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# Phytochemical Profiling and Antimicrobial Potential of Fagonia Indica Solvent Extracts, Highlighting Biofilm Suppression

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## Abstract

This study investigates the phytochemical composition and antimicrobial properties of aerial part extracts of *Fagonia indica*, a medicinal plant widely used in traditional medicine. Sequential extraction was performed using solvents of increasing polarity (n-hexane, chloroform, ethyl acetate, acetone, methanol, and water). Qualitative phytochemical screening was coupled with quantitative analyses of total phenolic content (TPC) and total flavonoid content (TFC). Multidrug-resistant (MDR) Gram-positive and Gram-negative bacteria as well as a fungal strain were tested for antimicrobial activity utilizing disk diffusion, minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC) assays. Additionally, anti-biofilm potential was studied. Methanol and ethyl acetate extracts yielded the highest TPC (125.7 ± 5.2 mg GAE/g) and TFC (68.4 ± 3.8 mg QE/g), correlating with broad-spectrum antimicrobial activity, particularly among polar extracts, which produced larger inhibition zones and lower MIC values. Importantly, despite lower overall phytochemical yield, the chloroform extract exhibited superior anti-biofilm activity, inhibiting *Staphylococcus aureus* (71.8%) and *Klebsiella pneumoniae* (70.8%). These findings confirm that solvent polarity differentially influences both antimicrobial potency and anti-biofilm efficacy. Overall, this study underscores the chloroform extract of F. indica as a novel and promising source of targeted anti-biofilm agents, offering valuable insights for developing innovative, plant-based therapeutics against resistant and biofilm-associated infections.

**Keywords:** Fagonia indica; Antimicrobial; Biofilm; Phytochemicals; Solvent-extraction.

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## Introduction

Antimicrobial resistance (AMR) has emerged as one of the most pressing global health threats of the 21st century. The World Health Organization (WHO, 2022) has identified AMR as a priority area, warning that bacterial pathogens resistant to multiple drugs are projected to cause nearly 10 million deaths annually by 2050 if new solutions are not developed. Conventional antibiotics are increasingly ineffective due to the rapid acquisition of resistance mechanisms such as efflux pumps, enzymatic inactivation, and target modification (Naghavi, et al., 2024). Compounding the problem, the rise of biofilm-associated infections has made clinical management even more challenging. Biofilms are structured communities of bacterial cells embedded in an extracellular polymeric matrix that confer tolerance to antimicrobials and host defenses, rendering biofilm-related infections up to 1,000 times more resistant to antibiotic treatment than planktonic bacteria (Uruén et al., 2020). This dual crisis of resistance and biofilm persistence necessitates alternative strategies, particularly the exploration of natural bioactive agents with multifaceted mechanisms of action.

Medicinal plants represent a valuable reservoir of structurally diverse phytochemicals with antimicrobial and anti-biofilm properties. Historically, natural products have been central to drug discovery, with over 60% of current antibiotics derived directly or indirectly from natural sources (Bernardini et al 2018). Phytoconstituents such as flavonoids, terpenoids, alkaloids, and phenolics disrupt microbial membranes, inhibit quorum sensing, and interfere with biofilm formation. Unlike synthetic antibiotics with narrow targets, plant extracts often act through multiple mechanisms, reducing the likelihood of resistance development. Importantly, traditional knowledge provides valuable leads by highlighting plants that have been safely and effectively used for centuries in folk medicine (Khanashyam et al, 2023).

Fagonia indica Burm. f. (family Zygophyllaceae), locally known as "Dhamasa," is one such plant with a historically medicinal use in South Asia and the Middle East. Traditionally, F. indica has been prescribed for treating infections, inflammatory disorders, and wound healing (Sultana et al., 2018). Phytochemical studies reveal that it contains flavonoids, saponins, alkaloids, and phenolic acids, many of which are associated with antimicrobial and antioxidant activities (Ali, K. and Khan et al., 2021). Recent in vitro and in vivo studies have also reported its cytoprotective, hepatoprotective, and anticancer properties. Despite these promising findings, systematic evaluation of its antimicrobial and anti-biofilm efficacy across extracts of varying solvent polarity remains limited (Górniak etal, 2019).

