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In Vitro Antifungal Efficacy of Acetone Leaf Extracts From Selected Aromatic Plants Against Phytopathogenic Fungi Associated With *Carica Papaya* L.

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Abstracts: Papaya (*Carica papaya* L.) cultivation is severely affected by fungal diseases, resulting in significant yield losses and increased reliance on chemical fungicides. However, the extensive use of synthetic fungicides raises serious environmental and health concerns highlighting the need for safer and sustainable alternatives. The present study investigated the phytochemical composition and in vitro antifungal efficacy of acetone leaf extracts of five aromatic medicinal plants, namely *Pongamia pinnata* (L.) Pierre, *Melia azedarach* (L.), *Vitex negundo* (L.), *Ocimum sanctum* (L.) and *Tagetes erecta* (L.). Preliminary phytochemical screening confirmed the presence of diverse bioactive compounds, including alkaloids, flavonoids, glycosides, steroids, terpenoids, phenols and tannins, with noticeable variation among species. Antifungal activity was evaluated using the agar well diffusion method against major papaya pathogens, viz., *Fusarium solani*, *Oidium caricae*, *Mycosphaerella caricae*, *Phytophthora palmivora* and *Pythium aphanidermatum*. Among the tested extracts, Vitex negundo exhibited the highest inhibitory activity, producing zones of inhibition ranging from 15.33 ± 0.88 to 20.00 ± 0.57 mm, followed closely by Melia azedarach 15.66 ± 1.45 to 19.66 ± 0.57 mm. Moderate antifungal effects were observed for *Tagetes erecta*, *Pongamia pinnata* and *Ocimum sanctum*. Although the standard fungicide Bavistin showed superior inhibition, the activity of V. negundo and M. azedarach was comparable against key pathogens. Overall, the strong antifungal efficacy associated with rich phytochemical content highlights these medicinal plants as promising eco-friendly alternatives to synthetic fungicides for sustainable papaya disease management.

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INTRODUCTION

Carica papaya L. (papaya) is a highly valued tropical fruit crop cultivated extensively in India and other tropical and subtropical regions due to its rapid growth, high productivity and exceptional nutritional and medicinal value. India ranks among the leading global producers of papaya contributing substantially to domestic consumption and international trade. Papaya fruits are rich sources of essential vitamins (A, C, and E), minerals, dietary fiber and bioactive compounds, including carotenoids, phenolics and digestive enzymes such as papain which confer antioxidant, anti-inflammatory, digestive and immunomodulatory properties (Saran et al., 2014; Fuentes and Santamaria et al., 2014; Sanikommu, et al., 2021). Owing to these attributes papaya occupies a prominent position in nutritional security, traditional medicine, and agro-based economies.

Despite its economic and dietary significance, papaya production is severely constrained by fungal diseases that cause considerable pre- and post-harvest losses, leading to deterioration in fruit quality, reduced shelf life and significant economic setbacks for farmers. Major phytopathogenic fungi associated with papaya include *Fusarium solani, Oidium caricae, Mycosphaerella caricae, Phytophthora palmivora* and *Pythium aphanidermatum* which are responsible for destructive diseases such as root rot, damping-off, powdery mildew, fruit rot, leaf spot and stem canker (Rawal, 2010; Srikantharajah, et al., 2021; Guang Heng, et al., 2023; Getnet, et al., 2024). These infections may result in yield losses ranging from 30% to 70%, depending on climatic conditions, cultivar susceptibility, and disease severity (Fenta, et al., 2023; Peja et al., 2025). The intensification of fungal epidemics under changing climatic patterns has further exacerbated the need for effective sustainable disease management strategies.

Conventional disease control in papaya largely relies on chemical fungicides which, although effective in the short term, pose serious concerns including environmental pollution, accumulation of toxic residues in fruits, disruption of beneficial soil microflora and the emergence of fungicide-resistant pathogen strains (Chavez-Quintal, et al., 2011; Ademe, 2013; Atul et al., 2021; Deresa, and Diriba, 2023). These drawbacks have intensified global efforts to develop eco-friendly, biodegradable and plant-based alternatives for crop protection. Botanical extracts derived from medicinal and aromatic plants have emerged as promising natural fungicides due to their broad-spectrum antimicrobial activity, environmental safety and minimal risk to human health (Zaker, 2016; G Sandhya, et al., 2024).

