

Isolation, Characterization and Antifungal Evaluation of Flavonoid-Rich Fractions from *Datura metel* Leaves Against *Candida albicans*

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ABSTRACT:

Background: Fungal infections caused by pathogenic microorganisms, particularly *Candida albicans*, have become a major global health concern due to the rapid emergence of antimicrobial resistance and reduced effectiveness of existing antifungal agents. Medicinal plants rich in bioactive phytoconstituents have gained considerable attention as potential sources of novel antifungal compounds.

Objective: The present study was undertaken to evaluate the antifungal potential of *Datura metel* leaf extracts and to isolate and characterize bioactive flavonoid compounds responsible for antifungal activity against *Candida albicans*.

Materials and Methods: Powdered leaves of *Datura metel* were subjected to successive solvent extraction using petroleum ether, chloroform, ethyl acetate, acetone, methanol, and hydroalcoholic solvents. Preliminary phytochemical screening was performed using standard qualitative tests. Antifungal activity of the extracts and isolated fractions was evaluated against *Candida albicans* ATCC No. 10231 using agar well diffusion method with fluconazole as the standard drug. Bioactive fractions were isolated by flash chromatography and characterized using FTIR, and C13 NMR spectroscopic techniques.

Results: Among all extracts, the chloroform extract exhibited maximum antifungal activity with a zone of inhibition of 18 mm at 10 mg/mL concentration, followed by ethyl acetate extract showing 16 mm inhibition. Phytochemical screening confirmed the presence of flavonoids, alkaloids, phenolic compounds, tannins, glycosides, and terpenoids in the active extracts. Flash chromatographic separation yielded fractions DCH FII, and DME FII, among which DCH FII demonstrated comparatively superior antifungal activity with MIC 62.5 µL/mL. FTIR analysis confirmed the presence of hydroxyl, aromatic, ether, and conjugated carbonyl functional groups characteristic of flavonoid compounds. Further structural elucidation using C13 NMR spectroscopy confirmed the presence of aromatic and oxygenated carbon signals typical of flavonoid skeletons.

Conclusion: The present study demonstrated that *Datura metel* possesses significant antifungal activity due to the presence of flavonoid phytoconstituents. The chloroform extract and its isolated fractions exhibited promising antifungal potential against *Candida albicans*. The findings scientifically validate the traditional medicinal use of *Datura metel* and suggest that flavonoid-rich fractions may serve as potential natural antifungal agents for future pharmaceutical development.

Keywords: *Datura metel*, antifungal activity, flavonoids, *Candida albicans*, phytochemical screening...

INTRODUCTION

Fungal infectious diseases are among the major causes of global morbidity and mortality, particularly due to the rapid emergence of antimicrobial resistance against currently available therapeutic agents [1,2]. Antimicrobial resistance has become a serious public health concern worldwide and is no longer restricted to a particular geographical region, as it affects both developed and developing countries alike [3]. The alarming increase in resistance among pathogenic fungi has significantly reduced the effectiveness of existing antifungal therapies and has created an urgent need for the discovery of novel and safer antifungal agents. Among the clinically important fungal pathogens, species belonging to the genera *Candida*, *Cryptococcus*, *Pneumocystis*, and *Aspergillus* are

responsible for a substantial number of opportunistic infections associated with increased morbidity and mortality [4]. Among these, *Candida* species are considered one of the leading causes of superficial as well as life-threatening systemic fungal infections, especially in immunocompromised individuals [5].

Candida species are commonly present as normal microbiota of the oral cavity, gastrointestinal tract, and genitourinary tract of healthy individuals. However, under favourable conditions such as immunosuppression, prolonged antibiotic therapy, diabetes mellitus, or hospitalization, these opportunistic fungi can cause various clinical manifestations ranging from superficial mucosal infections to invasive candidiasis. The most clinically significant species include *Candida albicans*, *Candida tropicalis*, *Candida krusei*, and *Candida parapsilosis* [7,8]. The increasing prevalence of drug-resistant *Candida* strains has become a major therapeutic challenge in clinical practice.

Currently available antifungal agents are mainly derived from microbial or synthetic sources and include azoles, polyenes, and echinocandins. Although these drugs are effective, their prolonged use is associated with several limitations such as toxicity, adverse effects, high treatment cost, limited spectrum of activity, and development of resistant fungal strains [1,4]. Consequently, researchers are increasingly focusing on medicinal plants as alternative sources of antifungal compounds because plants contain a wide variety of bioactive phytoconstituents that can serve as promising lead molecules for the development of new therapeutic agents.