Solvent polarity plays a pivotal role in phytochemical extraction. Polar solvents (e.g., methanol, ethanol, water) are more effective in extracting phenolics, tannins, and glycosides, whereas non-polar or semi-polar solvents (e.g., hexane, chloroform, ethyl acetate) often yield terpenoids, steroids, and other lipophilic compounds (Samsuri et al, 2020). Since these chemical classes differ in their antimicrobial mechanisms, the solvent system employed may significantly alter biological outcomes. For instance, phenolic-rich extracts commonly show strong bactericidal activity against planktonic cells, while lipophilic

compounds may preferentially interfere with bacterial adhesion, signaling, and biofilm development (Górniak etal, 2019). Yet, few studies have explicitly compared antimicrobial and anti-biofilm activities of F. indica extracts across a polarity gradient, leaving a critical knowledge gap in understanding its full therapeutic potential.

Despite increasing reports on the antimicrobial activity of medicinal plants, little is known about how solvent polarity shapes the biological efficacy of extracts, particularly with respect to anti-biofilm activity. Fagonia indica, a plant with deep roots in ethnomedicine, provides an ideal model to investigate this aspect owing to its chemically diverse phytoconstituents. While polar solvents are generally associated with higher phenolic and flavonoid recovery that contribute to antimicrobial activity, non-polar or semi-polar solvents may concentrate terpenoids and lipophilic compounds that interfere with biofilm formation. Therefore, this study was designed to systematically evaluate the phytochemical profiles and biological activities of sequential solvent extracts of Fagonia indica, with the central hypothesis that solvent polarity differentially influences antimicrobial potency and anti-biofilm efficacy.

**Preparation of Plant Extracts**

Aerial parts of *Fagonia indica* were collected from their natural habitat, taxonomically authenticated by a botanist faculty, and a voucher specimen (Voucher No. UAF-PL-2023-009) was deposited in the herbarium, Department of Botany, University of Agriculture, Faisalabad (UAF Herbarium) Pakistan (Figure 1). The material was shade-dried at room temperature for two weeks and ground into fine powder using an industrial blender. Sequential maceration was performed with increasing solvent polar characteristics (n-hexane, chloroform, ethyl acetate, acetone, methanol, and distilled water) by soaking 50 g of plant powder in 500 mL solvent for 72 h with intermittent shaking. Extracts were filtered, concentrated under reduced pressure using a rotary evaporator, weighed to determine yield, and stored at 4 °C until further analysis (Shehab et al., 2020).

Figure 1: Flower of Fagonia Indica

**2.2 Phytochemical Analysis**

**2.2.1 Qualitative Screening**

Standard colorimetric assays were employed to identify major phytochemical groups. Saponins were confirmed by the froth test, tannins and phenolics by ferric chloride, alkaloids by Mayer's and Wagner's reagents, and flavonoids by the alkaline reagent test (Pratap et al., 2024).

**2.2.2 Quantitative Determination**

Total Phenolic Content (TPC) was determined by the Folin–Ciocalteu method using gallic acid as standard, with absorbance measured at 765 nm. Total Flavonoid Content (TFC) was assessed via aluminum chloride colorimetry using quercetin as standard, with absorbance read at 421 nm. Results were expressed as mg GAE/g for TPC and mg QE/g for TFC (Begum et al., 2023).

**2.3 Antimicrobial Assays**

**2.3.1Test Microorganisms and Their Culture Conditions**

A panel of reference strains, including Salmonella typhi (ATCC 14028), Candida

albicans (ATCC 10231), Escherichia coli (ATCC 25922), Pseudomonas aeruginosa (ATCC 27853), Klebsiella pneumoniae (ATCC 13883), and Staphylococcus aureus (ATCC 25923), were used to assess the extracts' antibiotic activity. The fungus was cultivated in Sabouraud dextrose agar/broth, whereas the bacteria were cultivated in Mueller–Hinton broth/agar (MHB/MHA) (Sulieman et al., 2023).

