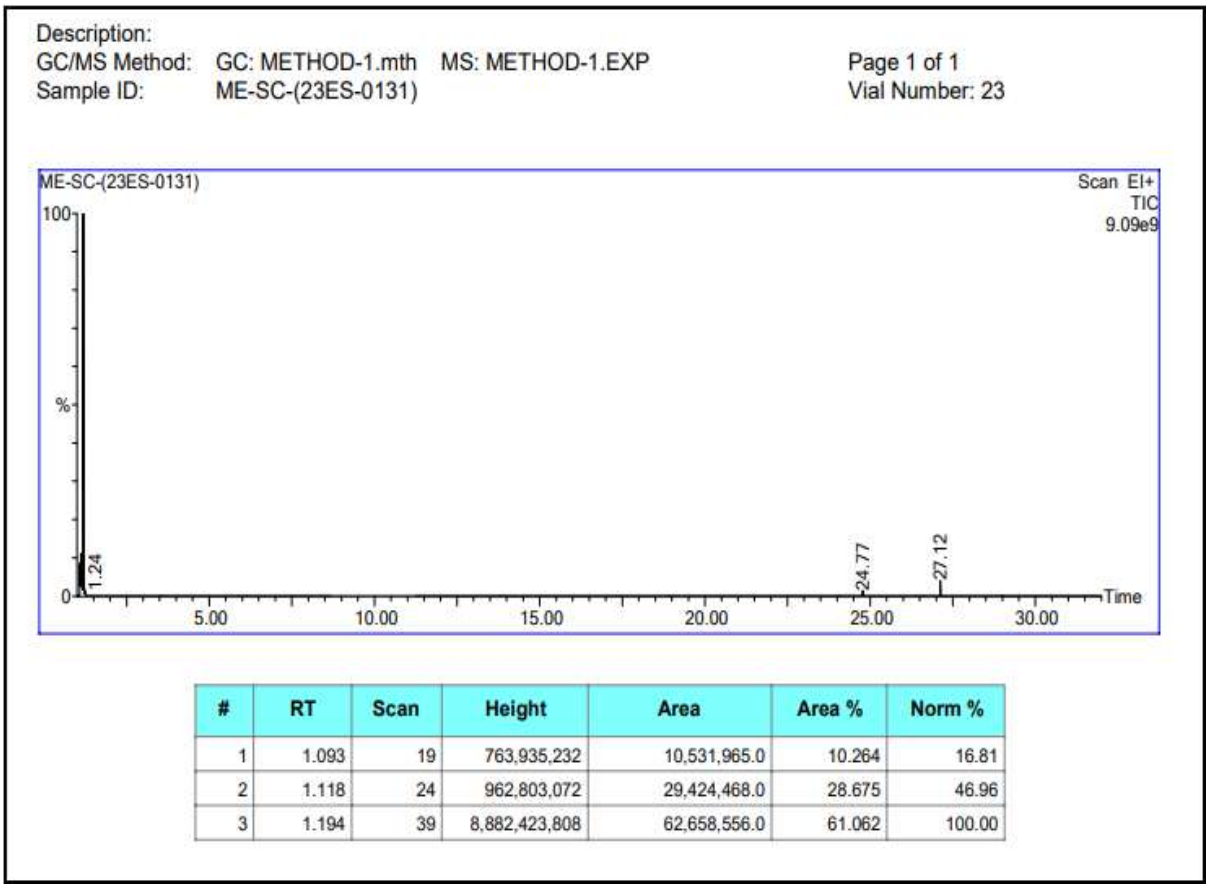
spectrum of known components stored in the GC-MS NIST (2008) library.