Medicinal plants have been extensively used in traditional systems of medicine for the treatment of infectious diseases. These plants are rich in secondary metabolites such as flavonoids, alkaloids, tannins, glycosides, terpenoids, and phenolic compounds possessing significant antimicrobial properties. Herbal remedies are generally considered safer, cost-effective, and readily accessible, particularly in rural and resource-limited regions. These advantages have encouraged scientific investigations aimed at validating the traditional claims of medicinal plants through *in vitro* and *in vivo* studies [3,9].

Datura metel, a perennial herbaceous plant belonging to the family Solanaceae, is widely recognized in traditional medicine for its diverse pharmacological activities including antimicrobial, anti-inflammatory, analgesic, and antioxidant properties. Several studies have reported that different parts of the plant contain biologically active compounds capable of inhibiting the growth of pathogenic microorganisms [6,8]. In particular, flavonoids isolated from medicinal plants have attracted considerable attention due to their potent antimicrobial and antifungal activities.

Therefore, the present investigation was undertaken to isolate potent antifungal flavonoids from *Datura metel* and to evaluate the antifungal efficacy of the plant extract and isolated flavonoid fractions against pathogenic fungal isolates at different concentrations. The study aims to provide scientific evidence supporting the traditional use of *Datura metel* as a potential source of natural antifungal agents for combating resistant fungal infections.

2. MATERIALS AND METHODS

2.1 Collection and Authentication of Plant Material

Fresh leaves of *Datura metel* were collected from Satara district, Maharashtra, India, during the flowering season. The collected plant material was washed thoroughly with distilled water to remove adhering soil and foreign particles, shade dried at room temperature, and pulverized into coarse powder using a mechanical grinder. The plant specimen was authenticated as voucher specimen number 997502, and a voucher specimen was preserved in the departmental herbarium for future reference.

2.2 Preparation of Extracts

The powdered leaves of *Datura metel* were subjected to successive solvent extraction using solvents of increasing polarity, namely petroleum ether, chloroform, ethyl acetate, acetone, methanol, and hydroalcoholic solvents. The extraction procedure was carried out for the isolation of bioactive phytoconstituents, particularly flavonoids, possessing antifungal activity. The obtained extracts were concentrated under reduced pressure and subjected to phytochemical screening for the identification of major secondary metabolites [9].

2.3 Successive Solvent Extraction

Approximately 100 g of dried powdered leaves of *Datura metel* were packed in a Soxhlet apparatus and successively extracted with petroleum ether, chloroform, ethyl acetate, methanol, and hydroalcoholic solvents for 6 h each. Before extraction with the next solvent, the marc was air dried to remove residual solvent. The extracts obtained were concentrated using a rotary vacuum evaporator at low temperature to obtain semisolid masses. The percentage yield, color, and consistency of each extract were recorded [10,11].

2.4 Phytochemical Evaluation

The various extracts obtained from *Datura metel* leaves were subjected to preliminary phytochemical screening using standard qualitative chemical tests for the detection of alkaloids, flavonoids, glycosides, tannins, saponins, terpenoids, steroids, proteins, carbohydrates, and phenolic compounds [12].

2.5 In Vitro Antifungal Activity

The antifungal activity of different extracts of *Datura metel* was evaluated by agar well diffusion method against *Candida albicans* ATCC No. 10231 using fluconazole as the standard antifungal agent. The method was based on the diffusion of antifungal compounds through agar medium resulting in the formation of a zone of inhibition around the well containing the test sample [13-14].

2.5.1 Test Organism

The fungal strain *Candida albicans* ATCC No. 10231 was used for the antifungal screening. The culture was maintained on Sabouraud dextrose agar slants and subcultured periodically under sterile laboratory conditions.

2.5.2 Preparation of Culture Medium

Antibiotic Assay Medium No. 19 was used for antifungal screening. The medium consisted of peptone (9.4 g), dextrose (10 g), sodium chloride (10 g), and agar (23.5 g) dissolved in 1000 mL distilled water, and the pH was adjusted to 5.1 ± 0.1 after sterilization. The prepared medium was sterilized by autoclaving at 121°C under 15 lbs pressure for 15 min.

2.5.3 Preparation of Standard Solution

Fluconazole was used as the reference standard antifungal drug. Accurately weighed 10 mg of fluconazole was dissolved in 10 mL dimethyl sulfoxide (DMSO) to obtain a final concentration of 1 mg/mL. The solution was sonicated and vortexed to ensure complete dissolution.

2.5.4 Preparation of Sample Solutions

Different extracts of *Datura metel* were dissolved in DMSO to obtain concentrations of 5 mg/mL and 10 mg/mL. The prepared solute

ions were vortexed properly to obtain uniform sample solutions before antifungal evaluation.

