Antifungal Activity of Secondary Metabolites from *Lantana Camara* L. against Bio-Deteriorating Fungi of *Oryza Sativa* L. (Nigerian Local Rice)

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ABSTRACT

Fungal contamination in rice can result in the production of mycotoxins, posing significant health risks to humans. This study investigated the antifungal efficacy of selected secondary metabolites, terpenoids, saponins, alkaloids, and flavonoids, as well as the antioxidant properties of *Lantana camara* L. against fungal pathogens in locally cultivated rice. The plant extract was obtained, and fungal pathogens were isolated and identified using standard microbiological techniques. Antioxidant activity was assessed using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and Ferric Reducing Ability of Plasma (FRAP) assays, while antifungal susceptibility was evaluated via the agar diffusion plating method. Statistical analysis was conducted using ANOVA with Tukey's HSD test at $p < 0.05$.

The fungal species identified in the rice samples included *Aspergillus niger, Nigrospora oryzae, Fusarium oxysporum, Rhizopus* sp., *Alternaria alternata*, and *Rhizopus oryzae*. A total metabolite yield of 63.18% was obtained from 1300 g of plant material, with phytochemical screening confirming the presence of terpenoids, flavonoids, saponins, tannins, cardiac glycosides, anthraquinones, steroids, alkaloids, and phenols. Antioxidant assays revealed a concentration-dependent increase in radical scavenging activity, whereas absorbance values declined at higher metabolite concentrations. Among the tested metabolites, alkaloids exhibited the highest antifungal activity, effectively inhibiting the radial growth of all fungal isolates, whereas saponins demonstrated the least potency, inhibiting only *Rhizopus oryzae*.

These findings indicate that secondary metabolites from *L. camara* exhibit significant antifungal activity at minimal effective concentrations. Further activity-guided fractionation is recommended to isolate and characterize the most potent antifungal compounds for potential application in rice pathogen management.

Keywords: Antimicrobial, Antioxidant, Fungal infection, Rice, Secondary metabolites,

INTRODUCTION

Nature has long served as a source of therapeutic agents, with a substantial proportion of modern pharmaceuticals being derived from natural compounds (Prince & Prabakaran, 2018). The presence of diverse phytochemicals in various plant tissues highlights their potential as reservoirs of bioactive molecules with medicinal applications (Fayaz *et al.,* 2017). Plant-derived extracts and their active constituents have been

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extensively investigated for their antimicrobial properties and exhibit significant therapeutic potential. Medicinal plants, in particular, are rich in secondary metabolites, many of which possess potent antimicrobial activities (Mahesh & Satish, 2018).

Plants remain a valuable resource for novel antibiotic discovery, with many species demonstrating considerable antioxidant properties that confer protective benefits. The medicinal efficacy of plant materials is often attributed to the synergistic interactions of various secondary metabolites, including alkaloids, steroids, tannins, phenolics, flavonoids, resins, and fatty acids, which elicit diverse physiological responses.

Lantana camara L., a small perennial shrub of the Verbenaceae family, grows up to 2 meters in height and forms dense thickets across a range of ecological zones. Native to the American tropics (Kalita *et al.,* 2017), the plant has been widely utilized in traditional medicine for the treatment of numerous ailments (Sorkhabi-Abdolmaleki *et al.,* 2018). It is a rich source of bioactive compounds, including steroids, flavonoids, oligosaccharides, triterpenoids, glycosides, and naphthoquinones (Sorkhabi-Abdolmaleki *et al.,* 2018; Begum *et al.,* 2018). Phytochemicals isolated from *L. camara* have been reported to exhibit a broad spectrum of biological activities, including anticancer, antioxidant, hepatoprotective, leishmanicidal, antibacterial, nematicidal, and antiulcer properties (Sathish *et al.,* 2018; Begum *et al.,* 2018).

Rice (*Oryza sativa* L.) is a staple food for over half of the global population and represents the most extensively cultivated cereal crop worldwide (Dogara & Jumare, 2018). Annually, approximately 480 million metric tons of milled rice are produced, with China and India contributing nearly 50% of global production. In Nigeria, rice consumption per capita is estimated at 32 kg, with an annual increase of 4.7% over the past decade, leading to a total consumption of 6.4 million metric tons in 2017, compared to an annual domestic production of 3.7 million metric tons. Cereal grains, including rice, are highly susceptible to fungal contamination, particularly in regions where cultivation involves flood irrigation, creating high-moisture conditions that favor fungal proliferation and mycotoxin biosynthesis (Majeed *et al.,* 2018). Additionally, inadequate post-harvest handling practices exacerbate the risk of fungal invasion during storage and transportation (Zhao *et al.,* 2019). Given the extensive consumption of rice in Nigeria, fungal contamination presents a significant public health concern due to the potential production of toxic secondary metabolites, known as mycotoxins.

Mycotoxins are toxic secondary metabolites synthesized by various fungal species, persisting in food commodities throughout storage and processing (WHO, 2018). The majority of mycotoxigenic fungi belong to the genera *Aspergillus, Fusarium, Talaromyces*, and *Penicillium*, which are known to produce well-characterized mycotoxins (Phoku *et al.,* 2017; Omotayo *et al.,* 2019). Fungal contamination of rice can occur both pre-harvest, due to environmental exposure, and post-harvest, during handling and storage (Omotayo et al., 2019). Rice grains are typically harvested with high moisture content, providing a conducive environment for fungal colonization and subsequent mycotoxin production. Although mycotoxin contamination in rice has been extensively investigated in several countries (Ferre, 2016), studies on its occurrence in Nigerian rice remain limited (Sinphithakkul *et al.,* 2019).

The increasing prevalence of antifungal resistance among foodborne pathogens necessitates the exploration of alternative, plant-based antimicrobial compounds for food preservation. Many plants produce phytochemicals with medicinal properties, which have historically been used in disease treatment and control. Contemporary research highlights not only the direct antimicrobial effects of plant-derived compounds but also their antioxidant properties, which contribute to human health benefits. Due to concerns regarding the toxicity of synthetic food preservatives, the use of natural antimicrobial agents has gained increasing attention. The presence of synthetic preservatives in food products, as indicated by labeling requirements, remains a major consumer concern. Consequently, the search for effective, plant-derived antimicrobial agents continues to be a priority.