Medicinal plants such as *Pongamia pinnata, Melia azedarach, Vitex negundo, Ocimum sanctum* and *Tagetes erecta* are well recognized in traditional Indian medicine and agriculture for their antimicrobial, insecticidal and therapeutic properties. Their antifungal efficacy is attributed to diverse phytochemicals, including alkaloids, flavonoids, phenols, tannins, terpenoids and coumarins which disrupt fungal cell membrane integrity, inhibit spore germination and interfere with metabolic and enzymatic processes (Tiwari and Prajapati, 2024; Pattanayak, et al., 2010; Vaibhav et al., 2024; Madhavi and Revathi, 2025; Singh, et al., 2020). Recent studies have demonstrated significant antifungal activity of plant extracts against major phytopathogens, including *Fusarium, Colletotrichum, Phytophthora* and *Pythium* species underscoring their potential in sustainable agriculture (Honnesh and Saha, 2021; Fugare, et al., 2021; Deresa and Diriba, 2023).

Plants are rich natural sources of bioactive compounds with proven antimicrobial potential and long-standing use in traditional medicine. Growing concerns over environmental safety and fungicide resistance have accelerated the search for eco-friendly alternatives to synthetic pesticides. Fungal diseases cause major yield and economic losses in papaya cultivation, highlighting the urgent need for sustainable disease management strategies. Plant-derived phytochemicals offer a promising, biodegradable, and safer approach for controlling phytopathogenic fungi and improving crop protection (Oniha, et al., 2021; Ayilara, et al., 2023; Santra et al., 2020).

Although numerous plant species have been individually screened for antifungal properties comparative studies focusing on acetone leaf extracts of selected aromatic plants against major papaya phytopathogens remain limited. Acetone is considered an efficient extraction solvent capable of dissolving a wide spectrum of polar and non-polar phytochemicals, thereby enhancing antifungal efficacy (Ademe, 2013; Dissanayake, et al., 2019; Sharmila, et al., 2020). Therefore, the present study was undertaken to evaluate the in vitro antifungal activity and phytochemical composition of acetone leaf extracts of *Pongamia pinnata*, *Melia azedarach, Vitex negundo, Ocimum sanctum* and *Tagetes erecta* against key fungal pathogens associated with *Carica papaya* L. The findings aim to contribute toward the development of eco-friendly, sustainable and economically viable plant-based fungicides for integrated disease management in papaya cultivation.

MATERIALS AND METHODS

Collection of plant materials

The leaves of five selected aromatic plant species namely Karanj (*Pongamia pinnata* L.) Pierre, Nimbora (*Melia azedarach* L.), Nirgudi (*Vitex negundo* L.), Tulsi (*Ocimum sanctum* L.), and Zendu (*Tagetes erecta* L.), were collected from the rural regions of Kandhar, District Nanded, Maharashtra, India, during their active growing season. Only fresh and healthy leaves were selected to ensure maximum phytochemical integrity. The collected plant materials were carefully identified and authenticated in the department of botany Yeshwant Mahavidyalaya, Nanded (Kokate, 2010; Harborne, 1998). After collection, the leaves were thoroughly washed under running tap water followed by rinsing with distilled water to eliminate dust and surface impurities. The cleaned samples were then shade-dried at ambient room temperature for a period of 7–10 days until complete dehydration was achieved. The dried leaves were finely powdered using a sterile mechanical grinder to obtain a uniform particle size. The powdered samples were transferred into airtight containers and stored at 4 °C to prevent degradation and preserve their bioactive constituents until further experimental use (Sasidharan et al., 2011).

Preparation of plant extracts

50 g of powdered plant materials was extracted using 250 mL of acetone in a Soxhlet extraction apparatus for 6–8 hours with the temperature maintained at 56°C (boiling point of acetone). (Harborne, 1998; Azwanida, 2015). The extract was filtered through Whatman No. 1 filter paper and the solvent was evaporated under reduced pressure using a rotary evaporator to obtain crude extracts. The dried extracts were weighed and stored in sterile glass vials at 4 °C. Stock solutions (100 mg/mL) were prepared using dimethyl sulfoxide (DMSO) and sterilized through a 0.22 μm membrane filter and concentrated using appropriate method (Nostro et al., 2000).

