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RESEARCH ARTICLE

MJM BIOLABS

Antimicrobial Activity of *Opuntia monacantha* Cladode Extract Combined with Underground Honey Against Common Pathogens Found on Specimens from Patients with Oral Thrush

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ABSTRACT

The burden of *candidiasis* on an individual is significantly huge, particularly among those living with specific chronic illnesses and those immunocompromised. With the growing incidences of the latter primary conditions especially in developing countries, there is need to find preventive measures that are affordable, common and locally available to the people. Because natural products are believed to be readily available, safe, effective, and affordable; herbal treatments are still widely used all over the world. Consequently, this study sought to investigate whether *Opuntia monacantha* cladode extract combined with underground honey have antimicrobial activities towards oral *candidiasis*. This research used an experimental study design. The target population was the Elgeyo Marakwet community of Kenya, where *O. monacantha* and underground honey are commonly, easily, and readily available. Samples were collected by the researchers and their taxonomy validated by a verified herbalist. Afterwards, phytochemical screening and extraction of *O. monacantha* were done. In the microbiology laboratory, test for the activity of the mixture against *Staphylococcus aureus, Escherichia coli,* and *Candida albicans* was carried out. The Kirby - Bauer disk diffusion method was used to determine the antibacterial and antifungal activities of the plant extract. By using the broth microdilution approach, minimum inhibitory concentrations (MIC) were identified. The extraction of *Opuntia monacantha* yielded 29.92%. Antimicrobial tests showed that the mixture of *O. monacantha* extract and underground honey exhibited moderate inhibition against *C. albicans, E. coli, and S. aureus*, with inhibition increasing with concentration. The methanolic extract and underground honey alone also displayed antimicrobial activity, though less pronounced. Phytochemical analysis confirmed the presence of alkaloids, tannins, flavonoids, and saponins in *O. monacantha*, while underground honey contained alkaloids, tannins, and saponins but lacked flavonoids. In conclusion, *O. monacantha* and underground honey possess bioactive compounds with antimicrobial potential, supporting their traditional medicinal use.

Keywords: *Opuntia monacantha,* Underground Honey, Oral Thrush, Alkaloids, Flavonoids, Tannins.

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INTRODUCTION

Oral *candidiasis*, commonly known as oral thrush, is an opportunistic infection that commonly affects immunocompromised people and those living with certain chronic illnesses. *Candidiasis* is a fungal infection caused by several species of the fungi Candida-most commonly, *Candida albicans* (Al-Garawi et al., 2022). The fungus is a normal flora that normally lives on the skin and inside the body, in moist places such as the mouth, throat, gut, and vagina. It rarely causes infections, but in cases of immune deficiency and when the normal flora balance is disrupted, it multiplies and causes infections such as oral thrush and candida vaginitis. Although majority of incidences of oral thrush are due to proliferation and growth of Candida species, there are isolated cases of non-Candida etiologies; thus the synonymous use of the terms oral *candidiasis* and oral thrush may not be entirely true (Lu, 2021). Pathogen specimens isolated from patients with oral thrush have consistently shown co-existence of the fungi with specific bacteria such as *Staphylococcus aureus*, and *Escherichia coli* that may be construed also as etiological factors. For instance, Bose et al. (2023) found that *S. aureus* promoted the growth and hyphal transition of *C. albicans* while *E. coli* inhibited fungal growth. Nonetheless, and albeit the fact that it starts as a mild illness, being an opportunistic infection means it can grow and spread quickly to become fatal.

Candidiasis has a prevalence of between 30% to 50% in the general population yet it carries a 40 to 60% mortality rate (Sharma & Chakrabarti, 2023). It is common in young children, especially in those less than one month, people with a reduced immune response such as HIV patients, leukaemia patients and patients undergoing cancer treatment with chemotherapy or radiation, and those who use steroid anti-asthmatic sprays without proper oral care. Globally, nearly 2,000,000 cases of oral *candidiasis* are recorded annually and is expected to grow (Bongomin et al., 2017). In the United States alone, it is the fourth most prevalent nosocomial infection affecting every 10 per 100,000 people annually as reported by Bongomin et al. (2017). Moreover, approximately 37% of new-borns are affected by oral thrush in the United States (Clair-Brown et al., 2018). Similar trends are observed in other developed countries such as those in Europe where *candidiasis* is the sixth most prevalence nosocomial infection. In Africa, the prevalence of oral *candidiasis* is significantly high though it varies from country to country with countries such as South Africa reporting as high as 80% particularly among people living with HIV/AIDS (Ndiaye, 2005). Although only a small number of studies have been done here in Kenya to examine oral *candidiasis*, the prevalence points a worrisome picture with the commonest causing-species are *C. auris* and *C. albicans*. This is in part due to the growing prevalence of specific chronic illnesses, immunocompromising diseases, irrational use of specific medications e.g. broad spectrum antibiotics and corticosteroids, inadequate resources for screening, diagnosis and treatment (Taylor et al., 2023). As such, it is important for the general population to be able to prevent themselves against such infections using locally available resources such as plants and underground honey. Therefore, this study sought to investigate whether *Opuntia monacantha* cladode extract combined with underground honey, commonly and readily available amongst the people of the Elgeyo Marakwet community of Kenya, can be used as preventive measures towards managing oral thrush.

