Exploring antimicrobial potential of *Achillea wilhelmsii* dry powder against microbes(fungus) to enhance taste and life span of (Barfi) sweets.

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Abstract

The key objective of this study is to minimize dependence on synthetic preservatives and antifungal agents in the manufacturing of traditional local desserts. We selected two substances from *Achillea wilhelmsii* to evaluate the plant's association with antifungal properties. The study involved three analytical approaches: biological, computational, and chemical FTIR and GC-MS spectrophotometry. We sought to pinpoint the primary bioactive elements accountable for the antifungal effect. We then produced various batches of barfi, a traditional milk-based confection, by integrating differing concentrations (0%, 0.5%, 1%, and 1.5%) of *Achillea wilhelmsii* dry powder. The indole alkaloid extracts exhibited considerable antifungal efficacy, suggesting their appropriateness as natural antifungal agents. There is a forceful positive correlation between the concentration of *Achillea wilhelmsii* and the number of days before fungal growth appears. The regression equation suggests that each 1% increase in powder delays fungal growth by 0.48 days. After ten days, this rule does not apply. This observation shows that although using more *Achillea wilhelmsii* dry powder helps slow down fungal growth at first, there might be a limit, where adding more doesn't make much difference.

Keywords: *Achillea wilhelmsii*, Dairy products, Asteraceae, Dessert fusion

Introduction

The dairy value chain is built around the connection between livestock and secondary milk products, like milk barfi. This process not only benefits producers by increasing profits but also provides consumers with diverse options to enjoy the nutritional benefits of dairy. The typical tastes and health benefits of barfi make it rather popular (Mathur 2000). One of the challenges in the confectionery industry (Subramaniam 2016), however, is determining how to extend the shelf life of these products without compromising their taste or quality (Lurie 2019). A popular sweet in the subcontinent, barfi, is also known as burfi. Burfi is particularly found in Pakistan, India, Bangladesh, and Nepal. Referring to its normally pale, milk-based look, its name, barf, refers to "snow" in Persian (Jana et al, 2019) and reflects the deep-rooted custom of celebrating with sweets from the subcontinent (Jain 2020). Basically, barfi has evolved into a cultural emblem representing hospitality, festivity, and happiness. People on the subcontinent prefer locally made sweets (Jana et al 2019), mostly because of cultural heritage and changing consumer tastes. The current study aims to enhance the shelf life of barfi, ensuring it remains fresh and safe for consumers over extended periods, while also reducing the demand for synthetic preservatives in the local sweets industry. This locally accessible plant, *Achillea wilhelmsii*, was chosen because its yellow hue further accentuates the eye-catching appearance of barfi and eliminates the need for food coloring. Pakistan's yearly output exceeds 70 million tons as of 2023 (Aranguiz and Spoelstra). Pakistan ranks fourth among milk producers worldwide. Fungal infections cause damage and degradation of food goods (Copetti et al, 2025), which affects the food industry. This damage and loss of food products resulting from fungal contamination causes economic losses, food waste resulting from spoilage, and health hazards (Sánchez-Torres 2025). Particularly in emerging nations like Pakistan, India, and others in tropical climes, fungus infection is a major concern in agriculture, storage, transit, and retail(Swami et al, 2025). Mycotoxins (Kushwaha et al. 2025) and aflatoxicosis toxins (Goossens et al. 2025) are carcinogenic for both humans and animals. Every year, developing nations lose up to 20–30% of food goods from post-harvest fungal degeneration.

Materials and Methods

Preparation of sweet

There are different types of barfi like Kaju Barfi, Pista Barfi, Coconut Barfi, Besan Barfi and Milk Barfi the most basic form, made from khoya (milk solids). Targeting here basic and initial form of barfi. Prepare barfi with varying concentrations (e.g., 0%, 0.5%, 1%, 1.5%) of *Achillea wilhelmsii* powder. After this store under controlled conditions (28–30°C, moderate humidity). Fungal growth monitor with visually observation, sensory attributes (taste, smell, texture) and shelf life over 5-10 days.

