Brazilian Dental Science

unesp
UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
Instituto de Ciência e Tecnologia
Campus de São José dos Campos

ORIGINAL ARTICLE

DOI: https://doi.org/10.4322/bds.2025.e4742

The impact of *cumin* seed extracts with antifungal properties on *Candida albicans* and adhesion resistance in soft denture liners

O impacto dos extratos de sementes de cominho como antifúngicos sobre *Candida albicans* e na resistência ao cisalhamento em reembasadores macios para próteses dentárias

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How to cite: Abdul Nour AN, Rashid AAL, Al-Saadi AS. The impact of *cumin* seed extracts with antifungal properties on *C. albicans* and adhesion resistance in soft denture liners. Braz Dent Sci. 2025;28(3):e4742. https://doi.org/10.4322/bds.2025.e4742

ABSTRACT

Objective: Soft liner materials, while beneficial for denture stability, often harbor fungal growth, particularly at sites where the bond between denture base and the liner weakens, creating favorable conditions for colonization by *C. albicans*. Herbal plant extracts offer a novel therapeutic approach due to their antifungal properties, which are typically safer and associated with fewer side effects compared to conventional antifungal drugs. This study aims to evaluate the antifungal impact of ethanol and oil extracts of *cumin* seeds and their impact on the shear bond strength of soft denture liners. **Material and Methods:** *C. albicans* strains were isolated and identified. Extracts of *cumin* seeds (ethanol and oil) were prepared and evaluated for their antifungal activity using microdilution tests. Thirty specimens of soft lining material were prepared for testing shear bond strength testing using a Universal Instron machine, both with and without *cumin* extracts incorporation. **Results:** Both ethanol and oil extracts of *cumin* demonstrated inhibition of *C. albicans* growth. Significant increases in shear bond strength were observed with ethanol *cumin* extract in comparison to the control group, whereas the oil *cumin* extract did not significantly affect shear bond strength. **Conclusion:** Oil and ethanol extracts from *cumin* seeds exhibit antifungal properties versus *C. albicans*. While ethanol extract enhances shear bond strength, the oil extract shows no significant impact on bond strength. These findings highlight the potential of ethanol *cumin* extract as a dual-function material for enhancing denture hygiene and mechanical stability.

KEYWORDS

Antifungal; *Cumin* extracts; Shear; Soft liners; Strength.

RESUMO

Objetivo: Materiais de reembasamento resiliente embora benéficos para a estabilidade de próteses dentárias, frequentemente abrigam crescimento fúngico, particularmente em locais onde a ligação entre a base da prótese e o reembasador enfraquece, criando condições favoráveis para a colonização por *C. albicans*. Extratos de plantas medicinais oferecem uma abordagem terapêutica inovadora devido às suas propriedades antifúngicas, que geralmente são mais seguras e associadas a menos efeitos colaterais em comparação com os medicamentos antifúngicos convencionais. Este estudo tem como objetivo avaliar o efeito antifúngico dos extratos etanólico e oleoso das sementes de cominho e seu impacto na resistência de união ao cisalhamento de reembasadores resilientes. **Material e Métodos:** Cepas de *C. albicans* foram isoladas e identificadas. Extratos de sementes de cominho (etanólico e oleoso) foram preparados e avaliados quanto à sua atividade antifúngica utilizando testes de microdiluição. Trinta corpos de prova de material de reembasamento resiliente foram preparados para o teste de resistência de união ao cisalhamento utilizando uma máquina Universal Instron, tanto com quanto sem a incorporação dos extratos de cominho. **Resultados:** Ambos os extratos de cominho, etanólico e oleoso, demonstraram inibição do

crescimento de *C. albicans*. Aumentos significativos na resistência de união ao cisalhamento foram observados com o extrato etanólico de cominho em comparação ao grupo controle, enquanto o extrato oleoso de cominho não afetou significativamente a resistência de união. **Conclusão:** Os extratos oleoso e etanólico das sementes de cominho apresentam propriedades antifúngicas contra *C. albicans*. Enquanto o extrato etanólico melhora a resistência de união ao cisalhamento, o extrato oleoso não mostra impacto significativo na resistência de união. Esses achados destacam o potencial do extrato etanólico de cominho como um material de dupla função para melhorar a higiene das próteses e a estabilidade mecânica.