Plants have been used for medicinal purposes long before the prehistoric period. Traditional forms of medicine continue to be practiced on many accounts (Salmerón-Manzano et al., 2020). According to Gakuya et al. (2019), medicinal plants contain phytoconstituents like tannins, flavonoids, and alkaloids that have antimicrobial properties. In Kenya,more than 70 % percent of the total population depends on traditional medicine for their health needs while more than 90% use herbal products at one time to treat or cure a disease.

Opuntia monacantha also known as the “prickly pear” or “cochineal prickly pear” belongs to the *Cactaceae* family. It is a succulent shrub or tree that grows to about 5 meters in height and has a well-developed trunk with clusters of long spines (Ahmed et al., 2021). It has been used for many years as a medicine in the Elgeyo Marakwet community to manage oral and oropharyngeal *candidiasis*, especially in young children. In addition, it has been used in the same community for the management of pancreatitis, blood cleansing, breast, and cervical cancer, and diabetes (Kigen et al., 2017). The plant parts used by the community are the cladodes which may be burnt to ash known as “Tusan” or boiled and the solution drank. It may be used solely or in combination with underground honey known as “Kusumia” that is obtained from insects in dry trees locally known as “Kiipchiom”.

The underground honey on the other hand, which is collected from the ground holes around trees is mainly added to improve palatability by masking the bland taste since it is mainly given to infants and toddlers. However, in this study, we aimed to verify if it might also be having antimicrobial activity. Furthermore, the Elgeyo Marakwet community at times also uses underground honey solely for the management of *candidiasis* by mixing it with drinking water or applying it on the lesions (Kigen et al., 2017). This is useful for the pharyngeal lesions as they cannot be painted using the paste of extract and underground honey.

Current research aimed to validate the claimed activity of *Opuntia monacantha* and underground honey in the treatment of oropharyngeal *candidiasis*. This is because research resources on the efficacy of different traditionally used remedies are becoming more readily available and such research may lead to the discovery of new remedies that may have

advantages over existing conventional medicine as well as push for evidence-based use of herbal remedies.

METHODS

Study Design

Current study employed an experimental research design. The study aimed to examine the phytochemical properties an afterwards the antimicrobial activity of *Opuntia monacantha* cladode extract combined with underground honey. This design was suitable for testing the hypothesis that: *Opuntia monacantha* cladode extract combined and underground honey possess bioactive compounds that have antimicrobial activity against *Candida albicans, Staphylococcus aureus,* and *Escherichia coli.*

Study Location

Elgeyo-Marakwet county in Kenya formed the study location for this study. The county is located within the Rift Valley region where the people of Marakwet community have used *Opuntia monacantha* and underground honey as a traditional medicine for *candidiasis*.

Plant Material Collection and Drying

The fleshy cladodes of *O. monacantha* were harvested from Elgeyo Marakwet County. The spines within the leaves were then physically removed. The leaves were cut into smaller pieces and sun-dried for two weeks. Underground honey was also harvested and refined by removing any organic dirt and the insects present then stored in a glass jar, with the help of an herbalist.

Materials, Reagents, and Apparatus

The materials used were *Opuntia monocantha* cladode extract, underground honey, fluconazole disks, and gentamicin disks. The microorganisms used were *Candida albicans, Staphylococcus aureus,* and *Escherichia coli.*

The apparatus used included a drying oven, Bunsen burner, round-bottomed flasks, autoclave, analytical balance, forceps, spatula, cotton swabs, stirring rod, wire loop, Whatman filter paper, conical flasks, 500 mL measuring cylinders, funnels, laminar flow cabinet, and Petri dishes.The reagents used were Mueller Hinton agar, Sabouraud dextrose agar, methanol, ethanol, 0.9 % NaCl, and distilled water.