Isolation and identification of fungal pathogens

Diseased papaya plant samples (leaves, fruits, and stems) showing typical fungal infection symptoms were collected from papaya fields. The samples were surface sterilized using 1% sodium hypochlorite for 2–3 minutes, followed by triple washing with sterile distilled water. Small tissue segments were aseptically inoculated onto Potato Dextrose Agar (PDA) medium and incubated at 27 ± 2 °C for 5–7 days (Dhingra and Sinclair, 1995). Pure cultures were obtained by subculturing actively growing hyphal tips and identified based on colony morphology and microscopic features using standard mycological manuals (Barnett and Hunter, 1998; Leslie and Summerell, 2006). The fungal species identified included *Fusarium solani*, *Oidium caricae*, *Mycosphaerella caricae*, *Phytophthora palmivora*, and *Pythium aphanidermatum*.

Antifungal activity assay

The antifungal activity of acetone leaf extracts was evaluated using the agar well diffusion method (Perez et al., 1990; Valgas et al., 2007). Sterile molten PDA medium was poured into Petri plates and allowed to solidify. A standardized fungal spore suspension (10^6 spores/mL) was uniformly spread on the surface of the medium. Wells of 6 mm diameter were aseptically punched, and 100 μL of plant extract at different concentrations (25, 50, 75, and 100 mg/mL) was added into each well. DMSO served as the negative control, while Bavistin (0.1%) was used as the standard control. Plates were incubated at 27 ± 2 °C for 72–96 hours. The antifungal activity was assessed by measuring the diameter of inhibition zones (mm). All experiments were performed in triplicate.

Phytochemical analysis

Preliminary phytochemical analysis of acetone leaf extracts was performed to detect the presence of glycosides, alkaloids, flavonoids, steroids, terpenoids, saponins, coumarin, proteins, reducing sugar, phenols and tannins using standard qualitative procedures (Harborne, 1998; Kokate, 2010; Sofowora, 2008). The analysis included tests for glycosides using Salkowski Test, alkaloids using Mayer's test, Flavonoids using Shinoda test, Steroids using Chloroform test, Terpenoids using Salkowaski test, Saponins using Foam test, Coumarin using Coumarin test, Proteins using Biuret test, Reducing Sugar using Fehling test, Phenols and Tannins using Ferric Chloride test.

Statistical Analysis

All experiments were conducted in triplicate, and data were expressed as mean ± standard deviation (SD). Statistical significance was determined using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test at $p < 0.05$ using SPSS software (Gomez and Gomez, 1984; Steel et al., 1997).

RESULT AND DISCUSSION

Phytochemical analysis of Selected aromatic plant extracts

Table 1: Phytochemical constituents of acetone leaf extracts of selected aromatic plants

Phytochemical Constituents	Test	*Pongamia pinnata* (L.) Pierre	*Melia azedarach* (L.)	*Vitex negundo* (L.)	*Ocimum sanctum* (L.)	*Tagetus erecta* (L.)
Glycosides	Salkowski Test	++	+	+	++	++
Alkaloids	Mayer's test	+++	++	++	+++	++
Flavonoids	Shinoda test	+++	+++	++	++	+++
Steroids	Chloroform test	+++	++	+	+	+
Terpenoids	Salkowaski test	++	++	++	+	++
Saponins	Foam test	+	+	+	+	+
Coumarin	Coumarin test	++	++	+	+	++
Proteins	Biuret test	+	+	+	++	+
Reducing Sugar	Fehling test	+	+	+	++	+
Phenols	Ferric Chloride test	++	++	++	+++	+++
Tannins	Ferric Chloride test	++	+++	+++	+++	+++

Where: - + In low concentration, ++ In moderate concentration, +++ In high concentration and *- Absent,*

The preliminary phytochemical screening of acetone leaf extracts from the selected aromatic plants Pongamia pinnata, Melia azedarach, Vitex negundo, Ocimum sanctum and Tagetes erecta revealed the presence of a wide range of bioactive constituents, although their distribution and relative abundance varied among species in Table:1).