Fungal contamination is particularly problematic in tropical climates such as Nigeria, where high temperature and humidity favor mycotoxin biosynthesis. These conditions, combined with the heavy reliance on rice as a dietary staple, elevate the risk of chronic mycotoxin exposure. Mycotoxin production may occur at various stages of the rice production cycle, including pre-harvest (e.g., deoxynivalenol, ergot alkaloids), post-harvest

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(e.g., fumonisins, ochratoxins), and storage (e.g., aflatoxins). Owing to their chemical stability, mycotoxins are resistant to degradation by conventional food processing methods, including autoclaving.

The emergence of antifungal resistance has driven the search for novel bio-fungicides, with plant-derived secondary metabolites offering promising alternatives. The objective of this study was to evaluate the antifungal activity of selected secondary metabolites—terpenoids, saponins, alkaloids, and flavonoids extracted from *Lantana camara* L., as well as their antioxidant properties, against toxigenic fungi associated with Nigerian local rice.

MATERIALS AND METHODS

Collection and Preparation of Plant and Rice Samples

Fresh leaves of *Lantana camara* were collected from various locations within the Ibadan metropolis and placed in well-labeled Ziploc bags. Botanical identification and authentication were conducted at the university herbarium before the samples were transported to the laboratory for further analysis. The leaves were surface-sterilized using a 10% sodium hypochlorite (NaOCl) solution for approximately 2 minutes to eliminate unwanted microorganisms, followed by three successive rinses with sterile distilled water to remove residual NaOCl (Olaomi, 2023). The sterilized leaves were then air-dried at room temperature until a constant weight was achieved.

Samples of infected rice, including leaves, roots, stems, and grains, were collected from a rice farm in Mokwa, Niger State, Nigeria. The plant materials were placed in well-labeled Ziploc bags and transported to the laboratory for subsequent analysis (Jonathan, 2019;).

Extraction of Plant Metabolites

A total of 1300 g of dried and pulverized *Lantana camara* leaf material was transferred into a glass container, followed by the addition of 5000 mL (5 L) of pure methanol. The mixture was stirred at 2-hour intervals and allowed to undergo maceration for 72 hours. The solvent, now containing the extracted metabolites, was separated using a muslin bag, and the filtrate was further purified by passage through Whatman No. 1 filter paper (1 mm pore size).

To maximize extraction efficiency, the residual plant material (marc) was subjected to a second extraction cycle using an additional 5000 mL (5 L) of pure methanol. The combined filtrates were subsequently concentrated using a rotary evaporator (Heidolph Laborota 400 Efficient, Germany, Model 517-01002-002) maintained at 40°C. Further concentration was performed in a vacuum oven at 40°C under a pressure of 700 mmHg to ensure complete solvent removal.

Isolation of Fungal Pathogens from Rice Grains

Approximately 10 g of rice grains were surface-sterilized using a 0.5% sodium hypochlorite (NaOCl) solution, followed by ten successive washes with 100 mL of sterile distilled water to eliminate residual disinfectant. Ten grains were randomly placed in each Petri dish containing potato dextrose agar (PDA) supplemented with chloramphenicol (500 mg/L) to inhibit bacterial contamination. The inoculated plates were incubated at room temperature and monitored daily for fungal growth over five days. Emerging fungal colonies were subcultured onto PDA slants in sterile media bottles and freshly prepared PDA plates for subsequent identification (Okpewho *et al.,* 2024).

Isolation of Fungal Pathogens from Plant Samples

The collected plant samples were rinsed with sterile distilled water for approximately 2 minutes to dislodge surface-associated microorganisms into sterile sampling bottles. The resulting suspension was subjected to serial dilution up to a 10^{-5} dilution factor. Aliquots of the diluted samples were inoculated onto Petri dishes containing potato dextrose agar (PDA) supplemented with chloramphenicol (500 mg/L) to inhibit bacterial growth. The inoculated plates were incubated at room temperature and monitored daily for fungal growth over

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five days. Emerging fungal colonies were subsequently subcultured onto PDA slants in sterile media bottles and freshly prepared PDA plates for further identification (Anakaa *et al.,* 2025).

Qualitative Phytochemical Screening

The qualitative phytochemical screening of the crude methanol extracts was performed using standard procedures as described by Sofowora (2008).

Tannins

A 0.1 g portion of the extract was mixed with 10 mL of distilled water, filtered, and treated with a 0.1% ferric chloride ($FeCl_3$) solution. The development of a brownish-green or blue-black coloration indicated the presence of tannins.

Saponins

A 0.1 g portion of the extract was boiled with 20 mL of distilled water in a water bath and filtered. A 10 mL aliquot of the filtrate was mixed with 5 mL of distilled water and vigorously shaken to generate a stable froth. The addition of three drops of olive oil and subsequent emulsion formation confirmed the presence of saponins.

Flavonoids

A few drops of 1% ammonia (NH_3) solution were added to the aqueous extract. A yellow coloration, which disappeared upon the addition of concentrated sulfuric acid, indicated the presence of flavonoids.

Terpenoids

A 0.1 g portion of the extract was dissolved in 2 mL of chloroform. Subsequently, 3 mL of concentrated sulfuric acid (H_2SO_4) were carefully added to form a distinct layer. The presence of terpenoids was confirmed by the appearance of a reddish-brown coloration at the interface.

Glycosides

A 0.1 g portion of the extract was diluted in 5 mL of distilled water, followed by the addition of 2 mL of glacial acetic acid containing one drop of ferric chloride ($FeCl_3$). The mixture was underlaid with 1 mL of concentrated sulfuric acid. The presence of glycosides was indicated by the formation of a brown ring at the interface, with the possibility of a violet ring below and a greenish ring in the acetic acid layer, which gradually dispersed throughout the solution.

Alkaloids

A 0.1 g portion of the extract was dissolved in 10 mL of acidified ethanol, boiled, and filtered. A 5 mL aliquot of the filtrate was treated with 2 mL of dilute ammonia, followed by 5 mL of chloroform. After gentle shaking, the chloroform layer was separated and further extracted with 10 mL of acetic acid. This solution was divided into two portions: Mayer's reagent was added to one, and Dragendorff's reagent to the other. The appearance of a cream-colored precipitate (Mayer's reagent) or a reddish-brown precipitate (Dragendorff's reagent) confirmed the presence of alkaloids.