**2.3.2 Disk Diffusion Assay**

Antimicrobial activity was screened using the Kirby–Bauer disk diffusion method. Microbial suspensions were adjusted to 0.5 McFarland standard and spread on MHA plates. After being impregnated with 20 µL of extract solution (50 mg/mL), sterile paper disks (6 mm) were put onto inoculation plates. After being incubated for 18 to 24 hours at 37 °C, the inhibition zones (mm) were measured. Solvent-impregnated disks served as negative controls, while standard antibiotic disks were positive controls (Aslam et al., 2021).

**2.3.3 Evaluation of Minimum Inhibitory and Bactericidal Concentrations**

MIC and MBC were determined for the most active extracts using broth microdilution in 96-well plates. Extracts were serially two-fold diluted, and wells were inoculated with bacterial suspensions ($10^7$ CFU/mL). Plates were incubated at 37 °C for 18–24 h. MIC was defined as the lowest concentration inhibiting visible growth. For MBC determination, aliquots from non-turbid wells were subcultured on fresh MHA plates and incubated. The MBC corresponded to the lowest concentration producing ≥99.9% bacterial killing. The MBC/MIC ratio was used to classify bactericidal (≤4) or bacteriostatic (>4) effects (Mahmoud et al., 2025).

**2.4 Biofilm Inhibition Assay**

The anti-biofilm activity of *F. indica* extracts was evaluated using the microtiter plate crystal violet assay with slight modifications (Khan et al., 2018). A standardized inoculum of *Staphylococcus aureus* (ATCC 25923) and *Klebsiella pneumoniae* (ATCC 13883) equivalent to 0.5 McFarland standard (~1 × $10^7$ CFU/mL) was prepared in Mueller-Hinton broth (MHB). Aliquots of 100 µL bacterial suspension were dispensed into sterile 96-well flat-bottom polystyrene plates along with 100 µL of extract solutions at final concentrations of 25, 50, 100, and 200 µg/mL. Negative controls contained bacteria with solvent only, while wells with broth alone served as sterility controls.

To enable the production of biofilms, plates were statically incubated for 48 hours at 37 °C. Planktonic cells were carefully aspirated after incubation, and non-adherent cells were eliminated by washing wells three times with sterile phosphate-buffered saline (PBS, pH 7.4). After being fixed with 200 µL of methanol for 15 minutes, the adhering biofilm was allowed to air dry before being stained for 20 minutes with 0.1% (w/v) crystal violet. After washing with running distilled water to get rid of extra dye, 200 µL of 33% (v/v) glacial acetic acid was used to resolubilize the bound crystal violet.

A microplate reader was used to detect absorbance at 595 nm. The following formula was used to determine the percentage inhibition of biofilm formation:

$$\textbf{\textit{Biofilm Inhibition}}\ (\%) = ((\textbf{\textit{OD\_control}} - \textbf{\textit{OD\_treated}}) / \textbf{\textit{OD\_control}}) \times \mathbf{100}$$

OD = Optical Density (also called absorbance).

OD_control → absorbance of biofilm formed in the control wells (bacteria without extract).

OD_treated → absorbance of biofilm in the presence of extract.

All experiments were performed in triplicate, and results are presented as mean ± SD.

**2.5 Statistical Analysis**

All experiments were conducted in triplicate, and results are expressed as mean ± standard deviation (SD). Differences among groups for TPC, TFC, and inhibition zones were analyzed by one-way ANOVA with Tukey's HSD post-hoc test. Values of $p < 0.05$ were considered statistically significant (Ullah et al., 2017).

## 3. Results

### *3.1 Phytochemical Profile of Fagonia indica Extracts*

The qualitative phytochemical screening of the Fagonia indica extracts validated the existence of several important secondary metabolites. Table 1 illustrates the presence of flavonoids, saponins, tannins, and phenolic compounds in the methanol, aqueous, and ethyl acetate extracts and the presence of alkaloids only in the more polar methanol extract. The n-hexane and chloroform extracts were less polar, and this confirmed the rational association of the extraction solvent with phytochemical profile.

**Table 1.** Qualitative phytochemical screening of *Fagonia indica* solvent extracts showing the presence (+) or absence (−) of major secondary metabolite classes.

| Phytochemical Class | n-Hexane | Chloroform | Ethyl Acetate | Acetone | Methanol | Aqueous |
|---|---|---|---|---|---|---|
| Flavonoids | - | - | + | + | + | + |
| Saponins | - | + | + | + | + | + |
| Alkaloids | - | - | - | - | + | - |
| Tannins | - | - | + | + | + | + |
| Phenolic Compounds | - | - | + | + | + | + |

Note: '+' denotes presence, '-' denotes absence.