Preparation of Plant Extracts

Plant extracts were prepared following procedure outlined by Rasul (2018). Therefore, 11.88g of the dried cladodes were weighed and macerated in one litre of methanol for 72hrs then evaporated using a rotary evaporator to obtain a concentrated extract for further analysis. The underground honey extract on the other hand was obtained by macerating 15g of the underground honey in 500ml of methanol for 72hrs and the resultant solution evaporated using a rotary evaporator to obtain a concentrated extract.

Phytochemical Screening

Opuntia monocantha extract and underground honey were subjected to various qualitative chemical tests to determine the presence of various active metabolites, specifically of alkaloids, saponins, flavonoids, and tannins.

Detection of alkaloids

To confirm the presence of alkaloids in the methanolic extract, three different chemical tests were used: Mayer's test, Wagner's test, and Dragendorff test.

- In Mayer's test, a small amount of the extract and underground honey were separately dissolved in 2 ml of distilled water, after which a few drops of Mayer's reagent (potassium mercuric iodide solution) were added. The presence of alkaloids was determined by checking for the formation of a cream-colored precipitate in the resultant mixture (Alamgir, 2018).
- Wagner's test involved separately dissolving a small quantity of the extract and underground honey in 2 ml of distilled water before adding a few drops of Wagner's reagent (iodine solution in potassium iodide). The formation of a reddish-brown precipitate indicated the presence of alkaloids (Kale, 2020).
- In Dragendorff test, a small amount of the extract and underground honey were separately dissolved in 2 ml of distilled water, after which a few drops of Dragendorff reagent (solution of bismuth potassium iodide) were added. The mixture was then observed for the formation of an orange or red precipitate, which indicated that alkaloids were present.

Each of the three tests was carried out in duplicate, and the results were recorded. To ensure the accuracy and reproducibility of the tests, the extract's concentration was adjusted to standard concentrations.

Detection of flavonoids

Three methods were used to detect the presence of flavonoids in the extract and underground honey.

- The first method was the Shinoda test, 2g of *Opuntia monocantha* dried powder and 2 ml of underground honey were separately dissolved in ethanol, which was then warmed and filtered, before adding 0.1g of magnesium ribbon. A few drops of concentrated HCl were then added. According to Garg & Garg (2019), a yellow-orange colouration indicated that flavonoids were present.
- The second method used was the Alkalinized Shinoda test, where 2 drops of NaOH were separately added to 2g of *Opuntia monocantha* dried powder and 2 ml of underground honey. A yellow colour was formed at the beginning, which gradually became colourless after adding a few drops of dilute hydrochloric acid (Kale, 2020). The presence of flavonoids was indicated by the disappearance of the yellow colour.
- The third method used was the lead acetate test. 10% lead acetate solution was added to 2g of *Opuntia monocantha* dried powder and 2ml of underground honey. The formation of a yellow precipitate indicated that flavonoids were present (Kale, 2020).

Detection of saponins

The froth test was used to test for the presence of saponins. 2g of *Opuntia monocantha* dried powder and 2 ml of underground honey were each mixed with 5 ml of distilled water in a test tube, then shaken vigorously and left to stand for 15 minutes. The presence of saponins, was indicated by the formation of a stable foam (Alamgir, 2018).

Test for tannins

The two tests that were used to detect the presence of tannins were Ferric chloride and lead acetate tests. In both tests, 2g of *Opuntia monocantha* dried powder and underground honey were each mixed with 10 ml of water and heated in a water bath, followed by filtration of the mixture. For the first method, 2 drops of ferric chloride were added, while in the second method, 2 drops of lead acetate were added to the filtrate. A black precipitate in the Ferric chloride test and a brown puffy precipitate in the lead acetate test indicated the presence of tannins (Kale, 2020).

Preparation of Antifungal and Bacterial Test strains

Sub-cultures of *Staphylococcus aureus, Candida albicans,* and *Escherichia coli* were prepared from pure cultures at the Kabarak University Microbiology Laboratory.