Anthraquinones

A 1.1 g portion of the extract was boiled with 10 mL of sulfuric acid (H_2SO_4) and filtered while hot. The filtrate was extracted with 5 mL of chloroform, and the chloroform layer was transferred into a separate test tube. The addition of 1 mL of dilute ammonia resulted in color changes indicative of anthraquinones.

Steroids

A 0.1 g portion of the extract was dissolved in chloroform, shaken, and filtered into a separate test tube. A few

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drops of acetic anhydride were added, followed by the addition of concentrated sulfuric acid. The development of a green coloration confirmed the presence of steroids.

Phenols

A 0.1 g portion of the extract was dissolved in ethanol, shaken, and filtered into a separate test tube. The addition of a few drops of ferric chloride ($FeCl_3$) resulted in a blue-black coloration, indicating the presence of phenolic compounds.

Isolation of Selected Secondary Metabolites and Antifungal Susceptibility Testing

Isolation of Alkaloids

The extraction of alkaloids was conducted following the method described by Krishnaiah et al. (2009). A 10.0 g portion of the crude extract was transferred into a 250 mL beaker, and 200 mL of 10% acetic acid in ethanol was added. The mixture was covered and left to stand for 4 hours before filtration. The filtrate was concentrated in a water bath to one-quarter of its initial volume, after which concentrated ammonium hydroxide (NH_4OH) was added until complete precipitation was achieved. The resulting precipitate was allowed to settle, collected on a pre-weighed filter paper, and washed with dilute NH_4OH. The dried alkaloid fraction was collected, weighed, and the percentage yield was determined by difference.

Isolation of Flavonoids

Flavonoid extraction was performed using the method described by Bohm and Kocipai-Abyazan (1994). A 10 g portion of the plant extract was repeatedly extracted with 100 mL of 80% aqueous methanol at room temperature. The resulting solution was filtered through Whatman No. 42 filter paper (125 mm) and the filtrate was evaporated to dryness using a water bath. The dried extract was weighed to obtain a constant mass.

Isolation of Terpenoids

A 20 g portion of the crude extract was placed in a 250 mL beaker and mixed with 200 mL of absolute ethanol. The mixture was covered and allowed to stand for 4 hours before being filtered. The filtrate was then concentrated using a water bath until it reached one-quarter of its original volume. The concentrated extract was subjected to liquid-liquid partitioning with petroleum ether in a separating funnel. The petroleum ether layer was collected, dried, and weighed to determine the terpenoid content by difference.

Isolation of Saponins

A 20.0 g portion of the crude extract was introduced into a 250 mL conical flask, and 100 mL of 20% ethanol was added. The mixture was heated at approximately 55°C for 4 hours with continuous stirring, followed by filtration. The residue was re-extracted with an additional 200 mL of 20% ethanol, and the combined filtrates were concentrated over a water bath at approximately 90°C until the volume was reduced to 40 mL. The concentrated extract was transferred into a 250 mL separating funnel, and 20 mL of diethyl ether was added. After vigorous shaking, the aqueous layer was retained, while the ether layer was discarded. This purification process was repeated. A 60 mL volume of n-butanol was then added, and the n-butanol extract was washed twice with 10 mL of 5% sodium chloride (NaCl) solution. The final extract was heated in a pre-weighed 250 mL beaker using a water bath until complete evaporation. The residue was further dried in an oven to a constant weight, and the percentage saponin content was determined by difference.

Antifungal Susceptibility Testing

A 20 mL aliquot of sterilized Sabouraud Dextrose Agar (OXOID) was aseptically poured into sterile Petri dishes and allowed to solidify for approximately 10 minutes. The plates were further dried in a hot air oven to remove residual moisture from both the agar surface and Petri dish covers. The spread plate method was employed, in which a 0.5 McFarland standard dilution of a 72-hour fungal broth culture was evenly spread across the agar surface using a sterile cotton swab. A sterile cork borer (6 mm in diameter) was flamed, cooled,

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and used to create wells in the inoculated agar medium. Pre-prepared concentrations of the plant extracts were dispensed into the wells, with proper labeling. Dimethyl sulfoxide (DMSO) was used as a negative control to verify the absence of antifungal activity. The plates were left undisturbed for approximately one hour to facilitate adequate diffusion of the extracts before being incubated at 25°C for 72 hours. Following the incubation period, the diameters of the inhibition zones were measured in millimeters and recorded.

Data Analysis

The inhibitory effects of varying concentrations of secondary metabolites were statistically evaluated using a one-way analysis of variance (ANOVA). All statistical analyses were performed utilizing GraphPad Instant Statistical Software (Version 3.0), with statistical significance established at $p < 0.05$.

RESULTS

Table 1: Fungal Isolates obtained from Rice Plant Parts

Sample	Plant Part	Isolated Fungi
A	Leaf	*Rhizopus oryzae*, *Aspergillus niger*, *Nigro oryzae, Fusarium oxysporum*
B	Stem	*Aspergillus niger*, *Fusarium oxysporum*, *Rhizopus sp*., *Alternaria alternate*
C	Root	*Rhizopus sp*., *Alternaria alternata*, *Aspergillus niger*
D	Grain	*Aspergillus niger*, *Rhizopus sp*., *Fusarium oxysporum*, *Alternaria alternate*
E	Root	*Aspergillus niger*, *Alternaria alternate*
F	Stem	*Alternaria alternata*, *Rhizopus sp*., *Nigro orzyae*
G	Leaf	*Rhizopus sp*., *Nigro oryzae*, *Aspergillus niger*, *Alternaria alternata*, *Rhizopus orzyae*
H	Grain	*Aspergillus niger*, *Fusarium oxysporum*
I	Stem	*Rhizopus sp*., *Aspergillus niger*
J	Grain	*Rhizopus oryzae Alternaria alternata*, *Aspergillus niger*, *Nigro oryzae*, *Fusarium oxysporum*, *Rhizopus sp*.