Chemical analysis. Follow by FTIR and GC-Ms analysis to identify plant extracts.

Computational method

Online database is used to obtain protein structures (Laskowski et al. 1997). After this unwanted residue is removed from the structure. The 3D structure of ligand submitted for analysis to Swiss docking server (Bugnon et al. 2024). Visualization of result was view on chimera (Pettersen et al. 2004).

Bioassay for anti-fungal

The agar well assay was used to determine the antifungal activity of selected plant extracts. For this purpose, a solution of Sabouraud dextrose agar (SDA) was prepared and autoclaved. About 20-25 mL of SDA solution was poured into sterilized petri plates under laminar air flow, and holes of 6 mm width were made in agar using sterile Pasteur pipettes. The fungal strain was placed in the center of the plates. First, the stock of crude plant extracts was prepared for the plant by dissolving 100 mg of plant extract in 1 ml of DMSO. Then, from the stock solution, plant extracts of 100 µL were added into each well. The activity was assessed after 72 hours at an incubation temperature of 28ºC. The diameter of the inhibitory zones was quantified in millimeters. The results were documented, and statistical analysis was conducted to assess the efficacy of each plant extract against the fungal strain. This information will elucidate the antifungal effects of the plant extracts examined.

Table 1. botanical information about plants.

Taxonomy of plants
Kingdom: Plantae
Phylum: Tracheophyta
Class: Magnoliopsida
Order: Asterales
Family: Asteraceae
Genus: Achillea
Species: *Achillea wilhelmsii*

Statistical calculation Pearson Correlation, Linear Regression and percentage formula applied to obtain results.

Result

FTIR results is presented as figure 1 and table 2. *Achillea wilhelmsii* FTIR suggests the presence of Phenolic compounds (O–H and aromatic rings), Terpenoids (C–H, C=O, and aliphatic chains), Flavonoids or glycosides (C–O and aromatic structures) and ester or fatty acid components (C=O stretch) in plant.

GC-MS results of Extract 1 are presented in figure 2 and table 3. GC-Ms result of extract 2 5,6-Dimethoxyindole are presented in fig 3 and table 4

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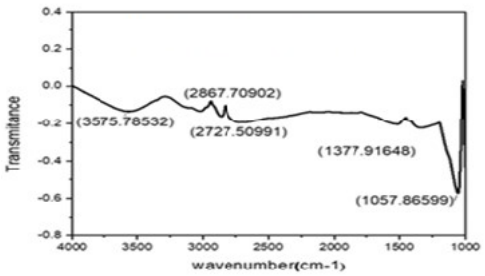


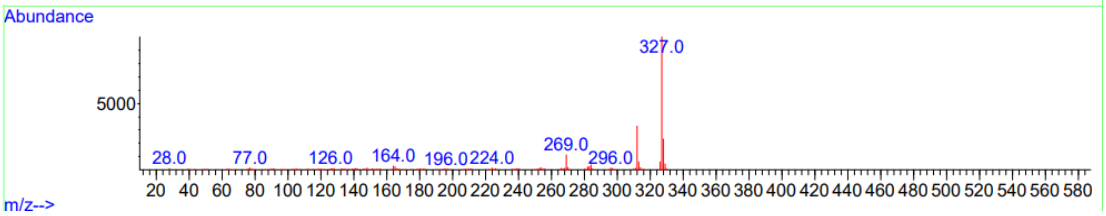
On X-axis wavelength in cm-1 on Y-axis transmission

Figure 1. Spectrogram of FTIR of whole plant methanol extract of *Achillea wilhelmsii*