2.5.5 Preparation of Fungal Inoculum

A fresh culture of *Candida albicans* was inoculated on Sabouraud dextrose agar slants and incubated at 20–25°C for 72 h. The fungal suspension was prepared in sterile saline solution, and the turbidity was adjusted according to McFarland standards to obtain approximately 1.5×10^6 cells/mL.

2.5.6 Agar Well Diffusion Method

Approximately 15–20 mL of sterilized molten agar medium was poured into sterile Petri plates and allowed to solidify. The prepared fungal inoculum was uniformly spread on the agar surface. Wells of 8–10 mm diameter were made aseptically using a sterile cork borer. About 100 µL of standard fluconazole solution, test extract solution, and DMSO (negative control) were introduced into the respective wells. The plates were kept at 2–8°C for 15–20 min to facilitate diffusion and then incubated at 20–25°C for 24–48 h. After incubation, the zones of inhibition were measured in millimeters using a zone reader.

% Growth Inhibition = $\frac{\text{Colony diameter in control} - \text{Colony diameter in treated sets}}{\text{Colony diameter in control}} \times 100$

2.5.7 MIC

The minimum inhibitory concentration (MIC) of DCH FII against *Candida albicans* ATCC 10231 was determined using the broth microdilution method. Serial dilutions ranging from 1000 to 62.5 µg/mL were prepared and tested against standardized fungal inoculum. After incubation for 48 h, optical density was measured at 600 nm and MIC was defined as the lowest concentration producing at least 50% inhibition of fungal growth.

Culturing of microorganism, inoculum development, and MIC determination were carried out in laminar air flow. Samples were prepared 1 mg/ml and then appropriately diluted at different serial dilutions ranging from 1000(µg/ml) to 62.5(µg/ml). The inoculum of cultures (single cultures) was developed in broth medium. The cultures were then incubated and subsequently, serially diluted to reach the density of 2×10^4 cells per ml. Cell counting was done using hemocytometer. Nutrient broth was dispensed in tubes, and 100 µL of cell culture was inoculated in it. Then, 100 µL of different concentration of extract was added to each tube. Each experiment was carried out in a triplicate set. Growth control was run in parallel with every experiment. All the experimental tubes were incubated in incubator for 48h. After completion of incubation period, the optical density was measured at 600 nm using spectrophotometer. MIC was defined as the minimum concentration of extract that caused 50% inhibition in growth of test microorganism [15-16].

Percent inhibition calculated by $\frac{\text{Control Abs} - \text{Test Abs}}{\text{Control Abs}} \times 100$

3. RESULTS AND DISCUSSION

3.1 Percentage Extractive Yield of *Datura metel* Leaves

Successive solvent extraction of powdered leaves of *Datura metel* was carried out using solvents of increasing

polarity, namely petroleum ether, chloroform, ethyl acetate, methanol, acetone, and hydroalcoholic solvents. The extracts obtained showed variations in color, consistency, and percentage yield depending upon the solvent polarity and solubility of phytoconstituents present in the plant material.

Table 1 Percentage Extractive Yield of *Datura metel* Leaf Extracts

Sr. No.	Extract	Color and Consistency	Percentage Yield (% w/w)
1	Petroleum Ether Extract	Dark green sticky mass	2.84
2	Chloroform Extract	Greenish black mass	3.96
3	Ethyl Acetate Extract	Brown semisolid mass	5.42
4	Acetone Extract	Brownish black residue	11.63
5	Methanolic Extract	Dark brown semisolid mass	9.75
6	Hydroalcoholic Extract	Dark brown semisolid mass	13.24

The hydroalcoholic extract showed the highest percentage extractive yield (13.24% w/w), followed by acetone extract (11.63% w/w) and methanolic extract (9.75% w/w). Petroleum ether extract exhibited the lowest yield (2.84% w/w). The higher yield observed in polar solvents indicates the abundance of polar phytoconstituents such as flavonoids, glycosides, tannins, and phenolic compounds in *Datura metel* leaves.

The difference in extractive values among solvents may be attributed to the polarity-dependent solubility of phytochemicals. Polar solvents are known to extract phenolic and flavonoidal constituents more efficiently, whereas non-polar solvents mainly extract lipophilic compounds such as sterols and terpenoids.

3.2 Preliminary Phytochemical Screening

Preliminary phytochemical evaluation of different solvent extracts of *Datura metel* leaves revealed the presence of several biologically active secondary metabolites including alkaloids, flavonoids, tannins, phenolic compounds, glycosides, steroids, terpenoids, carbohydrates, and saponins.