Various fungal pathogens were consistently isolated from different parts of the rice samples, including *Aspergillus niger*, *Nigro oryzae*, *Fusarium oxysporum*, *Rhizopus* sp., *Alternaria alternata*, and *Rhizopus oryzae*. The frequency of occurrence varied among the fungal species.Among the identified fungi, *Aspergillus niger* exhibited the highest frequency of occurrence at 23.5%, followed by *Rhizopus* sp. and *Alternaria alternata* at 20.6%. *Fusarium oxysporum* had a frequency of 14.7%, while *Nigro oryzae* was recorded at 11.8%. The least frequently occurring species was *Rhizopus oryzae*, with a frequency of 8.8% (Table 1).

Table 2: Phytochemical constituents of crude methanol extract of *Lantana camara*

Metabolites	Presence
Saponins	+
Tannins	+
Flavonoids	++
Cardiac glycosides	+

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Anthraquinones	+
Terpenoids	++
Steroids	+
Alkaloids	+
Phenol	+

+ = present; ++ = abundant; - = absent

A total yield of 63.178% was obtained from 1300 g of crude methanolic extract of *Lantana camara*. The percentage yields of selected secondary metabolites were determined using specific quantities of the crude extract. From 10 g of the crude extract, the percentage yields of alkaloids and flavonoids were 3.532% and 4.923%, respectively. Additionally, 20 g of the crude extract was utilized to determine the yields of saponins and terpenoids, which were found to be 7.192% and 7.141%, respectively.

Qualitative phytochemical screening of the methanolic extract confirmed the presence of a diverse array of bioactive constituents. Terpenoids and flavonoids were detected in high abundance, while saponins, tannins, cardiac glycosides, anthraquinones, steroids, alkaloids, and phenols were also present in appreciable amounts. The findings indicate a higher concentration of terpenoids and flavonoids than saponins, tannins, cardiac glycosides, anthraquinones, steroids, alkaloids, and phenols (Table 2).

Table 3: Absorbance values of FRAP determination for Alkaloids

Concentration	Abs1	Abs2	Abs3	Mean	Mean Abs blank
20ug/ml	0.574	0.577	0.578	0.576	0.443
40ug/ml	0.662	0.661	0.665	0.663	0.530
60ug/ml	0.726	0.722	0.720	0.723	0.590
80ug/ml	0.774	0.773	0.770	0.772	0.639
100ug/ml	0.804	0.800	0.804	0.803	0.670
Blank	0.133	0.133	0.133	0.133	***

Table 4: Absorbance values of FRAP determination for Flavonoids

Concentration	Abs1	Abs2	Abs3	Mean	Mean Abs blank
20ug/ml	0.675	0.677	0.671	0.674	0.541
40ug/ml	0.804	0.804	0.809	0.806	0.673
60ug/ml	1.506	1.500	1.504	1.503	1.370
80ug/ml	1.599	1.595	1.594	1.596	1.463
100ug/ml	1.807	1.801	1.800	1.803	1.670
Blank	0.133	0.133	0.133	0.133	***

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Table 5: Absorbance values of FRAP determination for Saponins

Concentration	Abs1	Abs2	Abs3	Mean	Mean Abs blank
20ug/ml	0.576	0.577	0.573	0.575	0.442
40ug/ml	0.841	0.842	0.844	0.842	0.709
60ug/ml	1.094	1.091	1.097	1.094	0.961
80ug/ml	1.109	1.105	1.106	1.107	0.974
100ug/ml	1.111	1.119	1.117	1.116	0.983
Blank	0.133	0.133	0.133	0.133	***

Table 6: Absorbance values of FRAP determination for Terpenoids

Concentration	Abs1	Abs2	Abs3	Mean	Mean Abs blank
20ug/ml	0.551	0.555	0.559	0.555	0.422
40ug/ml	0.607	0.605	0.604	0.605	0.472
60ug/ml	0.672	0.677	0.670	0.673	0.540
80ug/ml	0.883	0.880	0.878	0.880	0.747
100ug/ml	1.084	1.082	1.088	1.085	0.952
Blank	0.133	0.133	0.133	0.133	***

Table 7: Absorbance values for FRAP determination for ascorbic acid/ standard

Concentration	Abs 1	Abs 2	Abs 3	Mean	Mean Abs Blank
0.5ug/ml	0.122	0.124	0.124	0.124	0.013
1ug/ml	0.312	0.311	0.312	0.312	0.201
2.5ug/ml	0.439	0.440	0.438	0.439	0.328
5ug/ml	0.467	0.467	0.467	0.467	0.356
10ug/ml	0.480	0.486	0.489	0.485	0.374
20ug/ml	0.692	0.693	0.694	0.693	0.582
40ug/ml	1.057	1.078	1.077	1.071	0.960
60ug/ml	1.562	1.562	1.563	1.562	1.451
80ug/ml	1.661	1.662	1.664	1.662	1.551

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100ug/ml	1.714	1.715	1.716	1.715	1.604
Blank	0.111	0.111	0.111	0.111	**

Table 8: FRAP content for the different isolated secondary metabolites at ascorbic acid equivalence

Concentration	Alkaloids	Flavonoids	Saponins	Terpenoids
20ug/ml	3.93	4.44	3.92	3.81
40ug/ml	4.39	5.14	5.33	4.08
60ug/ml	4.70	8.80	6.65	4.44
80ug/ml	4.96	9.29	6.72	5.53
100ug/ml	5.12	10.38	6.77	6.60

The antioxidant activity of selected secondary metabolites (alkaloids, flavonoids, saponins, and terpenoids) was evaluated using the 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) radical scavenging assay. The results indicated a concentration-dependent increase in the percentage scavenging activity of the metabolite extracts. However, a slight decrease in absorbance with increasing metabolite concentration was observed, although this variation was negligible. Similarly, ascorbic acid, used as the standard, exhibited an increasing antioxidant activity with increasing concentration, accompanied by a corresponding decrease in absorbance.

The Ferric Reducing Antioxidant Power (FRAP) assay demonstrated an increase in absorbance for both the metabolite extracts and the standard ascorbic acid. However, a comparative analysis revealed that the metabolite extracts exhibited a higher percentage increase in antioxidant activity than ascorbic acid, indicating their superior free radical scavenging potential (Tables 3 – 8).