This was further confirmed by quantitative analysis of the extracts. According to Table 2, the highest Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) of the methanol extract were 125.7 + 5.2 mg GAE/g and 68.4 + 3.8 mg QE/g, respectively. This was then succeeded by the aqueous and ethyl acetate extract and both extracts presented high levels of both classes of compounds. TPC and TFC of the non-polar n-hexane and chloroform extracts were both insignificant, which confirms that polar solvents are much more efficient in extracting both types of phytochemicals.

**Table 2.** Quantitative estimation of total phenolic content (TPC) and total flavonoid content (TFC) in *Fagonia indica* solvent extracts, expressed as mg GAE/g and mg QE/g of extract, respectively.

| Extract | TPC (mg GAE/g) | TFC (mg QE/g) |
|---|---|---|
| n-Hexane | 5.1±1.2 | 1.3±0.5 |
| Chloroform | 8.9±1.8 | 2.7±0.8 |
| Ethyl Acetate | 85.3±4.1 | 45.6±3.1 |
| Acetone | 71.2±3.5 | 39.5±2.6 |
| Methanol | 125.7±5.2 | 68.4±3.8 |
| Aqueous | 110.5±4.8 | 59.1±3.4 |

### *3.2 Antimicrobial Activity of Fagonia indica Extracts*

#### 3.2.1 Zone of Inhibition

The six solvent extracts of F. indica all exhibited considerable broad-spectrum antimicrobial activity, judged by the zones of inhibition reported in Table 3. In line with the quantitative phytochemical

results, the ethyl acetate and methanol extracts had the broadest zones of inhibitory activity to Gram-positive and Gram-negative bacterium. This shows a close relationship between the large proportion of polar compounds including flavonoids and phenolics and the overall inhibitory effect. Antimicrobial activity of S. aureus was markedly great where the methanol extract yielded a zone of 22.5 mm and compared to other studies that showed the 15.0 mm.

**Table 3.** Antimicrobial activity of *Fagonia indica* solvent extracts measured as zone of inhibition (mm) against selected pathogenic microorganisms.

| Pathogen | n-Hexane | Chloroform | Ethyl Acetate | Acetone | Methanol | Aqueous |
|---|---|---|---|---|---|---|
| *S. aureus* | 9.1 ±1.2 | 11.8 ±1.5 | 20.4± 1.9 | 18.6± 1.7 | 22.5 ±2.1 | 17.9±1.6 |
| *P. aeruginosa* | 7.5 ±0.8 | 8.9± 1.0 | 16.1± 1.5 | 14.5± 1.3 | 18.2 ±1.6 | 15.5±1.4 |
| *E. coli* | 8.3 ±0.9 | 9.5± 1.1 | 17.3± 1.6 | 16.8± 1.5 | 19.4 ±1.7 | 18.1±1.6 |
| *K. pneumoniae* | 7.1 ±0.7 | 8.2± 0.9 | 15.8± 1.4 | 13.9± 1.2 | 17.6 ±1.5 | 16.3±1.4 |
| *C. albicans* | 6.5 ±0.6 | 7.8± 0.8 | 14.2± 1.3 | 12.1± 1.1 | 15.8 ±1.4 | 14.5±1.3 |

All values represent the Mean ± SD of three independent experiments.

### 3.2.2 Minimum Inhibitory and Bactericidal Concentrations

MIC and MBC tests of the strongest extract, methanol, were important to give quantitative information on the potency. Table 4 displays the MIC values (between 125 and 500 µg/mL) and MBC values (between 500 and 1000 µg/mL), which is in line with earlier literature findings. The MBC/MIC ratio was determined in each bacterial strain. For S. aureus, the ratio was 2, which means there was a bactericidal effect (the extract did not only stop growth but it also killed bacteria). However, P. aeruginosa had a ratio of 4 indicating a bacteriostatic effect. This demonstrates that the mode of action of extract may be strain specific, a subtle observation with serious consequences on therapeutic usage.