Table 2. FTIR spectroscopy result of whole plant

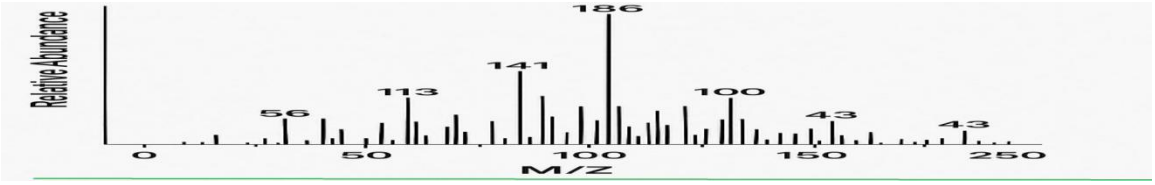
S.N.	Peak	Reason	Interpretation
1	3400 cm^{-1} (Broad peak)	O–H stretching (hydroxyl groups)	Indicates presence of alcohol or phenols
2	~2920 cm^{-1} &~2850 cm^{-1}	C–H stretching (alkyl groups)	Presence of aliphatic hydrocarbons, possibly fatty acids or terpenes
3	~1740 cm^{-1}	C=O stretching (ester, aldehyde, or ketone)	Esters or carboxylic acid derivatives
4	~1630 cm^{-1}	C=C stretching (alkenes or aromatic rings)	Flavonoids, phenolic compounds,
5	~1510–1450 cm^{-1}	Aromatic ring vibrations	Aromatic compounds consistent with polyphenols
6	~1250–1000 cm^{-1} (Multiple peaks)	C–O stretching	Strong indication of carbohydrates, glycosides
7	~800–600 cm^{-1}	Aromatic ring bending	Indicate the aromatic structures

Extract 1



Y-axis (Abundance) this shows the relative intensity, while X-axis show (m/z)

Fig 2. GC-Ms result of 1H-Indole, 5,6-dimethoxy-2-(3,5-dimethoxyphenyl)-3-methyl



Y axis (Abundance) this shows the relative intensity, while X-axis show (m/z)

Figure 3. GC-Ms result of extract 2 5,6-Dimethoxyindole Extract 2

Table 3. Explanation of GC-Ms analysis of extract 1(1H-Indole, 5,6-dimethoxy-2-(3,5-dimethoxyphenyl)-3-methyl).

m/z	Explanation
28.0	Common small fragment, possibly ethylene ($C_2H_4^+$) or CO^+.
77.0	Benzyl or phenyl ion ($C_6H_5^+$) common aromatic fragment.
126.0	Likely an aromatic fragment from partial indole core.
164.0	Possible intermediate fragment (dimethoxy-substituted indole).
196.0	Another possible fragment includes both methoxy groups.
224.0	May indicate loss of small groups (e.g., CH_3, CH_2).
269.0,	Heavier fragments, likely near molecular ion.
327.0 (Base Peak)	Likely the molecular ion [M^+] of 5,6-Dimethoxyindole, suggesting a molecular weight of 327 g/mol. This is the most abundant ion in the spectrum.

The H-Indole, 5,6-dimethoxy-2-(3,5-dimethoxyphenyl)-3-methyl at 327 m/z confirms the expected molecular weight of the compound. Fragmentation is consistent with loss of methoxy (–OCH_3, 31 Da) or methyl groups, and with the presence of aromatic systems (peaks at 77, 126, etc.).

Table 4. Explanation of GC-Ms of extract 2 (5,6-Dimethoxyindole).

m/z	Fragment Identity	Explanation
43	$C_3H_7^+$	Common alkyl fragment (from methyl or ethyl group).
56	$C_4H_8^+$ or $C_3H_4N^+$	Alkyl or nitrogen-containing ring fragment.
100	—	Fragment from ring cleavage or loss of CH_3/OCH_3.
113	$C_8H_9^+$ or similar	Aromatic fragments are likely with one methoxy.
141	$C_9H_{11}NO^+$	Large fragment, likely containing the indole core with substituents.
186	Base peak	This is the most abundant ion. Likely the molecular ion [$M]^+$ or a major fragment close to the molecular weight (177 + possible adduct or rearrangement).