Table 9: Effect of different concentrations of isolated metabolites on inhibition of *Alternaria alternata*

Treatment	Saponin	Crude	Flavonoid	Alkaloid	Terpenoid
50%	0^a	5^a	0^a	11^a	11^a
100%	0^a	13^a	10^a	15^a	12^a
Negative Control	4^a	4^a	4^a	4^a	4^a
Positive Control	10^a	10^a	10^a	10^a	10^a

Means with same superscript down with column have not significant difference based on Tukey's HSD test at p = 0.05; Positive control: Ridomilgold fungicide 40g/kg metalaxyl-M 650g/kg mancozeb; Negative control-dimenthyl sulphoxide

The antifungal activity of secondary metabolites extracted from *Lantana camara* against *Alternaria alternata* was assessed, as presented in Table 9. The results indicated that saponins, at both 50% and 100% concentrations, exhibited no significant inhibitory effect on fungal growth, with no statistically significant difference compared to the negative and positive controls. The crude extract demonstrated a mean inhibition zone of 5 mm at 50% concentration and 13 mm at 100% concentration, with no significant difference from the controls. Flavonoids had no inhibitory effect at 50% concentration, whereas at 100%, a 10 mm inhibition zone was observed, which was not significantly different from the controls. Alkaloids exhibited inhibition zones of 11 mm at 50% and 15 mm at 100% concentration, while terpenoids showed inhibition zones of 11 mm at 50%

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and 12 mm at 100%. However, the inhibitory effects of both metabolites were not significantly different from those of the negative and positive controls.

Table 10: Effect of different concentrations of isolated metabolites on inhibition of *Rhizopus oryzae*

Treatment	Saponin	Crude	Flavonoid	Alkaloid	Terpenoid
50%	9^{a}	0^{b}	0^{a}	11^{ab}	0^{b}
100%	12^{a}	10^{a}	5^{a}	14^{a}	10^{a}
Negative Control	0^{b}	0^{b}	0^{a}	0^{c}	0^{b}
Positive Control	9^{a}	9^{a}	9^{a}	9^{b}	9^{a}

Means with same superscript down with column have not significant difference based on Tukey's HSD test at p = 0.05; Positive control: Ridomilgold fungicide 40g/kg metalaxyl-M 650g/kg mancozeb; Negative control-dimenthyl sulphoxide

Table 10 presents the effect of secondary metabolites on the growth of *Rhizopus oryzae*. Saponin exhibited an inhibition zone of 9 mm at 50% concentration and 12 mm at 100% concentration. These values were not significantly different from the positive control (9 mm) but were significantly different from the negative control (0 mm). Crude extract showed no inhibitory effect on fungal growth at 50% concentration (0 mm), which was not significantly different from the negative control (0 mm). However, at 100% concentration, the crude extract produced a 10 mm inhibition zone, which was not significantly different from the positive control (9 mm). Flavonoid had no inhibitory effect at 50% concentration, while at 100% concentration, it exhibited a 5 mm inhibition zone. This inhibition was not significantly different from those observed with the negative and positive controls. Alkaloid demonstrated an inhibition zone of 11 mm at 50% concentration, which was not significantly different from that at 100% concentration (14 mm). The highest inhibition was observed at 100% concentration (14 mm), which was also not significantly different from the positive control (9 mm). Terpenoid showed no inhibitory effect at 50% concentration. At 100% concentration, it produced a 10 mm inhibition zone, which was not significantly different from the positive control (9 mm). Overall, the statistical analysis indicates that the inhibitory effects of the tested secondary metabolites varied, with some exhibiting significant antifungal activity against *Rhizopus oryzae*, particularly at higher concentrations.

Table 11: Effect of different concentrations of isolated metabolites on inhibition of *Aspergillus niger*

Treatment	Saponin	Crude	Flavonoid	Alkaloid	Terpenoid
50%	0^{b}	5^{a}	0^{b}	10^{ab}	0^{b}
100%	0^{b}	12^{a}	0^{b}	12^{a}	10^{a}
Negative Control	0^{b}	0^{a}	0^{b}	0^{c}	0^{b}
Positive Control	9^{a}	9^{a}	9^{a}	9^{b}	9^{a}

Means with same superscript down with column have not significant difference based on Tukey's HSD test at p = 0.05; Positive control: Ridomilgold fungicide 40g/kg metalaxyl-M 650g/kg mancozeb; Negative control-dimenthyl sulphoxide

The effect of different secondary metabolites of *Lantana camara* on the inhibition zone of *Aspergillus niger* is presented in Table 11. The results indicate that saponin and flavonoid, at both 50% and 100% concentrations, exhibited no inhibitory effect on *A. niger* growth, showing no significant difference from the negative control (0 mm). Crude extract demonstrated an inhibition zone of 5 mm at 50% concentration and 12 mm at 100%

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concentration. However, these values were not significantly different from those of the negative (0 mm) and positive (9 mm) controls. Alkaloid at 100% concentration exhibited the highest inhibition zone (12 mm), which was statistically significant for *A. niger* growth suppression. This effect was not significantly different from that observed at 50% concentration (10 mm), which, in turn, was also not significantly different from the inhibition caused by the positive control (9 mm). Terpenoid at 50% concentration showed no inhibitory effect on *A. niger* growth, with no significant difference from the negative control (0 mm). However, at 100% concentration, it exhibited a 10 mm inhibition zone, which was not significantly different from the positive control (9 mm). Overall, the findings suggest that among the tested metabolites, alkaloid exhibited the most pronounced antifungal activity against *A. niger*, particularly at higher concentrations.