Table 2 Preliminary Phytochemical Screening of *Datura metel* Extracts

Test	Petroleum ether	Chloroform	Ethyl Acetate	Acetone	Methanol	Hydroalcoholic
Alkaloids						
Hager's test	+	-	-	+	-	+
Mayer's	+	+	+	+	+	+
Wagner's test	+	+	+	+	+	+
Picric acid test	-	-	-	-	-	-
carbohydrates						
Barfoed's test	+	-	-	-	+	+
Molish's test	-	+	+	+	-	+
Reducing sugars						
Benedict's test	+	+	-	-	+	-
Fehling's test	-	+	+	+	-	-
Glycosides						
Borntrager's test	+	+	+	-	-	+
10% NaOH test	+	+	+	+	+	-
Aqueous NaOH test	-	+	+	-	-	+
Cardiac Glycosides						
Baljet test	+	+	+	+	-	-
Test for Cardenolides	-	-	-	-	+	-
Proteins and Amino acids						

Biuret test	-	-	+	+	+	+
Xanthoproteic test	+	+	+	-	+	+
Millon's test	+	-	-	+	-	-
Flavonoids						
Ammonia test	+	+	+	+	+	+
Ferric chloride test	-	+	-	+	-	+
Lead acetate test	+	+	+	-	+	-
Phenolic compounds						
Iodine test	-	+	+	+	-	+
Ferric chloride test	+	+	-	+	+	-
Hot water test	+	+	+	+	+	+
Tannins						
Braymer's test	+	+	-	-	+	+
10% NaOH test	-	+	+	+	-	-
Saponins						
Foam test	+	-	+	-	-	+
Olive oil test	-	-	-	+	+	-

(+): Present, (-): Absent

The phytochemical screening results demonstrated that alkaloids were present in all extracts, while flavonoids and phenolic compounds were predominantly detected in chloroform, ethyl acetate, methanolic, acetone, and hydroalcoholic extracts. Tannins and glycosides were mainly observed in polar extracts.

The presence of flavonoids, alkaloids, phenolic compounds, and terpenoids may contribute significantly to the antifungal activity observed in the plant extracts. Flavonoids and phenolics are well known for their antimicrobial properties due to their ability to disrupt fungal cell membranes, inhibit nucleic acid synthesis, and interfere with fungal enzyme systems.

3.3 Antifungal Activity of Different Extracts of *Datura metel*

The antifungal activity of different solvent extracts of *Datura metel* leaves was evaluated against *Candida albicans* ATCC No. 10231 using the agar well diffusion method. Fluconazole was used as the standard antifungal agent.

Table 3 Antifungal Activity of Different Extracts Against *Candida albicans*

Sr. No.	Extract	Concentration (mg/mL)	Zone of Inhibition (mm)
1	Standard (Fluconazole)	1	20
2	Acetone Extract	5	12
		10	14
3	Methanolic Extract	5	12
		10	14
4	Petroleum Ether Extract	5	11
		10	15
5	Ethyl Acetate Extract	5	10
		10	16
6	Chloroform Extract	5	12
		10	18
7	Hydroalcoholic Extract	5	11

		10	14
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The results demonstrated that all extracts exhibited varying degrees of antifungal activity against *Candida albicans*. Among the extracts tested, the chloroform extract showed maximum antifungal activity with an inhibition zone of 18 mm at 10 mg/mL concentration, followed by ethyl acetate extract showing 16 mm inhibition. Petroleum ether extract demonstrated moderate activity, whereas methanolic, acetone, and hydroalcoholic extracts showed comparatively lower activity.