Disk preparation

The disks were sterilized in a hot air oven at 121 degrees Celsius for 15 minutes. To the Petri dishes media was added to about three-quarters of the dish and allowed to solidify in a laminar air flow chamber. Nine disks were prepared: *O. monacantha* extract to *C. albicans*, another underground honey extract to *C. albicans, O. monacantha* to *S. aureus*, underground honey to *S. aureus*, the mixture on *S. aureus* and the mixture on *C. albicans*. Finally, *O. monacantha* to *E. coli*, underground honey extract to *E. coli*, and the mixture extract to *E. coli*. Therefore, three plates had SDA and six had MHA.

Application of impregnated disks

A sample microbe was removed from the stock inoculum using sterile cotton swabs and scattered evenly throughout the surface of the solidified culture media. C.albicans was introduced to the SDA and *S. aureus* was introduced to the MHA. In this technique, the plate was divided into four quadrants and an isolated colony was picked from an agar plate using a sterilized inoculation loop and spread over the first quadrat using close streaks, from the edges of the first quadrant. The streaks extended to the second quadrant, and the process was repeated for the third and fourth quadrants. To one SDA Petri dish O. monocantha extract was introduced and then to another the underground honey extract was added and to another, the mixture was added. To one of the MHA containing *S. aureus*, the disk with *O. monacantha* extract was placed, to the other uunderground honey extract disk was added and to the third, a disk containing a mixture of the extract was added. To another set of MHA *E. coli* was introduced. To the MHA plates, gentamicin discs were added and to the SDA fluconazole discs were added at a distance from the test extracts. The Petri dishes were well labeled and incubated at 37 degrees Celsius for 24 hours for bacteria and at 30 degrees for 72-96 hours for the fungi while observing for growth and inhibition of growth of the microbes.

Data Analysis

This study used both qualitative and quantitative approaches for data analysis. Qualitative approaches were employed to analyze data from phytochemical screening while quantitative approaches were used to analyze data from antimicrobial activity and percentage yield. For the phytochemical screening, the presence of each phytochemical was confirmed based on observable color changes or precipitate formation as outlined in standard phytochemical testing procedures.

Disk diffusion method was used to assess antimicrobial activity whereby inhibition zones formed around each impregnated disk were measured using a calibrated ruler. The inhibition zone diameters were recorded for each test microorganism—*Candida albicans*, *Staphylococcus aureus*, and *Escherichia coli*—treated with *Opuntia monacantha* extract, underground honey, and their combination. The standard antibiotic disks (fluconazole for fungi and gentamicin for bacteria) served as positive controls, while disks without any extract acted as negative controls. The mean inhibition zone diameters for each treatment were calculated, and comparisons were made between the antimicrobial effects of *Opuntia monacantha* extract, underground honey, and their combination against the test organisms.

Ethical Considerations

Permission to conduct this study was obtained from the school of pharmacy, Kabarak university. Ethical approval was sought from Kabarak University Research Ethics committee (KUREC-071222). Permission to collect data was obtained from the National Commission for Science, Technology, and Innovation (License NO. NACOSTI/P/23/24910). No human or animal subject was involved in this study as test subject. Finally, permission was also obtained from local authority prior to data collection within the study location.

RESULTS

Calculation of the Percentage Yield of the Extract

The percentage yield is a measure of the extraction process efficiency and provides an estimation of the quantity of extract that can be obtained from a given amount of plant material. In this study, the percentage yield was found to be 29.92% as calculated below.

Percentage Yield = Actual Yield/ Theoretical Yield x 100

Percentage Yield = 3.554g/11.88g X 100

Percentage Yield = 29.92%

Antimicrobial Susceptibility and Activity

Table 1 shows the results of antimicrobial testing of the mixture of *Opuntia monacantha* and underground honey.

Table 1:

Antimicrobial Susceptibility Testing of the Mixture of O. monacantha Extract and Underground Honey at Various Concentrations

Plates	Concentration(mg/ul)	Average Zone of Inhibition (mm)		
		C. albicans	*E.coli*	*S. aureus*
1	0.8895	7.46	7.51	6.29
2	0.4448	6.17	6.43	6.03
3	0.2224	5.77	5.825	5.94
4	0.1112	5.30	5.12	5.32
	Negative Control	4.00	4.00	4.00
	Positive Control	9.40	22.71	24.40

Table 2 shows the inhibition results of *O. monacantha* methanolic extract at various concentrations.

Table 2:
Antimicrobial Susceptibility Testing of the Methanolic Extract of O. monacantha at Various Concentrations

Plates	Concentration(mg/ul)	Average Zone of Inhibition (mm)		
		C. albicans	*E.coli*	*S. aureus*
1	0.1777	7.31	7.18	6.37
2	0.0885	5.80	6.33	5.52
3	0.0444	5.33	5.50	5.45
4	0.0222	4.95	4.94	5.00
	Negative Control	4.00	4.00	4.00
	Positive Control	9.40	22.71	24.40

Table 3 shows the results of the antimicrobial testing of underground honey.