Table 12: Effect of different concentration of isolated metabolites on inhibition of *Fusarium oxysporum*

Treatment	Saponin	Crude	Flavonoid	Alkaloid	Terpenoid
50%	0^{b}	5^{a}	0^{b}	13^{ab}	9^{a}
100%	0^{b}	12^{a}	10^{a}	16^{a}	12^{a}
Negative Control	0^{b}	0^{a}	0^{b}	0^{c}	0^{b}
Positive Control	9^{a}	9^{a}	9^{a}	9^{b}	9^{a}

Means with same superscript down with column have not significant difference based on Tukey's HSD test at p = 0.05; Positive control: Ridomilgold fungicide 40g/kg metalaxyl-M 650g/kg mancozeb; Negative control-dimenthyl sulphoxide

Table 12 presents the effect of secondary metabolites on the growth of *Fusarium oxysporum*. Saponin exhibited no inhibitory effect on fungal growth, showing no significant difference from the negative control (0 mm) but a significant difference from the positive control (9 mm). Crude extract at 50% concentration produced a 5 mm inhibition zone, while at 100% concentration, the inhibition zone was 12 mm. These values were not significantly different from those of the negative (0 mm) and positive (9 mm) controls. Flavonoid at 50% concentration exhibited no inhibitory effect on *F. oxysporum*, with no significant difference from the negative control. However, at 100% concentration, it produced an inhibition zone of 10 mm, which was not significantly different from the positive control (9 mm). Alkaloid at 100% concentration exhibited the highest inhibition zone (16 mm), which was significantly different from both the negative (0 mm) and positive (9 mm) controls. However, this effect was not significantly different from that observed at 50% concentration.Terpenoid at 50% concentration exhibited an inhibition zone of 9 mm, while at 100% concentration, the inhibition zone increased to 12 mm. These inhibition effects were not statistically significant when compared to the positive control (9 mm). Overall, the results suggest that alkaloids demonstrated the strongest antifungal activity against *F. oxysporum*, particularly at higher concentrations.

Table 13: Effect of different concentrations of isolated metabolites on inhibition of *Nigro oryzae*

Treatment	Saponin	Crude	Flavonoid	Alkaloid	Terpenoid
50%	0^{b}	13^{b}	0^{a}	12^{a}	0^{b}
100%	0^{b}	1^{6}a	5^{a}	14^{a}	10^{a}
Negative Control	0^{b}	0^{d}	0^{a}	0^{b}	0^{b}
Positive Control	10^{a}	10^{c}	10_{a}	10^{a}	10^{a}

Means with same superscript down with column have not significant difference based on Tukey's HSD test at p = 0.05; Positive control: Ridomilgold fungicide 40g/kg metalaxyl-M 650g/kg mancozeb; Negative control-

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dimenthyl sulphoxide

Table 13 presents the effect of secondary metabolites on the growth of *Nigrospora oryzae*. Saponin, at both 50% and 100% concentrations, exhibited no inhibitory effect on fungal growth, resulting in inhibition zones significantly lower than that of the positive control (9 mm). Crude extract at 100% concentration exhibited the highest inhibition zone (16 mm), which was significantly different from all other treatments. This was followed by crude extract at 50% concentration, which produced a 13 mm inhibition zone, significantly different from both the negative (0 mm) and positive (10 mm) controls. Flavonoids at 50% concentration had no significant inhibitory effect on *N. oryzae* growth, similar to the negative control. At 100% concentration, flavonoids produced an inhibition zone of 5 mm, which was also not significantly different from the positive control. Alkaloids exhibited inhibition zones of 12 mm at 50% concentration and 14 mm at 100% concentration. These values were not significantly different from the inhibition zones of the negative (0 mm) and positive (10 mm) controls. Terpenoid at 50% concentration exhibited no inhibitory effect on *N. oryzae* growth, showing no significant difference from the negative control. However, at 100% concentration, terpenoid produced an inhibition zone of 10 mm, which was comparable to the positive control, indicating no significant difference between the two. Overall, the crude extract exhibited the strongest antifungal activity against *N. oryzae*, particularly at higher concentrations.

Table 14: Effect of different concentration of isolated metabolites on inhibition of *Rhizopus sp*.

Treatment	Saponin	Crude	Flavonoid	Alkaloid	Terpenoid
50%	0^{b}	10^{b}	0^{b}	10^{b}	0^{c}
100%	0^{b}	13^{a}	0.5^{b}	14.5^{a}	15^{a}
Negative Control	0^{b}	0^{c}	0^{b}	0^{c}	0^{c}
Positive Control	10^{a}	10^{b}	10^{a}	10^{b}	10^{b}

Means with same superscript down with column have not significant difference based on Tukey's HSD test at p = 0.05; Positive control: Ridomilgold fungicide 40g/kg metalaxyl-M 650g/kg mancozeb; Negative control-dimenthyl sulphoxide

Table 14 presents the inhibitory effects of secondary metabolites from *Lantana camara* on the growth of *Rhizopus* sp. The results indicate that saponin, at both 50% and 100% concentrations, exhibited no inhibitory effect on fungal growth. This response was not significantly different from the negative control (0 mm) but was significantly lower than the inhibition observed with the positive control (10 mm). Crude extract at 100% concentration exhibited an inhibition zone of 13 mm, which was significantly different from all other treatments. At 50% concentration, crude extract produced a 10 mm inhibition zone, which was not significantly different from the positive control (10 mm) but was significantly higher than the negative control (0 mm). Flavonoid at 100% concentration exhibited a minimal inhibition zone of 0.5 mm, while no inhibition was observed at 50% concentration. These values were not significantly different from the negative control but were significantly lower than the inhibition caused by the positive control (10 mm). Alkaloid at 50% concentration exhibited a 10 mm inhibition zone, which was not significantly different from the positive control (10 mm). At 100% concentration, alkaloid exhibited a 14.5 mm inhibition zone, which was significantly different from all other treatments.Terpenoid at 100% concentration exhibited the highest inhibition zone against *Rhizopus* sp., which was significantly different from all other treatments, including the positive control (Ridomil Gold fungicide, 40 g/kg metalaxyl-M, 650 g/kg mancozeb). Overall, crude, alkaloid, and terpenoid extracts demonstrated notable antifungal activity against *Rhizopus* sp., with terpenoid exhibiting the strongest inhibitory effect.