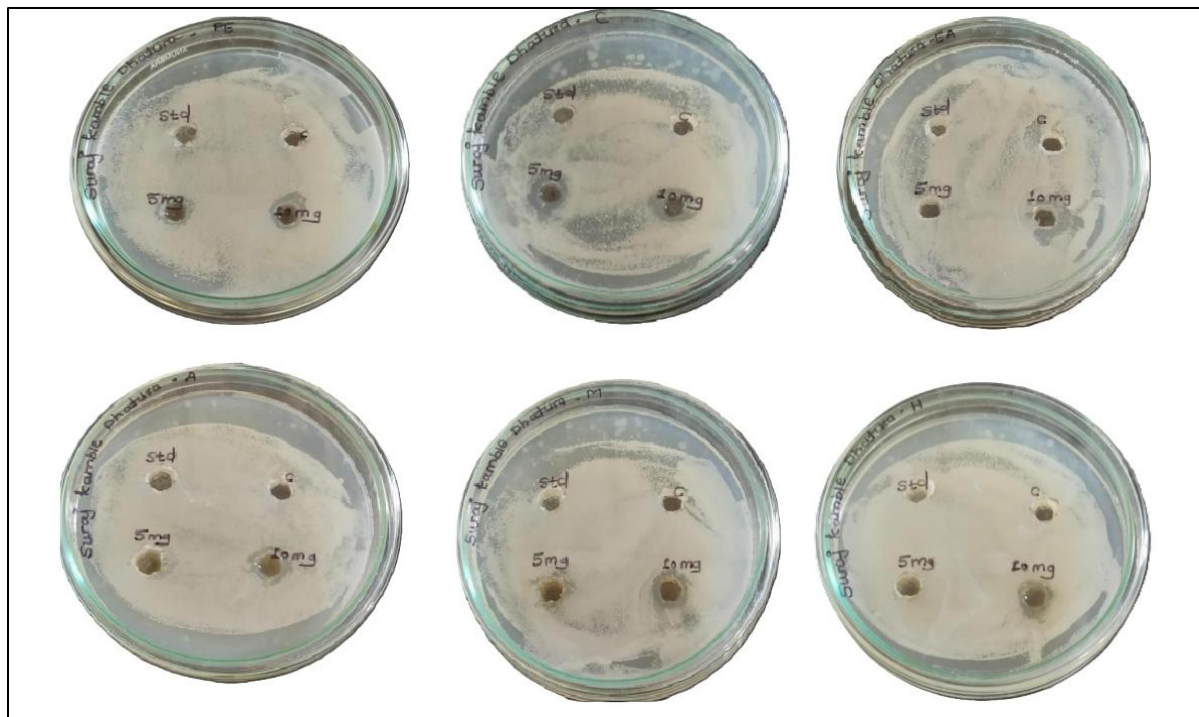


Figure 1 Antifungal activity of extracts

The antifungal activity increased with increasing concentration of extracts, indicating a concentration-dependent response. The superior activity of the chloroform extract suggested the presence of bioactive moderately non-polar phytoconstituents with potent antifungal properties.

3.4 Isolation of Bioactive Fractions by Flash Chromatography

The chloroform and methanolic extracts showing antifungal activity were subjected to flash chromatographic separation for isolation of bioactive fractions. Flash chromatography was carried out using silica gel columns with suitable solvent systems.

Major isolated fractions obtained included DCH FII, and DME FII. The isolated fractions were concentrated and further evaluated for antifungal activity against *Candida albicans*.

3.5 Antifungal Activity of Isolated Fractions

The isolated fractions were screened for antifungal activity against *Candida albicans* ATCC No. 10231 using agar well diffusion method. Fluconazole (1 mg/mL) was used as the standard drug.

Table 4 Antifungal Activity of Isolated Fractions

Sr. No.	Sample	Concentration	Zone of Inhibition (mm)
1	Control (DMSO)	—	—
2	Standard Fluconazole	1 mg/mL	29
4	DCH FII	100 µL	10
		200 µL	19
6	DME FII	100 µL	11
		200 µL	12

The results revealed that chloroform-derived fractions exhibited superior antifungal activity compared to

methanolic fractions. DCH FII also showed significant activity with inhibition zones increasing from 10 mm to 19 mm at higher concentration.

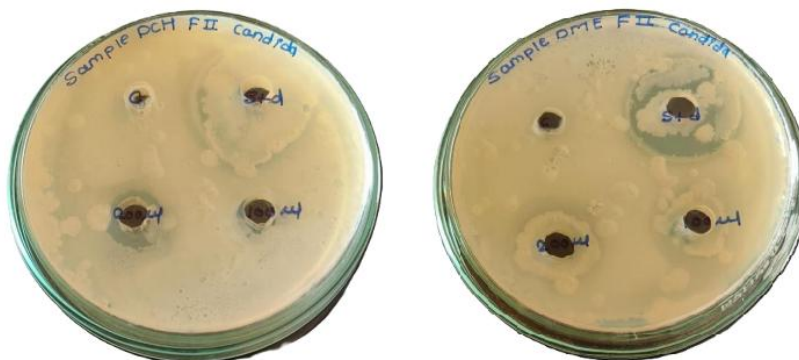


Figure 2 Antifungal activity of isolated flavonoids

In contrast, DME FII showed comparatively weak antifungal activity. The results suggested that active antifungal constituents were predominantly concentrated in the chloroform-derived fractions.

MIC:

The antifungal activity of DCH II against *Candida albicans* was evaluated using the broth microdilution method at 600 nm. The untreated control showed a mean absorbance of 0.80, indicating normal fungal growth.