Strong base peak near expected molecular weight (m/z 186), Presence of aromatic and alkyl fragmentation pattern consistent with an indole core and methoxy groups.

Extract 1 Stereochemistry

The compound has a molecular weight of 327.4 g/mol and the molecular formula $C_{19}H_{21}NO_4$. It consists of two benzene rings, four oxygen atoms, and one indole ring. Two of the oxygen atoms are located at terminal positions with the attachment of methyl atom respectively. The SMILES notation for the molecule is:

CC1=C(NC2=CC(=C(C(C=C12) OC) OC) C3=CC(=CC(=C3) OC) OC

The molecule comprises a total of 45 atoms, including 24 heavy atoms. It possesses one hydrogen bond donor and four hydrogen bond acceptors, along with five rotatable bonds. This molecular does not currently have a market name. According to IUPAC nomenclature, it is named 1H-Indole, 5,6-dimethoxy-2-(3,5-dimethoxyphenyl)-3-methyl. The molecular structure is illustrated in Table 5.

Extract 2 Stereochemistry

The compound has a molecular weight of 177 g/mol and the molecular formula $C_{10}H_{11}NO_2$. The structure contains one benzene ring and one indole ring. The SMILES notation for the molecule is: COC1=C(C=C2C(=C1) C=CN2) OC The molecule consists of a total of 24 atoms, including 13 heavy atoms. It has one hydrogen bond donor and two hydrogen bond acceptors, with two rotatable bonds. The canonicalized structure is presented in Table 6. This compound has no known market name. According to IUPAC nomenclature, it is named 5,6-Dimethoxyindole.This compound is a type of indole that has two methoxy groups (–OCH_3) added to the benzene part of the indole structure, specifically at positions 5 and 6. These substitutions can influence the compound's electronic and chemical properties.

Computational result

Two chemical structures of indole alkaloid are selected from the extract of *Achillea wilhelmsii* for computational analysis at molecular level. Used the PDB structure 5ESJ to achieve the molecular docking result for 5,6-Dimethoxyindole with the anti-fungal enzyme Lanosterol 14-demethylase (Zarei, Ahmadi, and Ramazani 2025). This enzyme is a key target in anti-fungal drug design because it's essential in ergosterol biosynthesis in fungi (analogous to cholesterol in humans). Inhibition of this enzyme disrupts fungal cell membrane integrity. The docking results suggest that the best binding pose for the indole compound to Lanosterol 14α-demethylase is slightly more favorable energetically (-8.1664 kcal/mol).

Table 5. Show molecular docking outcomes.

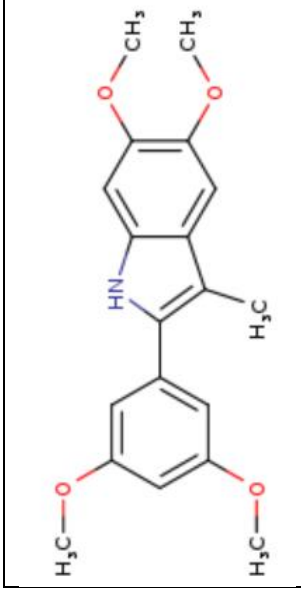
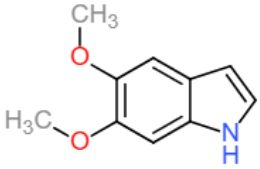
Name of ligand	Target	PDB	Top binding affinity in (kcal/mol)	Function
Extract 1 1H-Indole, 5,6-dimethoxy-2-(3,5-dimethoxyphenyl)-3-methyl 	Cytochrome P450 14α-Demethylase	1EA1	-6.634	Inhibiting CYP51 disrupts fungal cell membrane formation, making it a validated antifungal target.
	Cytochrome P450 3A4	5vcc	-7.624	CYP3A4 is a major enzyme in the liver involved in the metabolism of xenobiotics, including drugs and natural compounds.
	Lanosterol 14α-demethylase	5ESJ	-8.1587	key enzyme in fungal ergosterol biosynthesis, a common target for antifungal agents

Table 6. Show molecular docking outcomes of extract 2.