**Table 4.** Minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and MBC/MIC ratios of the methanol extract of *Fagonia indica* against pathogenic microorganisms.

| Pathogen | MIC (µg/mL) | MBC (µg/mL) | MBC/MIC Ratio |
|---|---|---|---|
| *S. aureus* | 250 | 500 | 2 |
| *P. aeruginosa* | 250 | 1000 | 4 |
| *E. coli* | 250 | 500 | 2 |
| *K. pneumoniae* | 500 | 1000 | 2 |
| *C. albicans* | 500 | 1000 | 2 |

### 3.2.3 Biofilm Inhibition

The biofilm inhibition assay revealed distinct differences among the F. indica solvent extracts (Table 5). The chloroform extract demonstrated the strongest antibiofilm effect, with inhibition rates of 71.8% against S. aureus and 70.8% against K. pneumoniae. Interestingly, this extract had a relatively lower phytochemical yield and smaller inhibition zones in the disk diffusion assay, indicating that its antibiofilm activity may be due to compounds specifically enriched in chloroform rather than general antimicrobial constituents. Methanol extract showed 65.4% and 62.7% inhibition against S. aureus and K. pneumoniae, respectively, consistent with its high

phenolic and flavonoid content. Ethyl acetate also exhibited strong antibiofilm activity (55.6% and 52.1%) while aqueous extract demonstrated moderate inhibition (48.3% and 45.9%). Acetone extract displayed weaker activity (38.2% and 35.6%), and n-hexane was the least effective (12.4% and 10.8%). These findings highlight that while polar extracts (methanol, aqueous, ethyl acetate) show strong antimicrobial activity overall, the chloroform extract possesses unique antibiofilm properties that are disproportionate to its general antimicrobial profile. This suggests the presence of intermediate polarity metabolites possibly terpenoids or other hydrophobic compounds that preferentially interfere with biofilm formation rather than planktonic growth.

**Table 5. Percentage biofilm inhibition of *Fagonia indica* solvent extracts against selected biofilm-forming pathogens (Mean ± SD, n = 3).**

| *Extract* | *S. aureus (%)* | *K. pneumoniae (%)* | *P. aeruginosa (%)* | *E. coli (%)* |
|---|---|---|---|---|
| n-Hexane | 12.4 ± 1.1 | 10.8 ± 1.0 | 9.8 ± 1.0 | 11.3 ± 1.0 |
| Chloroform | 71.8 ± 2.4 | 70.8 ± 2.1 | 59.7 ± 2.3 | 63.4 ± 2.3 |
| Ethyl acetate | 55.6 ± 2.3 | 52.1 ± 2.2 | 48.6 ± 2.0 | 57.8 ± 2.0 |
| Acetone | 38.2 ± 1.9 | 35.6 ± 1.7 | 33.9 ± 1.8 | 36.8 ± 1.8 |
| Methanol | 65.4 ± 2.8 | 62.7 ± 2.6 | 55.2 ± 2.6 | 60.1 ± 2.6 |
| Aqueous | 48.3 ± 2.1 | 45.9 ± 2.0 | 45.0 ± 2.1 | 50.5 ± 2.1 |

***4. Discussion***

Solvent polarity strongly influences the extraction of bioactive compounds and their antimicrobial performance. The disparity between solvent polarity and antimicrobial potency suggests that polar solvents selectively extract compounds responsible for broad-spectrum bacterial inhibition. In this study, methanol and aqueous extracts, rich in phenolic compounds and flavonoids, exhibited the largest inhibition zones against all tested pathogens. Quantitative phytochemical analysis confirmed that these polar extracts contained the highest Total Phenolic Content (125.7 ± 5.2 mg GAE/g) and Total Flavonoid Content (68.4 ± 3.8 mg QE/g), which aligns with prior reports linking phenolic-rich extracts to strong antimicrobial activity (Rashid et al., 2013). The MIC and MBC values further supported this, showing bactericidal effects against *S. aureus* and *E. coli* (MBC/MIC = 2), confirming the direct role of these compounds in inhibiting planktonic bacterial growth.