The MIC value of DCH II was determined to be 62.5 $\mu\text{L}/\text{mL}$. These results indicate that DCH II possesses significant antifungal activity against *Candida albicans*. Although slightly less potent than Fluconazole, the comparable MIC value and concentration-dependent inhibition suggest that DCH II contains bioactive constituents with promising antifungal potential and warrants further investigation.

Table 5 Antifungal Activity of DCH II Against *Candida albicans* Determined by Broth Microdilution Assay (600 nm)

Sample Code	Concentration ($\mu\text{L}/\text{mL}$)	Abs1	Abs2	Abs 3	Mean Abs	% Inhibition
Control	–	0.79	0.80	0.82	0.80	–
Fluconazole	1000	0.14	0.15	0.12	0.13	82.91
	500	0.20	0.21	0.23	0.21	73.33
	250	0.24	0.25	0.23	0.24	70.00
	125	0.26	0.27	0.29	0.27	65.83
	62.5	0.30	0.32	0.31	0.31	61.25
DCH II	1000	0.24	0.25	0.23	0.24	70.00
	500	0.27	0.28	0.29	0.28	65.00
	250	0.30	0.31	0.32	0.31	61.25
	125	0.45	0.42	0.43	0.43	59.83
	62.5	0.49	0.50	0.51	0.50	57.50

3.6 FTIR Characterization of Optimized Isolated Fractions

The chloroform extract of *Datura metel* leaves (DCL Liquid) was subjected to Fourier Transform Infrared (FTIR) spectroscopy for identification of important functional groups present in the extract. The FTIR spectrum exhibited characteristic absorption peaks at 3297.96 cm^{-1} , 2944.65 cm^{-1} , 2833.23 cm^{-1} , 1409.24 cm^{-1} , and 1019.08 cm^{-1} , indicating the presence of phenolic, aromatic, and aliphatic functional groups.

The broad absorption peak observed at 3297.96 cm^{-1} confirmed the presence of hydroxyl groups characteristic of flavonoids and phenolic compounds. The peaks at 2944.65 cm^{-1} and 2833.23 cm^{-1} corresponded to aliphatic C–H stretching vibrations, indicating the presence of hydrocarbon chains or methoxy substituents.

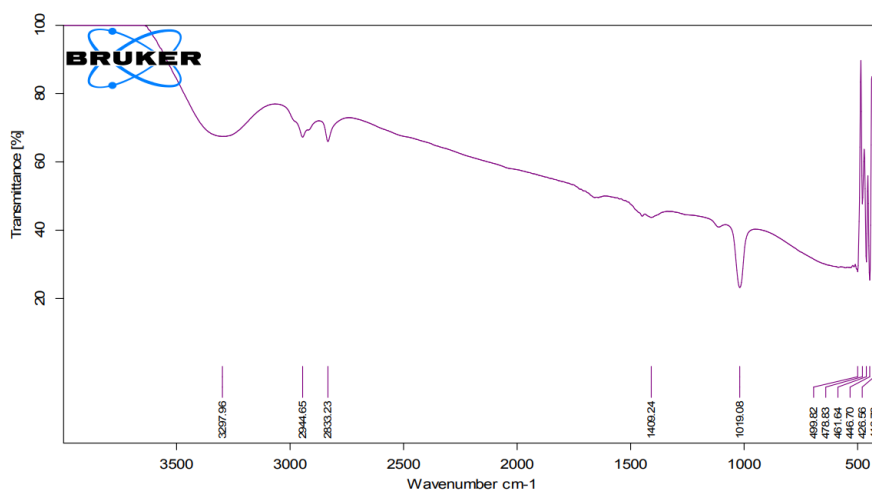


Figure 3 FTIR spectrum of Chloroform extract

The absorption peak at 1409.24 cm^{-1} indicated aromatic C=C stretching vibrations, confirming the presence of aromatic ring systems in the extract. The strong peak observed at 1019.08 cm^{-1} corresponded to C–O stretching vibrations associated with alcohols, ethers, and flavonoidal glycosidic linkages.

The FTIR spectral analysis therefore confirmed the presence of polyphenolic and flavonoidal constituents in the chloroform extract of *Datura metel*. These functional groups are known to contribute significantly toward antifungal activity due to their ability to interact with fungal cell membranes and intracellular proteins.