DISCUSSION

The isolation of multiple fungal pathogens from rice samples underscores potential threats to rice quality,

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yield, and food safety. The differential frequency of occurrence among the fungal species suggests variations in their colonization efficiency and persistence within rice environments. The high prevalence of *Aspergillus niger* (23.5%) is of particular concern due to its capacity to produce mycotoxins, notably ochratoxins, which pose significant health risks to consumers. Furthermore, its presence may contribute to post-harvest spoilage, leading to economic losses for farmers and stakeholders within the rice industry. The relatively high occurrence of *Rhizopus sp.* (20.6%) and *Alternaria alternata* (20.6%) further raises concerns, as these fungi are associated with the post-harvest deterioration of grains. *Alternaria alternata*, in particular, is known to synthesize secondary metabolites such as alternariol, which has recognized toxicological implications. *Fusarium oxysporum* (14.7%) is a well-documented phytopathogen implicated in Fusarium wilt, a disease capable of substantially reducing rice productivity. Additionally, certain *Fusarium* species are known producers of fumonisins and trichothecenes, both of which have been identified as significant food contaminants with adverse effects on human and animal health. The detection of *Nigrospora oryzae* (11.8%) suggests its potential role in rice seed discoloration and reduced seed viability, factors that may negatively impact germination rates and crop establishment. Although its impact is comparatively less severe than that of other pathogens, its presence indicates potential quality deterioration in stored rice. The relatively low occurrence of *Rhizopus oryzae* (8.8%) suggests that, while not a dominant contaminant, it remains a potential risk, particularly under storage conditions favorable for fungal proliferation. Given that *Rhizopus* species are opportunistic pathogens, their presence may indicate suboptimal post-harvest handling practices. These findings align with the reports of Uraguchi and Yamazaki (2018) and Garcia (2016), who documented the isolation of various fungal species from newly harvested rice and other crops. *Nigrospora*, *Aspergillus*, *Mucor*, and *Rhizopus* species are commonly recognized as storage fungi (Ominski *et al.,* 2014; Taligoola *et al.,* 2014). The findings of this study are consistent with those of Danazumi (2015), who reported the presence of four fungal genera in sorghum grain samples, and align with the study by Omaima (2018), which identified five fungal genera in different grain samples. The fungal species isolated from the grain samples in this study are well-documented spoilage organisms associated with various agricultural commodities, including cereals, fruits, and nuts (Muhammad, 2014;Aina *et. al*.,2012). The detection of *Aspergillus niger* further corroborates its widespread association with unmilled rice grain and storage conditions, where it may contribute to deterioration characterized by discoloration and the development of undesirable odors. Additionally, the growth of molds and other microorganisms can lead to a reduction in milling yield, a finding that is consistent with previous reports (Taligoola *et al.,* 2014; Wahyuningrum, 2021).

It has been established that the incidence of field fungi decreases significantly during storage due to their localization on the outer tissues of grains at the time of infection in the field (Mondal *et al.,* 2011). Furthermore, as the environmental conditions required for field fungi differ from those of storage fungi, field fungi are progressively eliminated during storage, leading to a concomitant increase in the population of storage fungi (Bankole *et al.,* 2019).

The qualitative phytochemical screening of the methanolic extract revealed the presence of various bioactive compounds, with terpenoids and flavonoids being the most abundant. This chemical composition is highly relevant to antioxidant activity, as flavonoids and terpenoids are well-documented for their strong free radical scavenging properties. Flavonoids, in particular, are Other detected phytochemicals, including phenols, alkaloids, tannins, and saponins, also contribute to the extract's antioxidant properties. Phenolic compounds, in particular, are known for their redox properties, which allow them to act as reducing agents and metal ion chelators, thereby preventing oxidative damage. Alkaloids and tannins have similarly been reported to exhibit radical-scavenging activity, while saponins may exert indirect antioxidant effects by modulating lipid metabolism and reducing oxidative stress-induced inflammation. The presence of cardiac glycosides, anthraquinones, and steroids, though in relatively lower concentrations, suggests potential synergistic effects that could enhance the overall bioactivity of the extract. Cardiac glycosides have been shown to influence oxidative stress pathways, while anthraquinones possess mild antioxidant activity through their ability to interact with ROS. The phytochemical composition of the methanolic extract, particularly its high content of flavonoids and terpenoids, suggests a significant antioxidant potential. Further quantitative analysis, along with *in vitro* and *in vivo* antioxidant assays, is required to confirm its efficacy in free radical scavenging and oxidative stress mitigation.

These findings align with the study by Bhakta and Ganjewala (2019), which identified flavonoids,

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carbohydrates, proteins, alkaloids, glycosides, saponins, steroids, terpenoids, and tannins as the major phytochemical groups present in *Lantana camara*. However, carbohydrates and proteins were not assessed in the present study. The presence of these bioactive compounds has been associated with antifungal activity, as supported by existing literature. Flavones extracted from *L. camara* have also demonstrated antibacterial and antifungal properties, as reported by Boughalleb *et al.* (2015) and Gujar and Talwankar (2012). The antioxidant properties of *L. camara* were further evidenced by a decrease in absorbance and an increase in antioxidant scavenging activity with rising concentration. This observation is consistent with the findings of Wahyuningrum *et al.* (2021), who reported that the ethanol extract of *L. camara* exhibited very strong antioxidant activity. Additionally, the soluble fraction of *n*-hexane was classified as strong, while the insoluble *n*-hexane fraction exhibited very strong antioxidant activity. Similarly, the soluble fraction of ethyl acetate was categorized as strong, whereas the insoluble ethyl acetate fraction and vitamin C demonstrated very strong antioxidant activity. Furthermore, Kalita *et al.* (2012) reported that the distilled water extract of *Lantana camara* leaves exhibited very strong antioxidant potential.

These results collectively suggest that the phytochemical constituents of *L. camara* contribute significantly to its antioxidant capacity, reinforcing its potential as a natural source of bioactive compounds with therapeutic applications.

Crude plant extracts, such as those derived from *Lantana camara*, contain a complex mixture of active and nonactive compounds, offering significant advantages as antifungal agents. Their natural origin makes them environmentally friendly and safer for consumers, while their diverse bioactive components reduce the risk of resistance development in fungal pathogens (Kosanic et al., 2016; Wang et al., 2016). Given the increasing concerns over synthetic fungicide resistance and environmental hazards, integrating plant-derived antifungal agents into rice pathogen management strategies is a promising alternative.