PALAVRAS-CHAVE

Antifúngico; Extratos de cominho; Cisalhamento; Reembasadores resilientes; Resistência.

INTRODUCTION

Soft liner materials take a decisive role in enhancing the stability, retention, and adaptation correction of dentures [1,2]. Over time, however, bonding between the soft lining and denture base material can weaken and creating an environment conducive to colonization by *C. albicans*, a fungus commonly found in the oral cavity. This fungal colonization can lead to infections that are challenging to manage, particularly among denture wearers [3,4]. Traditional antifungal treatments, while effective, often come with side effects, including the development of resistant strains of fungi. This underscores the importance of exploring new therapeutic approaches, such as the use of herbal extracts, which offer a promising alternative with potentially fewer side effects [5-7].

Cumin has been recognized for its disinfectant and antiseptic properties for such applications [8]. Phytochemical analyses reveal that *cumin* seeds are rich in active compounds, including flavonoids, alkaloids, proteins, coumarins, glycosides, tannins, steroids, and saponins[9]. Additionally, *cumin* contains organic acids such as propionic, citric, tartaric, ascorbic, fumaric, oxalic, malic, and aspartic acids, all of which contribute to its antifungal effects[10,11]. Promising available data have demonstrated that extracts from *cumin* seeds exhibit significant antifungal activity [12,13], particularly against *C. albicans* strains [14,15]. This highlights the potential of *cumin* as a natural remedy in the management of fungal infections.

Therefore, the aim of this study was to estimate the antifungal impact of *cumin* seed extracts, both ethanol and oil-based, and to evaluate the shear bond strength of soft lining materials upon their incorporation. This research seeks to explore novel therapeutic avenues that leverage the natural properties of *cumin* to mitigate fungal infections associated with denture wear.

MATERIAL AND METHODS

Isolation and identification of *C. albicans*

The isolation and identification of *C. albicans* were conducted using Gram staining, microscopic examination, germ tube formation, and API (Analytic Profile Index) *Candida* systems [16,17].

Extraction of *cumin* seed

Cumin seeds were washed and cleaned then left to dry at room temperature (25°C). The dried seeds were then ground using an electric grinder (Braun, Germany) [18].

Weight of 50 g of *cumin* seeds were used for both ethanol and oil extraction, employing a Soxhlet extractor (Cole-Parmer, USA). For the ethanol extract, the seeds were covered with 400 ml of 98% ethanol and 100 ml of distilled water. For the oil extract, 500 ml of n-hexane was used. The extraction process lasted for 4 hours per day over four days. The extracts were then evaporated by use a rotary evaporator (Cole-Parmer, USA) at 50-60°C under pressure until completely dry and stored in a dark place until use [19,20].

Minimal inhibitory concentration (MIC) for *cumin* extracts

The microdilution method was applied to determine the minimal inhibitory concentration (MIC). Serial dilutions of stock extract solution were prepared (ranging from 100% to 10%) in 96-well microtiter plates using Sabouraud Dextrose Broth (SDB) media (Oxoid, UK) [21,22].

Dimethyl sulfoxide (DMSO) was utilized as a negative control [23]. *C. albicans* was suspended in the media to a cell density of 1.5×10^6 cells/ml, equivalent to 0.5 McFarland standards at a wavelength of 530 nm, measured

spectrophotometrically. The smallest dilution that fully inhibited the growth of *C. albicans* was recorded as the MIC [12,22].

Toxicity testing method

The toxicity study done in Ministry of Industry and Minerals / Corporation for Research and Industrial Development / Center of Al-Razi for Research and Medical Diagnostic Kits Production/ Baghdad /Iraq. Twenty laboratory male rabbits bred followed the guidelines for using and caring of laboratory animals approved by Ministry of Industry and Minerals / Corporation for Research and Industrial Development /Center of Al-Razi for Research and Medical Diagnostic Kits Production/ Baghdad /Iraq, No.110/228 in 22/11/2020. The study requested to study matters that include: the case of the laboratory animals before examination and absence of injury or symptoms that interfere with the study.