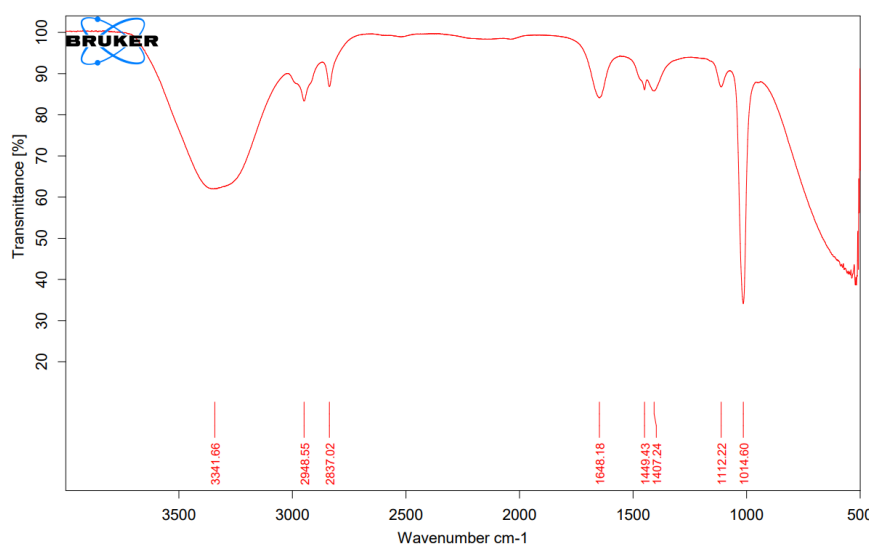


Figure 4 FTIR spectrum of DCH FII

Based on antifungal activity results, DCH FII was selected for spectral characterization. FTIR spectroscopy was carried out to identify major functional groups present in the isolated compounds.

Table 6 FTIR Interpretation

Absorption (cm ⁻¹)	Peak	Functional Group	Interpretation
FTIR Interpretation of Chloroform extract			
3297.96		O–H stretching	Phenolic hydroxyl group
2944.65		C–H stretching	Aliphatic hydrocarbons

2833.23	C–H stretching	Methoxy/aliphatic group
1409.24	Aromatic C=C stretching	Aromatic ring system
1019.08	C–O stretching	Alcoholic or ether linkage
499.82–418.78	Fingerprint region	Skeletal vibrations of aromatic compounds
FTIR Interpretation of DCH FII Fraction		
3341.66	O–H stretching	Phenolic hydroxyl group
2948.55	C–H stretching	Aliphatic hydrocarbons
1648.18	C=O stretching	Conjugated carbonyl group
1449.43	Aromatic C=C stretching	Aromatic ring
1112.22	C–O–C stretching	Ether linkage

The FTIR spectra confirmed the presence of hydroxyl, aromatic, carbonyl, and ether functional groups characteristic of flavonoidal compounds.

3.7 C13 NMR Analysis of Optimized Isolated Compound

Further structural elucidation of the optimized isolated compound was carried out using C13 NMR spectroscopy. The spectrum demonstrated characteristic aromatic and oxygenated carbon signals.

Table 7 C13 NMR Signals of Optimized Isolated Compound

Chemical Shift (δ ppm)	Probable Assignment
167.658	Carbonyl carbon
158.255–153.050	Oxygenated aromatic carbons
138.965–124.873	Aromatic carbons
115.523–112.154	Aromatic CH carbons