Name of ligand	Target	PDB	Top binding affinity in (kcal/mol)	Function
Extract 2 5,6-Dimethoxyindole 	Cytochrome P450 14α-Demethylase	1EA1	-5.914	A key enzyme involved in the ergosterol biosynthesis pathway in fungi.
	Cytochrome P450 3A4	5vcc	-6.577	Plays a major role in detoxification and breakdown of xenobiotics (foreign compound
	Lanosterol 14α-demethylase	5ESJ	-6.6282	Inhibition of this enzyme disrupts fungal cell membrane integrity.

Samples	*Candida albicans*
Achillea wilhelmsii aqueous extract	No activity
Achillea wilhelmsii methanolic extract	37mm

Bioassay anti-fungal result

As previously mentioned, evaluated the antifungal activity using the agar well assay and compared it with the standard drug nystatin (Maciel et al. 2025). The aqueous extract of *Achillea wilhelmsii* did not work against *Candida albicans* (Huang 2024*),* but the methanol extracts from the plant showed strong antifungal activity with a 37mm area. This finding indicates that the methanol extract of *Achillea wilhelmsii* possesses significant antifungal properties that could be further explored for potential therapeutic applications. We conduct further studies to identify the active compounds responsible for this activity and evaluate their effectiveness against fungal strains.

Preparation of sweet

Table 7. Preparation of different batches of sweet (milk barfi).

S.N.	Batch	Concentration of *Achillea wilhelmsii* Added dry powder in sweet	Weight of barfi sweet (mg)	Percentage of *Achillea wilhelmsii* dry powder adds in sweet	Visible fungal on the surface of Sweet in days
1	A	0	15	0%	5
2	B	0.5mg	15	3.3%	7
3	C	1mg	15	6.6%	8
4	D	1.5mg	15	10%	10

Percentage formula =

$$\left(\frac{\text{Weight of } \textit{Achillea wilhelmsii} \text{ dry powder add in sweet}}{\text{Weight of barfi}} \times 100 \right)$$

As the concentration of *Achillea wilhelmsii* dry powder increases, the number of days before visible fungal appears also increases. This suggests a dose-dependent antifungal effect, where higher concentrations of the powder extend the shelf life of the sweet (barfi). At 0% (control), fungus appears in 5 days, while at 10% concentration, it takes 10 days.

Statistical results

Pearson correlation analysis revealed a very strong positive correlation between *Achillea wilhelmsii* concentration and shelf life ($r = 0.99$, $p = 0.0077$), indicating statistical significance.

Linear regression analysis further confirmed this relationship. The regression model explained 98.8% of the variation in shelf life ($R^2 = 0.9883$, $p = 0.0059$). The regression equation indicated that each 1% increase in plant powder delayed fungal growth by approximately 0.48 days, although this effect plateaued beyond ten days.

Shelf-Life Evaluation of Barfi

Barfi samples fortified with increasing concentrations of *Achillea wilhelmsii* dry powder showed a clear delay in visible fungal growth. The control sample (0%) exhibited fungal growth after 5 days, whereas samples containing 10% powder showed fungal appearance only after 10 days, demonstrating a concentration-dependent preservation effect.

Conclusion

Achillea wilhelmsii dry powder shows tremendous natural antifungal ability for extending the shelf life of barfi (traditional sweet), as well as proves best for consumer safety due to its nontoxic nature. Additional considerations, we must examine infused oil extract (to mitigate bitterness) and plant-derived silver, iron, or zinc nanoparticles for the same objective.

Conflict of interest

The authors have declared no conflict of interest.

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