Rabbits were divided randomly into five groups: control, ethanol *cumin* swab, oil *cumin* swab, ethanol *cumin* ointment, and oil *cumin* ointment, with four rabbits in each group (Figure 1A, B). The test materials were applied simultaneously for 28 days [24]. For the swab method, swabs were immersed in 2 ml of extract and used to wipe the oral cavity of the rabbits. For the ointment method, mixed the extract concentrations with oils ointment (olive oil, cod liver oil, petroleum oil, lanolin oil) according to scientific sources [25], 2g of ointment was applied to a series of swabs and used to wipe the oral cavity (Figure 2 A, B). Rabbits were examined daily for clinical signs of toxicity or pharmacological effects throughout the study. After the experimental period, the oral cavities of the rabbits were examined for any redness, necrosis, swelling, or other signs of irritation [26].

Preparation of heat-cured acrylic specimens

Specimens of heat-cured acrylic(60 specimens) (Veracril, Colombia) were prepared and the dimensions were (75 mm length, 5 mm depth and 25 mm width, including a stopper measuring 3 mm, proportional to ADA (American Dental Association Specification) [27]. The conventional methods for flasking, curing, finishing, and polishing were followed [28]. Two of specimens of heat-cured were used to create a space measuring 25 mm length, 3 mm depth and 25 mm width[29] (Figure 2), which was filled with soft lining material (self-cured).

Figure 1 - (A) The extracts used in toxicity test, (B) Wiping the oral cavity with extract, (C) Oil ointment, (D) Wiping the oral cavity with oils ointment.

Soft liner specimens preparation

Soft lining material (self-cured, EZ-SOFT) was mixed corresponding to the instructions of manufacturers (P/L ratio 1g powder to 1ml liquid) for the control group. For the experimental groups, the percentages of *cumin* extract were incorporated: 30% for the oil extract and 70% for the ethanol extract, as ascertain by the microdilution test results. The *cumin* extract proportion were deducted from the liquid volume of the soft lining to achieve the appropriate powder-to-liquid ratio. A probe sonication apparatus (120 W and 60 kHz) was used to mix the monomer of the liner material with the *cumin* extracts for 20 seconds to ensure homogeneity [30]. The lining material powder was added and mixed to the dough stage before filling the space that was created between the two-heat cured acrylic specimens corresponding to the instructions of manufacturers.

Testing of shear bond strengh

Shear bond strength was tested by use a Universal Instron machine (Model 1195) with a load of (100 kg) at a speed of (0.5 mm/min). The maximum load was applied to specimens untill fracture and determined the shear bond strength, following American Society for Testing and Materials specifications [31].

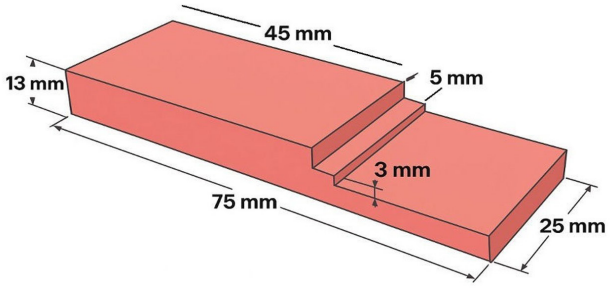

Figure 2 - Dimensions of the specimen for shear bond test.

$$Bond\ strength = \frac{F}{A}(N / mm^2) \quad (1)$$

Where F is the maximum load, and A is the cross-sectional area.

Scanning electron microscopy (SEM)

The morphological changes on randomly selected specimens surface were examined by use SEM (scanning electron microscope) [32]. Specimens were mounted on the SEM stage and analyzed, with photomicrographs taken at various magnifications. Analysis of elements of the specimens was conducted by using Energy dispersive spectroscopy (EDS) [33].