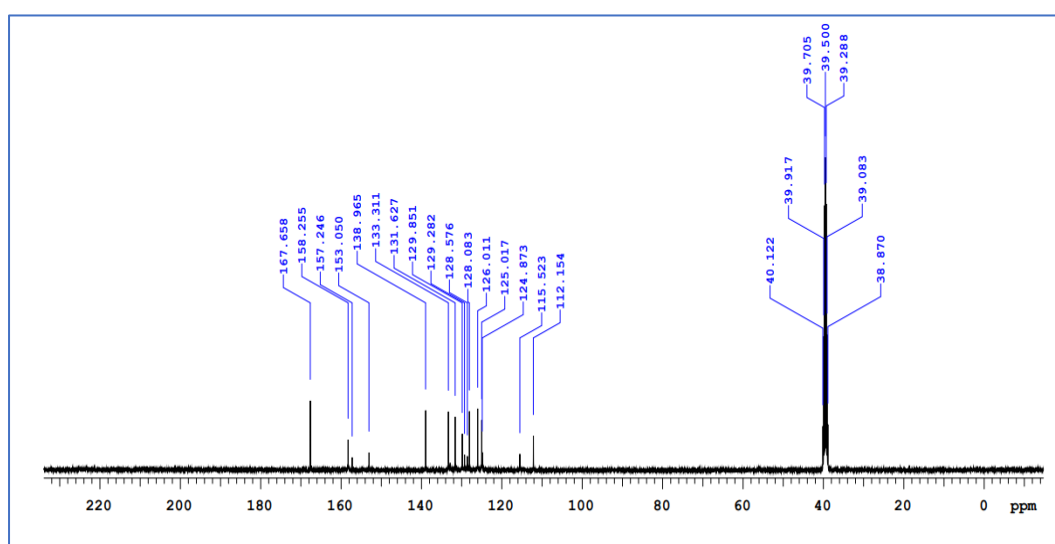


Figure 5 C13 NMR spectrum of DCH FII

The downfield signal at δ 167.658 ppm confirmed the presence of conjugated carbonyl carbon typical of flavonoid skeletons. Multiple oxygenated aromatic carbon signals further supported the polyphenolic flavonoidal structure of the isolated compound.

4. Discussion

The present investigation successfully demonstrated the antifungal potential of *Datura metel* leaf extracts and isolated flavonoidal fractions against *Candida albicans*. The chloroform extract exhibited maximum antifungal activity among all crude extracts, indicating the presence of potent moderately non-polar bioactive constituents.

Flash chromatographic separation yielded active fraction DCH FII, which demonstrated superior antifungal activity compared to methanolic fractions. FTIR analysis confirmed the presence of characteristic flavonoidal functional groups including hydroxyl, aromatic, ether, and conjugated carbonyl groups. The observed antifungal activity may be attributed to the synergistic action of flavonoids and phenolic compounds, which are known to inhibit fungal growth by disrupting fungal membrane integrity, interfering with ergosterol biosynthesis, and inhibiting fungal enzymatic systems.

Similar antifungal activity has been reported for flavonoid-rich fractions isolated from medicinal plants, where flavonoids exert their effects through membrane disruption, inhibition of ergosterol biosynthesis, and interference with fungal enzymatic systems. The present findings support these observations and suggest that the antifungal activity of DCH FII may be attributed to the presence of polyphenolic flavonoids acting either individually or synergistically.

Therefore, the present study scientifically validates the traditional medicinal use of *Datura metel* and suggests that flavonoid-rich fractions isolated from the chloroform extract may serve as promising natural antifungal agents for further pharmaceutical development.

5. CONCLUSION

The present investigation successfully demonstrated the antifungal potential of *Datura metel* leaf extracts and their isolated flavonoidal fractions against *Candida albicans*. Successive solvent extraction revealed that the hydroalcoholic extracts produced higher extractive yields, indicating the presence of substantial quantities of polar phytoconstituents. Preliminary phytochemical screening confirmed the presence of important secondary metabolites such as alkaloids, flavonoids, tannins, phenolic compounds, glycosides, terpenoids, and steroids, which are known to possess antimicrobial properties.

Among the various crude extracts evaluated, the chloroform extract exhibited the highest antifungal activity against *Candida albicans*, suggesting that moderately non-polar bioactive compounds were mainly responsible for the antifungal effect. Flash chromatographic separation of the active extracts resulted in the isolation of several fractions, among which DCH FII demonstrated comparatively superior antifungal activity when compared with methanolic fractions DME FII.

Spectral characterization studies further confirmed the flavonoidal nature of the isolated compounds. FTIR analysis revealed the presence of characteristic hydroxyl, aromatic, ether, and conjugated carbonyl functional groups associated with polyphenolic flavonoids. Further structural elucidation using C13 NMR spectroscopy confirmed the presence of aromatic and oxygenated carbon signals characteristic of flavonoid skeletons.

The observed antifungal activity of the isolated fractions may be attributed to the synergistic action of flavonoids and phenolic compounds, which are known to interfere with fungal membrane integrity, inhibit ergosterol biosynthesis, and suppress fungal enzymatic systems. Although the antifungal activity of the isolated fractions was comparatively lower than the standard drug fluconazole, the study confirmed that *Datura metel* is a promising natural source of antifungal phytoconstituents.

Overall, the present study scientifically validates the traditional medicinal use of *Datura metel* and highlights its potential for the development of novel plant-based antifungal agents. Further studies involving purification, structural elucidation, toxicity evaluation, and in vivo pharmacological investigations are recommended to establish the therapeutic applicability of the isolated flavonoidal compounds.

Declaration of Interest

The authors declare no conflict of interest.

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Author Contributions

Suraj Kamble: Investigation, Data Collection, Formal Analysis, Writing – Original Draft. Rita Chakole: Conceptualization, Supervision, Validation, Writing – Review and Editing.

Data Availability Statement

Data supporting the findings of this study are available from the corresponding author upon reasonable request.

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