Glycosides were detected in all five plant extracts with Pongamia pinnata, Ocimum sanctum and Tagetes erecta exhibiting moderate levels while Melia azedarach and Vitex negundo showed comparatively lower concentrations. Alkaloids were abundantly present in Pongamia pinnata and Ocimum sanctum whereas moderate levels were observed in Melia azedarach, Vitex negundo and Tagetes erecta. Flavonoids were prominently detected in Pongamia pinnata, Melia azedarach and Tagetes erecta, indicating their rich antioxidant potential, while moderate amounts were recorded in Vitex negundo and Ocimum sanctum.

Steroids were found in high concentration in Pongamia pinnata moderate levels in Melia azedarach and low levels in the remaining plant species. Terpenoids were moderately present in Pongamia pinnata, Melia azedarach, Vitex negundo and Tagetes erecta whereas Ocimum sanctum exhibited only a low concentration. Saponins were detected in low amounts across all the studied plant extracts suggesting their limited yet consistent presence.

Coumarins were moderately present in Pongamia pinnata, Melia azedarach and Tagetes erecta while Vitex negundo and Ocimum sanctum showed comparatively lower levels. Proteins and reducing sugars were detected in low to moderate concentrations with Ocimum sanctum showing relatively higher levels than the other plant species. Phenolic compounds were abundant in Ocimum sanctum and Tagetes erecta whereas moderate levels were observed in the remaining species. Similarly, tannins were found in high concentrations in Melia azedarach, Vitex negundo, Ocimum sanctum and Tagetes erecta, while Pongamia pinnata showed moderate content.

The present investigation revealed a rich and diverse phytochemical profile in acetone leaf extracts of *Pongamia pinnata, Melia azedarach, Vitex negundo, Ocimum sanctum* and *Tagetes erecta* with glycosides, alkaloids, flavonoids, steroids, terpenoids, phenols and tannins consistently detected. The relative abundance of these bioactive components among plant species appears to significantly influence their antifungal potential. In particular, Vitex negundo and Melia azedarach the high levels of phenols, flavonoids, alkaloids, and tannins found in *Melia azedarach* are strongly associated with its excellent antifungal efficacy which supports previous reports linking the potent antifungal and antibiofilm activities of this species to its phenolic-rich composition (Sharmila et al., 2020; Lakshmisha et al., 2020; Jaiswal and Kumar, 2025; Akacha et al., 2022). Similarly, the increased polyphenolic content in T. erecta reinforces its recognized antifungal and biopesticidal potential, as documented in recent studies showing inhibition of the growth of phytopathogenic fungi (Birson et al., 2022; Martinez-Ruiz et al., 2024). Although Pongamia pinnata and Ocimum sanctum exhibited relatively moderate antifungal activity, its rich content of flavonoids, phenols, tannins, and alkaloids underscores its therapeutic importance, corroborating previous findings on its antimicrobial properties and traditional medicinal relevance (Sharmila et al., 2020; Verma and Pandey, 2023). Taken together, the present findings confirm that the synergistic action of multiple secondary metabolites plays a significant role in antifungal efficacy and highlight the potential of this aromatic medicinal plant as an eco-friendly alternative to synthetic fungicides for sustainable plant disease management.

Overall, the rich diversity of phytochemical constituents observed in these plants supports their traditional medicinal use and suggests their strong potential as natural sources of antimicrobial, antioxidant and biopesticidal agents. The presence of key secondary metabolites such as alkaloids, flavonoids, phenols, tannins and terpenoids may be responsible for the observed biological activities of these plant extracts.