RESULTS

Minimal inhibitory concentration (MIC)

The microdilution test determined the MIC (mininal inhibitory concentration) as the minimal concentration that inhibited all the yeast cells. The MIC for *cumin* oil was found to be 10% (MFC10), while the MIC for *cumin* ethanol was 60% (MFC60). These concentrations were incorporated into the lining material [12,21]. Detailed results are presented in Tables I and II.

Toxicity test

The oral administration of *cumin* seed oil extract to rabbits did not cause any necrosis, unusual signs, or redness in the oral cavity. Furthermore, there were no observable changes in behavior, general health, weight, or appetite of the rabbits throughout the 28-day study period (Figure 3).

Oral cavities of the rabbits were examined for any signs of irritation based on Effraim et al. (2003) [26].

Table I - MIC of *cumin* oil extracts

Samples No.	Concentrations %										
	Control	10*	20	30	40	50	60	70	80	90	100
1	-	+	+	+	+	+	+	+	+	+	+
2	-	+	+	+	+	+	+	+	+	+	+
3	-	+	+	+	+	+	+	+	+	+	+
4	-	+	+	+	+	+	+	+	+	+	+

(+) represent no growth for *C. albicans*; (-) represent the growth of *C. albicans* [21,22]. *The MIC for *cumin* oil was 10% (MFC10). (1,2,3,4) number of *C. albicans* samples.

Shear bond strength test (N/mm²)

Table III shows mean value, standard deviation (SD), minimum, and maximum values of shear bond strength for each group. The highest mean value for shear bond strength was observed in the *cumin* ethanol group, while the control group had the lowest value.

A one-way ANOVA test showed significant differences in shear bond strength among groups ($F = 217.433$, $p = 0.001$). Table IV shows significant differences in pairwise comparisons between groups, except between control and *cumin* oil groups, which showed non-significant difference.