Distinct biofilm inhibition patterns indicate that moderately polar metabolites drive anti-biofilm activity. The disparity between antimicrobial and anti-biofilm activity suggests that different compounds, extracted by moderately polar solvents, are responsible for biofilm disruption. Despite its lower overall phytochemical yield, the chloroform extract exhibited the highest anti-biofilm activity, inhibiting 71.8% of *S. aureus* biofilms and 70.8% of *K. pneumoniae* biofilms. As reported previously, non-polar or moderately polar metabolites such as terpenoids, sterols, and lipophilic saponins can selectively disrupt bacterial adhesion, quorum sensing, and extracellular polymeric substance (EPS) matrix formation (Javed et al., 2021; Khalid et al., 2021, Afonoso et al 2023; Kha et al., 2021). Similar findings have been reported in *Azadirachta indica* and *Ocimum sanctum*, where terpenoid-rich fractions significantly inhibited *S. aureus* biofilms

(Singh et al., 2019; Khan et al., 2021). Such evidence reinforces the thesis that solvent polarity differentially influences antimicrobial versus anti-biofilm activity, emphasizing the importance of extraction optimization based on the intended biological mechanism.

The combined antimicrobial and anti-biofilm potential of *F. indica* enhances its therapeutic promise. Bactericidal activity is desirable to prevent reinfection and reduce resistance development, while the anti-biofilm effect targets persistent infections often refractory to conventional antibiotics. Together, these activities position *F. indica* as a potential source of novel therapeutic agents for multidrug-resistant (MDR) infections (Rahman et al., 2022). The findings underscore that plant-derived extracts can be tailored for specific biological effects through solvent-based extraction strategies, guiding future natural product drug discovery.

Future studies should focus on isolation and delivery optimization of active constituents**.** While crude extracts show promising activity, their complexity may limit solubility, bioavailability, and targeted delivery. Future work should involve bioassay-guided fractionation to isolate and structurally characterize anti-biofilm compounds using advanced analytical techniques such as HPLC or GC–MS (Talib & Aftab, 2021). Additionally, encapsulation in nanoparticles or advanced delivery systems could enhance stability and therapeutic efficacy. Overall, this study confirms that solvent polarity governs the nature of bioactive compounds in *Fagonia indica*, with polar solvents favoring broad-spectrum antimicrobial activity and moderately polar solvents optimizing anti-biofilm potential—thus substantiating the central thesis of this work.

## *5. Conclusion*

This study demonstrates that solvent polarity differentially governs the bioactivity of Fagonia indica extracts. Polar solvents (methanol and aqueous) efficiently extracted phenolic and flavonoid-rich fractions exhibiting strong broad-spectrum antimicrobial effects, whereas the moderately polar chloroform extract showed the most potent anti-biofilm activity against S. aureus (71.8%) and K. pneumoniae (70.8%). These findings establish that selective solvent extraction enables the enrichment of distinct phytochemical classes responsible for either antimicrobial or biofilm-inhibitory actions. Overall, the study validates the traditional medicinal relevance of F. indica and underscores its potential as a source of novel therapeutic agents targeting multidrug-resistant and biofilm-associated infections. Future research should emphasize bioassay-guided isolation of active constituents and optimized delivery systems to enhance clinical applicability.

## 6. Recommendations

This study provides several directions for future research. First, it is recommended to **isolate and characterize the bioactive compounds from the chloroform extract,** which demonstrated the strongest anti-biofilm activity. Second, the **molecular mechanisms underlying this anti-biofilm effect** should be elucidated to understand how specific phytochemicals interfere with biofilm formation. Third, the potential **synergistic effects between polar extracts (broad-spectrum antimicrobial) and mid-polar extracts (anti-biofilm)** should be explored, as this may enhance therapeutic efficacy against multidrug-resistant pathogens.

## 7. Innovation Statement

This study innovatively identifies solvent-specific bioactivity in Fagonia indica, revealing that a moderately polar

chloroform extract uniquely exhibits potent anti-biofilm effects distinct from general antimicrobial action. By demonstrating that selective solvent extraction can target specific phytochemical classes for tailored therapeutic outcomes, this work bridges traditional plant-based knowledge with modern biomedical applications. The findings contribute to the broader mission of leveraging natural resources to develop sustainable, plant-derived strategies against multidrug-resistant and biofilm-associated infections, empowering healthcare innovation through botanical research

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