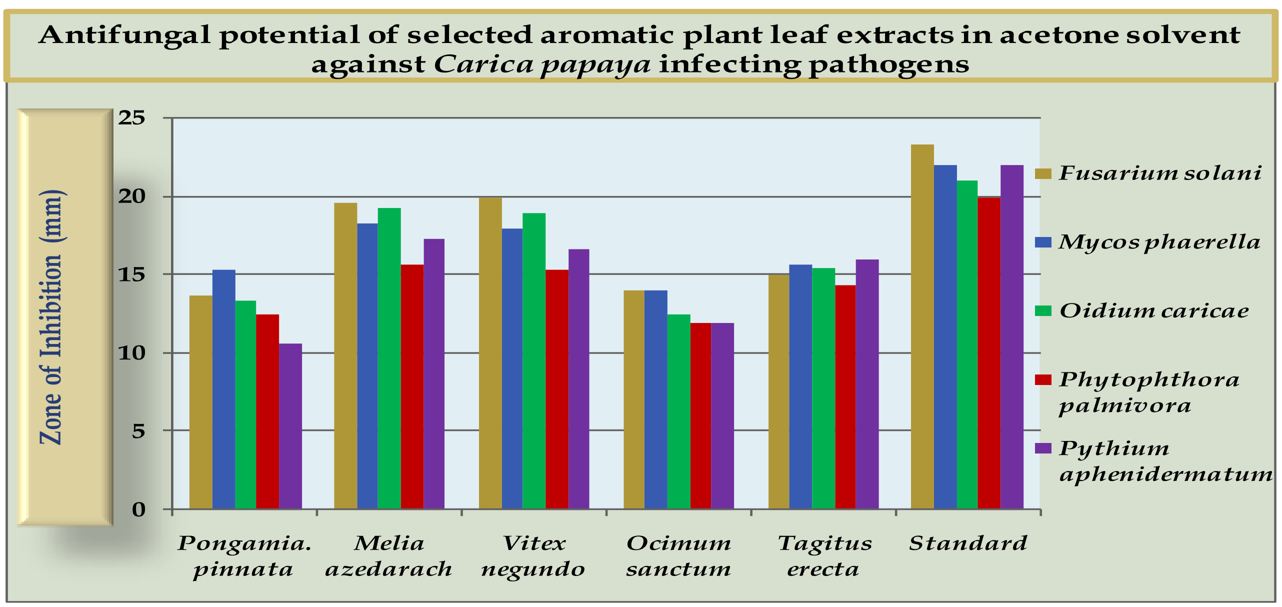
In vitro antifungal activity of selected aromatic plant extracts

Table 2: Antifungal potential of selected aromatic plant leaf extracts in acetone solvent against Papaya (*Carica papaya* L.) infecting pathogens.

Sr. No	Selected Medicinal Plants	Zone of Inhibitions in mm				
		Fusarium solani	*Oidium caricae*	*Mycosphaerella caricae*	*Phytophthora palmivora*	*Pythium aphenider-matum*
1	*Pongamia pinnata*	13.66±1.20	15.33±1.33	13.33±0.72	12.50±1.04	10.66±0.92
2	*Melia azedarach*	19.66±0.57	18.33±0.57	19.33±1.33	15.66±1.45	17.33±0.16
3	*Vitex negundo*	20.00±0.57	18.00±1.04	19.00±1.15	15.33±0.88	16.66±0.16
4	*Ocimum sanctum*	14.00±1.15	14.00±1.15	12.50±0.50	12.00±0.76	12.00±1.15
5	*Tagetus erecta*	15.00±1.15	15.66±0.66	15.50±1,04	14.33±0.57	16.00±0.76
6	Standard (Bavistin)	23.33±0.72	22.00±0.76	21.00±0.57	20.00±0.76	22.00±0.57

Note: Values are mean ± SD (n = 3). Different superscripts in a column indicate significant differences ($p < 0.05$).

Figure 1: Antifungal potential of selected aromatic plant leaf extracts in acetone solvent against Papaya (*Carica papaya* L.) infecting pathogens.



The antifungal activity of five aromatic plant leaf extracts prepared using acetone solvent, namely *Pongamia pinnata* (L.), Pierre, *Melia azedarach* (L.), *Vitex negundo* (L.), *Ocimum sanctum* (L.), *Tagetus erecta* (L.) and tested against five selected common fungal pathogens *Fusarium solani, Mycosphaerella caricae, Oidium caricae, Phytophthora palmivora* and *Pythium aphenidermatum* of *Carica papaya* plant and their fruits using the agar well diffusion assay. Bavistin had been used as a control (fungicide) to compare the effect of plant based treatments with synthetic fungicides. The data on the effect of aromatic plant extracts on plant pathogenic fungi of *Carica papaya* plant and their fruits are presented in Table 2.