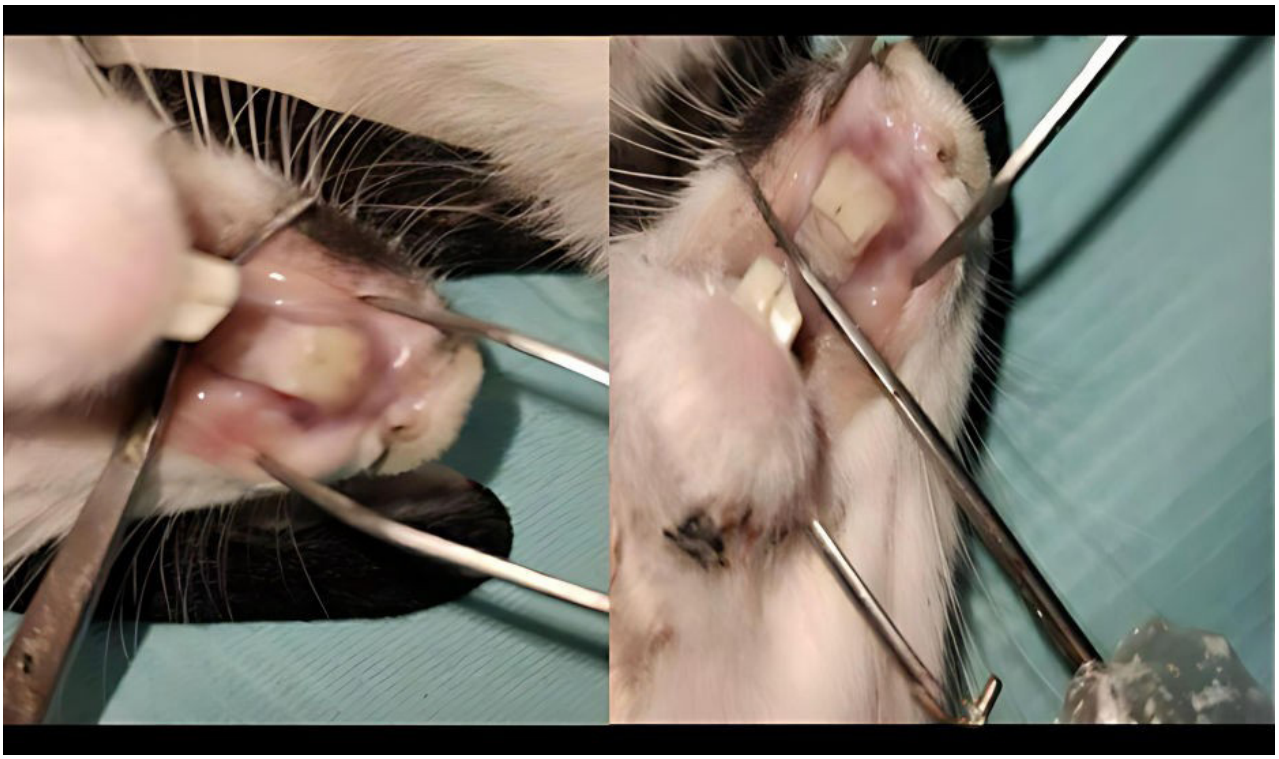

Figure 3 - Examination of the oral cavity of the animals.

Table II - MIC of *cumin* ethanolic extracts

Samples No.	Concentrations %										
	Control	10	20	30	40	50	60*	70	80	90	100
1	-	-	-	-	-	-	+	+	+	+	+
2	-	-	-	-	-	-	+	+	+	+	+
3	-	-	-	-	-	-	+	+	+	+	+
4	-	-	-	-	-	-	+	+	+	+	+

(+) represent no growth for *C. albicans*; (-) represent the growth of *C. albicans* [21,22].
*The MIC for *cumin* ethanolic extracts was 60% (MFC60).
(1,2,3,4) number of *C. albicans* samples.

Table III - Descriptive values of shear bond strength* for all groups

Group	Sample No.	Mean ± SD (N/mm²)	Min. (N/mm²)	Max. (N/mm²)	ANOVA
cumin Oil	10	0.126 ± 0.023	0.099	0.155	
cumin Ethanol	10	0.347 ± 0.076	0.284	0.474	F = 217.433, Sig. 0.001
Control	10	0.127 ± 0.022	0.104	0.159	

-*Cumin* Oil 10%, *Cumin* Ethanol 60%. *Shear bond strength were tested following American Society for Testing and Materials specifications (ASTM D-638m, 1986 [31]).