Table 3:
Antimicrobial Susceptibility Testing of Underground Honey at Various Concentrations

Plates	Concentration(mg/ul)	Average Zone of Inhibition (mm)		
		C. albicans	*E.coli*	*S. aureus*
1	1.0	6.45	6.47	5.70
2	0.5	5.39	6.09	5.32
3	0.25	5.33	5.32	5.19
4	0.125	4.79	4.92	4.83
	Negative Control	4.00	4.00	4.00
	Positive Control	9.40	22.71	24.40

Phytochemical constituent Analysis of *O. monacantha* Cladode Extract and Underground Honey

Table 4 below presents the results for the qualitative analysis of plant phytochemicals in the mixture of *O. monacantha* Cladode Extract and Underground Honey.

Table 4:
Phytochemical Analysis of O. monacantha Cladode Extract and Underground Honey

Phytochemical test	Observation (O.monacantha)	Inference (O.monacantha)	Intensity (O.monacantha)	Observation (Honey)	Inference (Honey)	Intensity (Honey)
			Alkaloids			
Meyers Reagent(acidic)	White brown buffy precipitate formed	Alkaloids present	+++	White brown buffy precipitate formed	Alkaloids present	+
Wegner's reagent	Brown precipitate formed	Alkaloids present	+++	Brown precipitate formed	Alkaloids present	+
Meyers Reagent	White buffy precipitate formed	Alkaloids present	+++	White buffy precipitate formed	Alkaloids present	+
Dragendorf's reagent	Orange precipitate formed	Alkaloids present	+++	Orange precipitate formed	Alkaloids present	++
			Tannins			
Ferric chloride test	Black precipitate formed	Tannins present	+	Black precipitate formed	Tannins present	+
Lead Acetate	White brown buffy precipitate formed	Tannins present	+	White brown buffy precipitate formed	Tannins present	+
			Flavonoids			
Shinoda test	Yellow color formed	Flavonoids present	+	No Yellow color formed	Flavonoids absent	-
Alkalinized Shinoda test	Yellow color formed that disappeared on adding HCL	Flavonoids present	++	No Yellow color formed	Flavonoids absent	-
Lead acetate	Yellow precipitate formed	Flavonoids present	+	No Yellow precipitate formed	Flavonoids absent	-
			Saponins			
Froth test	Formation of a froth that persisted for 15 minutes	Saponins present	+	Formation of a froth that persisted for 15	Saponins present	+

DISCUSSION

The phytochemical screening tests conducted on the *Opuntia monacantha* cladode extract revealed the presence of alkaloids as indicated by the change in colour in Meyer's, Wegner's, and Dragendorf's tests. Alkaloids are nitrogen-containing constituents that are frequently linked to biological actions like antibacterial, anti-inflammatory, and analgesic properties (Manske & Holmes, 2014). Very high concentrations of alkaloids were present in the cladode extract, suggesting that alkaloids are responsible for the biological activity of *O. monacantha*. Traces of alkaloids were present in underground honey as seen from the intensity of colour changes from Meyer's, Wegner's, and Dragendorf's tests. These traces of alkaloids can be linked to their biological activities.

The presence of tannins in the cladode extract was confirmed by the Ferric Chloride and Lead Acetate tests. Tannins are phenolic compounds that have a complex chemical structure, are either condensed or hydrolysable, and have antioxidant properties (Sieniawska & Baj, 2017). In addition to forming covalent bonds with proteins, they also complex with proteins through nonspecific forces like hydrophobic effects and hydrogen bonding. Therefore, the mechanism of their antimicrobial activity may be attributed to their ability to inactivate cell envelope transport proteins, enzymes, and microbial adhesins. Only traces of tannins were present in the cladode extract and underground honey, as confirmed by the colour intensity in the two tests. The presence of tannins in the two samples can be attributed to their biological activities.

Flavonoids' presence was confirmed by color change intensity in Ammonia, Lead acetate, Sulphuric acid, and Shinoda's tests. Due to their well-known antioxidant, anti-inflammatory, and antibacterial properties, flavonoids are helpful in the treatment of a variety of illnesses (Panche et al., 2016). The traces of flavonoids that were present in the cladode extract suggest a source of antimicrobial agents against *E. coli, C.albicans, and S. aureus*. On the other hand, there was no presence of flavonoids in the underground honey from all four tests, which suggests that the antimicrobial activity of underground honey is due to the other phytochemical groups such as tannins and alkaloids.