Anti-fungal Test by well diffusion method: Antibiotic susceptibility tests were determined by agar well diffusion (Kirby-Bauer) method. Fungal strains viz., A. niger and A. flavus were swabbed using sterile cotton swabs on SDA agar plate. Six wells (10mm diameter and about 2 cm a part) were made in each of these plates using sterile corkborer (6mm in diameter). About 100µl of samples with required concentrations (50ug/ml to 200ug/ml) were added using sterile pipette into the wells and allowed to diffuse at room temperature and the plates were kept for incubation at 37°C for 48 hours. At the end of incubation, inhibition zones were examined around the disc and measured with transparent ruler in millimetres. The size of the zone of inhibition (ZOI) was measured in millimetres. The absence of zone inhibition was interpreted as the absence of activity (16-17). The activities are expressed as resistant, if the zone of inhibition (ZOI) was less than 7 mm, intermediate (8-10 mm) and sensitive if more than 11 mm (18). Fluconazole with 150ug/ml was used as a std control for the current study.

Statistical Analysis: All the in vitro experimental data were presented as mean ± S.E.M (M± standard error) of three parallel measurements and data were evaluated by graph pad Statistical software (one way), p – values <0.05 were regarded as significant followed by posthoc

Results

GC-MS Analysis: S. Cumini extract GC–MS chromatogram shows 03 peaks representing the presence of 03 bio active compounds. Bioactive compounds are identified from the peak area, retention time and the molecular formula & weight. (Figure 1)



#	RT	Scan	Height	Area	Area %	Norm %
1	1.093	19	763,935,232	10,531,965.0	10.264	16.81
2	1.118	24	962,803,072	29,424,468.0	28.675	46.96
3	1.194	39	8,882,423,808	62,658,556.0	61.062	100.00

Fig. 1: GC-MS Chromatogram of S. Cumini Leaf Extract

Evaluation of S. Cumini Ethosomal Gel

Physical Evaluation: Ethosomal gel was studied for organoleptic Properties, grittiness, greasiness, ease of application and wash ability.

- **Color:** Slightly Greenish Brown
- **Physical Appearance:** The Gel Smooth in Appearance.
- **Greasiness:** Non-Greasy
- **Grittiness :** Free From Grittiness
- **Ease Of Application:** Easily/Smoothly Applied
- **Skin Irritation:** No Skin Irritation
- **Wash Ability:** Easily Washable

Fig. 2: S. Cumini Ethosomal Gel

Anti-fungal activity of S. cumini ethosomes gel:

Fig. 3: Anti-fungal activity of S. cumini against the A. flavus and A. niger in comparison to Positive control (Fluconazole-150ug/ml) and Negative control (Distilled water) and found that the S. cumini showed the anti-fungal activity on concentration dependent fashion after the 48hours of incubation.

Table.1: Zone of Inhibition (mm) values of S. cumini ethosomes with different concentrations against the A. niger and A. flavus after the 48hours of incubation period

Anti-fungal study-Well diffusion method-S. cumini ethosomes (ZOI in mm)		
Drug conc. (ug/ml)	**A. niger**	**A. flavus**
Negative control	0	0
Fluconazole -150ug	28	18
50ug	7	NZI
100ug	9	NZI
150ug	11	NZI
200ug	15	NZI

***NZI – No Zone Inhibition, ZOI – Zone of Inhibition**

Discussion: Natural sources, particularly the plants and herbs, have always been the scientist's first choice in the discovery and development of the therapeutic agents due to the presence of the phytoconstituent with various medicinal properties. Besides, medicinal agents obtained from plants are generally considered safe and are recommended for various ailments. Many plants have been studied and are reported to contain the phytoconstituent effective against various pathogens[16]. Resistance to antibiotics is of more significant concern worldwide due to rising incidences of resistance in the pathogens against the antimicrobials and antibiotics. This increase in the resistance is attributed to the random, indiscriminate use of the available antimicrobial drugs. Therefore, the scientists are continually looking for newer antimicrobial agents, particularly from the natural sources[17].

Conclusion: In this study, antimicrobial activity of given test compound viz., S. cumini ethosome with various concentrations was assessed by Agar well diffusion method. The observed results showed satisfactory anti-fungal effect of the molecule on concentration dependent fashion against the microbe tested, A. niger comparison to the std control used for the study. Fluconazole with 150ug/ml concentration was used as a reference std control for the current study.

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