The extracts of five aromatic medicinal plant leaves showed significant reduction in the growth of

Fusarium solani, Mycosphaerella caricae, Oidium caricae, Phytophthora palmivora and *Pythium aphenidermatum* and there was significant difference in the efficacy of these extracts. Among the acetone extracts of all five plants, two plants showed greater inhibition of mycelial growth of all pathogenic fungi tested than the control, *Vitex negundo* and *Melia azedarach* showed exceptionally prominent activity. The leaf extract of *Vitex negundo* showed maximum activity 20.00±0.57 mm zone of inhibition against *Fusarium solani* even at low concentrations and *Melia azedarach* also showed 19.66±0.57mm zone of inhibition. The following plants, such as *Tagetus erecta, Pongamia pinnata* and *Ocimum sanctum* exhibited fungicidal activity against all pathogenic fungi tested. Therefore, this study suggests that acetone extracts of the screened plants would be useful for treating plant and fruit diseases of *Carica papaya* caused by *Fusarium solani, Mycosphaerella caricae, Oidium caricae, Phytophthora palmivora* and *Pythium aphenidermatum*. The Bavistin used as a standard antifungal drug as control showed inhibition against the tested fungi, it also showed antifungal activity at 5 µg concentration.

Similar antifungal trends against phytopathogens associated with papaya using plant-based extracts have been studied by Patil et al., (2021), who reported strong inhibition of *Fusarium* and *Phytophthora* species under Indian agro-climatic conditions. Similarly, Karthikeyan et al., (2022) shows that acetone and methanol extracts of *Vitex negundo* and *Tagetes erecta* significantly suppressed fungal pathogens of tropical fruit crops, resulting in high phenolic and flavonoid contents. Jadhav et al., (2023) confirmed the efficacy of *Melia azedarach* extracts against soil and fruit-borne fungi, highlighting their role in sustainable disease management. Additionally, Deshmukh et al., (2020) reported remarkable antifungal activities of *Ocimum sanctum* and *Pongamia pinnata* against *Fusarium oxysporum* and *Pythium aphanidermatum*, supporting the broad-spectrum antifungal potential of these plants. Similarly, Pawar and Patil, (2022) observed significant inhibition of the growth of papaya phytopathogens using botanical extracts of medicinal plants highlighting their utility in biological disease control strategies. More recently, Chavan et al., (2024) reported that phytochemical-rich leaf extracts of indigenous plants effectively suppressed fungal pathogens of horticultural crops, further reinforcing the importance of botanical fungicides in reducing the dependence on chemical fungicides. Taken together, these findings strongly corroborate current results and underscore the growing scientific consensus that plant-based antifungal agents are environmentally friendly, biodegradable, and safe alternatives to synthetic fungicides for the sustainable management of papaya diseases.

It is clear that the acetone extract of leaves of *Vitex negundo* and *Melia azedarach* and other tested plants should be used as a potent biopesticide to treat plant diseases of *Carica papaya* plant and their fruits caused by fungi as it shows maximum activity even at almost the same low concentrations as standard antifungal agents.

CONCLUSION

The findings of this study clearly indicate that acetone leaf extracts of selected aromatic medicinal plants contain a broad spectrum of phytochemicals and exhibit strong antifungal activity against major fungal pathogens of papaya. The consistent detection of biologically active secondary metabolites, particularly alkaloids, flavonoids, phenols, tannins and terpenoids, appears to play a central role in mediating the fungicidal effects. Among the species evaluated, *Vitex negundo* and *Melia azedarach* exhibited excellent inhibitory potential, inducing fungicidal responses, underscoring their promise as effective natural fungicidal agents. The remarkable fungicidal efficacy of these plant extracts not only supports their traditional medicinal relevance but also emphasizes their practical value in developing sustainable plant disease management strategies. The use of plant-based formulations can significantly reduce the dependence on chemical fungicides, thereby reducing environmental pollution reducing the level of toxic residues in agricultural products, and reducing the risks of pathogen resistance development. Consistent with previous studies highlighting the effectiveness of botanical extracts and local biocontrol agents in crop protection, the current study further strengthens the scientific basis for the use of plant-derived antifungal agents as environmentally safe, biodegradable and economically viable alternatives. Overall, this study provides a strong scientific framework for advancing ecofriendly botanical fungicides and makes a significant contribution to the search for sustainable, safe and residue-free papaya production systems.

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