Saponins were also present in the cladode extract and underground honey as seen from the froth test results. According to Ashour et al., (2019), saponins kill microorganisms by cell lysis caused when they form stable complexes with lipids in the cell membrane. The traces of saponins in *Opuntia monacantha* and underground honey may explain the antimicrobial activity seen against *S. aureus, E. coli,* and *C. albicans*.

From Table 1, at 0.8895 mg/ul, the furthest zones of inhibition were recorded at 7.46mm, 7.51mm, and 6.29mm against *C. albicans, E. coli,* and *S. aureus* respectively. Comparatively, this was slightly lower than fluconazole's zone of inhibition (9.40) and those of gentamicin (22.71 against *E. coli*, 24.40 against *S. aureus*). As the concentration of the mixture was decreased, the average zone of inhibition also reduced. The zone of inhibition for the negative control was 4.00 mm.

From Table 2, at the highest concentration of 0.1777 g/ul, the highest zones of inhibition were recorded at 7.48mm, 7.13mm, and 6.38mm against *C. albicans, E. coli,* and *S. aureus* respectively. This was noticeably lower in comparison to the zones of inhibition of gentamicin (22.71mm against *E. coli* and 24.40mm against *S. aureus*) and fluconazole (9.40mm). Notably, the average zone of inhibition decreased as the concentration of the mixture decreased. The negative control zone of inhibition measured 4.00 mm.

From Table 3, at 1.0 ul/ml the furthest zones of inhibition were recorded at 6.46mm, 6.26mm, and 5.67mm against *C. albicans, E. coli,* and *S. aureus* respectively. Comparatively, this was slightly lower than fluconazole's zone of inhibition (9.40) and those of gentamicin (22.71 against *E. coli*, 24.40 against *S. aureus*) notably, as the concentration of the mixture was decreased, the average zone of inhibition also reduced. The zone of inhibition for the negative control was 4.00 mm.

In all the cases, the 100% dimethyl sulfoxide-impregnated negative control exhibited very little effect against the microbes, with a zone of inhibition of 4.00mm. These findings suggest that the antimicrobial activity that was detected was not considerably influenced by the solvent that was utilized during the extraction process. On the other hand, the positive controls, which were impregnated with gentamicin and fluconazole, exhibited the highest zones of

inhibition, suggesting that they are more effective than *Opuntia monacantha* extract and underground honey. Further studies are needed to determine the mechanism of action and potential use of the mixture of *O. monacantha* extract and underground honey in treating infections caused by *E. coli,S. aureus,*and *C. albicans*.

CONCLUSIONS

The findings of this study demonstrate that *Opuntia monacantha* cladode extract, underground honey, and their combination exhibit antimicrobial activity against *Candida albicans*, *Staphylococcus aureus*, and *Escherichia coli*. The antimicrobial susceptibility tests revealed that the mixture of *O. monacantha* extract and underground honey produced larger zones of inhibition compared to the individual extracts, suggesting a potential synergistic effect. However, while the antimicrobial activity of the mixture was evident, it remained lower than that of the positive controls (fluconazole and gentamicin), indicating that further optimization and concentration adjustments may be required to enhance efficacy.

The phytochemical analysis confirmed the presence of bioactive compounds, including alkaloids, tannins, flavonoids, and saponins, in both *O. monacantha* extract and underground honey, with the plant extract exhibiting higher concentrations of these secondary metabolites. The presence of these phytochemicals suggests that the observed antimicrobial activity may be attributed to their known bioactive properties, such as disruption of microbial cell membranes, inhibition of enzyme activity, and interference with microbial replication.

Overall, the results support the hypothesis that *O. monacantha* cladode extract and underground honey contain bioactive compounds with antimicrobial properties, reinforcing their traditional use in managing oral thrush.

RECOMMENDATIONS

Based on the findings of this study, we recommend comparative studies should also be carried out to evaluate the antimicrobial performance of *O. monacantha* and underground honey against conventional antifungal and antibacterial treatments, helping to determine their clinical applicability. Furthermore, given the promising results, efforts should be made to develop natural antimicrobial formulations, such as mouthwashes or topical applications, incorporating *O. monacantha* extract and underground honey.

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