The antifungal potential of secondary metabolites isolated from *L. camara* leaves was evaluated against fungal pathogens responsible for the deterioration of Nigerian local rice in this study. The results demonstrated significant inhibition of fungal growth, with alkaloids exhibiting the highest antifungal activity, effectively suppressing the radial growth of all tested fungal isolates. In contrast, saponins exhibited the least potency, inhibiting only *Rhizopus oryzae*. Other isolated metabolites demonstrated antifungal activity but required higher concentrations (100%) to achieve significant inhibition.

These findings align with previous reports by Singh and Srivastava (2012), who highlighted the biofungicidal activity of *L. camara* leaves against *Alternaria alternata*, a fungal species also examined in this study. Other studies have similarly documented the antifungal efficacy of *L. camara* leaf extracts (Boughalleb et al., 2015; Saraf et al., 2011; Gujar and Talwankar, 2012). The strong antifungal activity observed for alkaloids in this study may be attributed to the presence of isoquinoline alkaloids, such as berberine iodide, tetrahydropalmitine, berberine hydroxide, and bicuculline, all of which have been reported to exhibit antifungal properties at varying concentrations (Sarma et al., 2019; Maurya et al., 2011, 2012; Tuli et al., 2011; Basha et al., 2012, 2017; Pandey et al., 2017). Although alkaloids have not been extensively developed into commercial antifungal drugs (Wahyuningrum et al., 2021), the findings of this study suggest that they hold potential for novel antifungal compound discovery.

Conversely, the limited antifungal activity of saponins observed in this study contradicts earlier reports that identified saponins as effective antifungal agents (Sindambiwe et al., 2018). This discrepancy may be attributed to the specific antimicrobial behavior of saponins, which has been noted in previous studies to vary depending on fungal species, concentration, and environmental conditions (Sparg et al., 2014). The relatively low efficacy of saponins in this study may indicate that their antifungal action is highly dependent on structural modifications or synergistic interactions with other bioactive compounds.

known for their ability to donate hydrogen atoms or electrons, neutralizing reactive oxygen species (ROS) and preventing oxidative stress-related cellular damage. Their structural features, such as hydroxyl groups, contribute to their potent antioxidant activity, which has been linked to protective effects against degenerative diseases, including cardiovascular disorders and cancer. The high abundance of flavonoids in the extract suggests a significant antioxidant potential, making it a promising natural source of free radical inhibitors.

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Terpenoids also exhibit notable antioxidant properties, primarily through their ability to modulate oxidative stress by interacting with cellular signaling pathways and enhancing the activity of endogenous antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT). Their presence in high concentrations further reinforces the extract's potential as an effective antioxidant agent.

Other detected phytochemicals, including phenols, alkaloids, tannins, and saponins, also contribute to the extract's antioxidant properties. Phenolic compounds, in particular, are known for their redox properties, which allow them to act as reducing agents and metal ion chelators, thereby preventing oxidative damage. Alkaloids and tannins have similarly been reported to exhibit radical-scavenging activity, while saponins may exert indirect antioxidant effects by modulating lipid metabolism and reducing oxidative stress-induced inflammation. The presence of cardiac glycosides, anthraquinones, and steroids, though in relatively lower concentrations, suggests potential synergistic effects that could enhance the overall bioactivity of the extract. Cardiac glycosides have been shown to influence oxidative stress pathways, while anthraquinones possess mild antioxidant activity through their ability to interact with ROS. The phytochemical composition of the methanolic extract, particularly its high content of flavonoids and terpenoids, suggests a significant antioxidant potential. Further quantitative analysis, along with *in vitro* and *in vivo* antioxidant assays, is required to confirm its efficacy in free radical scavenging and oxidative stress mitigation.

These findings align with the study by Bhakta and Ganjewala (2019), which identified flavonoids, carbohydrates, proteins, alkaloids, glycosides, saponins, steroids, terpenoids, and tannins as the major phytochemical groups present in *Lantana camara*. However, carbohydrates and proteins were not assessed in the present study. The presence of these bioactive compounds has been associated with antifungal activity, as supported by existing literature. Flavones extracted from *L. camara* have also demonstrated antibacterial and antifungal properties, as reported by Boughalleb *et al.* (2015) and Gujar and Talwankar (2012). The antioxidant properties of *L. camara* were further evidenced by a decrease in absorbance and an increase in antioxidant scavenging activity with rising concentration. This observation is consistent with the findings of Wahyuningrum *et al.* (2021), who reported that the ethanol extract of *L. camara* exhibited very strong antioxidant activity. Additionally, the soluble fraction of *n*-hexane was classified as strong, while the insoluble *n*-hexane fraction exhibited very strong antioxidant activity. Similarly, the soluble fraction of ethyl acetate was categorized as strong, whereas the insoluble ethyl acetate fraction and vitamin C demonstrated very strong antioxidant activity. Furthermore, Kalita *et al.* (2012) reported that the distilled water extract of *Lantana camara* leaves exhibited very strong antioxidant potential. These results collectively suggest that the phytochemical constituents of *L. camara* contribute significantly to its antioxidant capacity, reinforcing its potential as a natural source of bioactive compounds with therapeutic applications.

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CONCLUSION

The study highlights the significant threat posed by multiple fungal pathogens to rice quality, yield, and food safety, with *Aspergillus niger* emerging as a major concern due to its mycotoxin production and spoilage potential. Other fungi, including *Rhizopus sp., Alternaria alternata,* and *Fusarium oxysporum*, contribute to post-harvest deterioration, economic losses, and potential health risks. The findings underscore the need for effective fungal management strategies to mitigate contamination. Additionally, the qualitative phytochemical analysis of Lantana camara revealed a rich composition of bioactive compounds, particularly flavonoids and terpenoids, which exhibit strong antioxidant properties. The antifungal screening demonstrated that alkaloids were the most potent bioactive agents, significantly inhibiting the growth of rice-deteriorating fungi. The findings support the potential application of plant-derived antifungal agents as sustainable alternatives to synthetic fungicides, offering a promising strategy for rice pathogen management and food safety enhancement. Further quantitative and in vivo studies are recommended to validate the efficacy and practical application of these bioactive compounds.

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