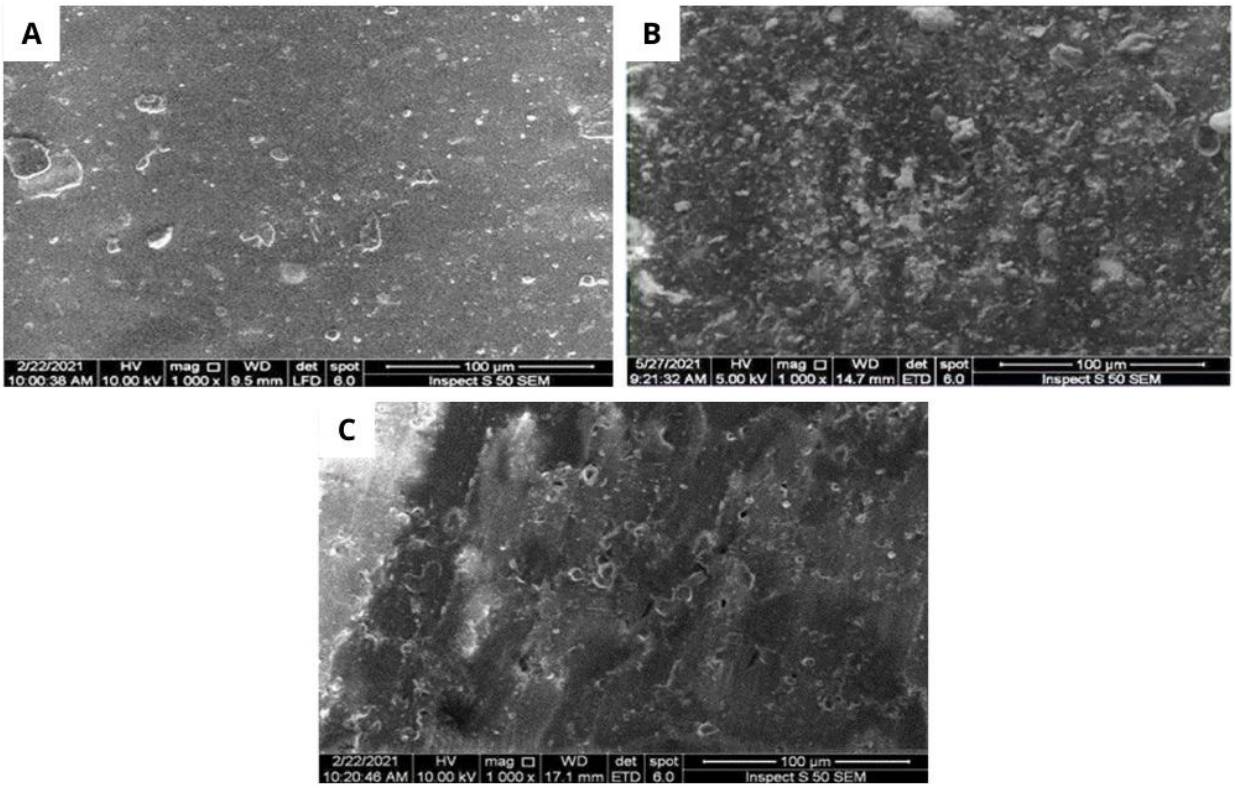

Figure 4 - SEM photomicrographs of denture lining material incorporated with *cumin* 1000x magnification A- pure soft denture liner B - 10% *cumin* oil, C - 60% *cumin* ethanol. Surface were examined by use SEM (scanning electron microscope) [32].

Table IV - Post-hoc Pairwise Comparison for Shear Bond Strength

Pairs Comparison*	Std. Error	Sig. (p-value)
Control - *cumin* Oil	0.010	1.000 (NS)
Control - *cumin* Ethanol	0.025	0.00003 (S)
cumin Ethanol - *cumin* Oil	0.025	0.00003 (S)

*Post-hoc Games-Howell test. Data were analyzed by SPSS V 0.25 (Statistical Package for Social Science) computer software.

Scanning electron microscopy (SEM) and energy dispersive spectroscopy (EDS)

Analysis of SEM (Figure 4) showed that the pure soft denture liner exhibited a regular surface morphology at 1000x magnification (Figure 4-A). In contrast, specimens incorporated with 10% *cumin* oil (Figure 4-B) and 60% *cumin* ethanol (Figure 4-C) displayed slight irregularities and porosity.

Energy dispersive spectroscopy (EDS) utilized to analyze the composition of denture lining specimens. Figure 5 A shows the composition of the lining material without extracts (C, H, O). Figure 5 B shows the composition with *cumin* oil (Na, Si, Ir, Cu, Co, Mg, Ni, Zn, C, H, O), and Figure 5 C shows the composition with *cumin* ethanol (C, O, H, Si, Na, Cu).

DISCUSSION

The use of soft liner materials serves to cushion and distribute stress on denture bases and supporting tissues [34]. Over time, however, these materials can become irregular and rough, potentially causing trauma to soft tissues and creating favorable conditions for *Candida* colonization, thus increasing the risk of denture stomatitis [35]. Various attempts have been made to reduce denture roughness [36], and prevent adherence of *C. albicans* such as nano additives[37,38], however some of these additives had no effect on its adherence [39], so incorporating medicinal plant extracts into soft lining materials as an antifungal approach is one such strategy [12].

cumin seed oil extract demonstrated antifungal effects against *C. albicans* at a concentration of 10%. This effect is attributed to compounds such as *cumin* *β*-pinene, aldehyde and p-cymene, which inhibit the growth of *Candida species* [40]. Carvacrol and p-cymene are also noted as the major components with antifungal properties against fungal species [20,41]. These findings were in agreement with previous studies by Mehdipour et al. (2019) [12], which reported strong inhibition of *C. albicans*

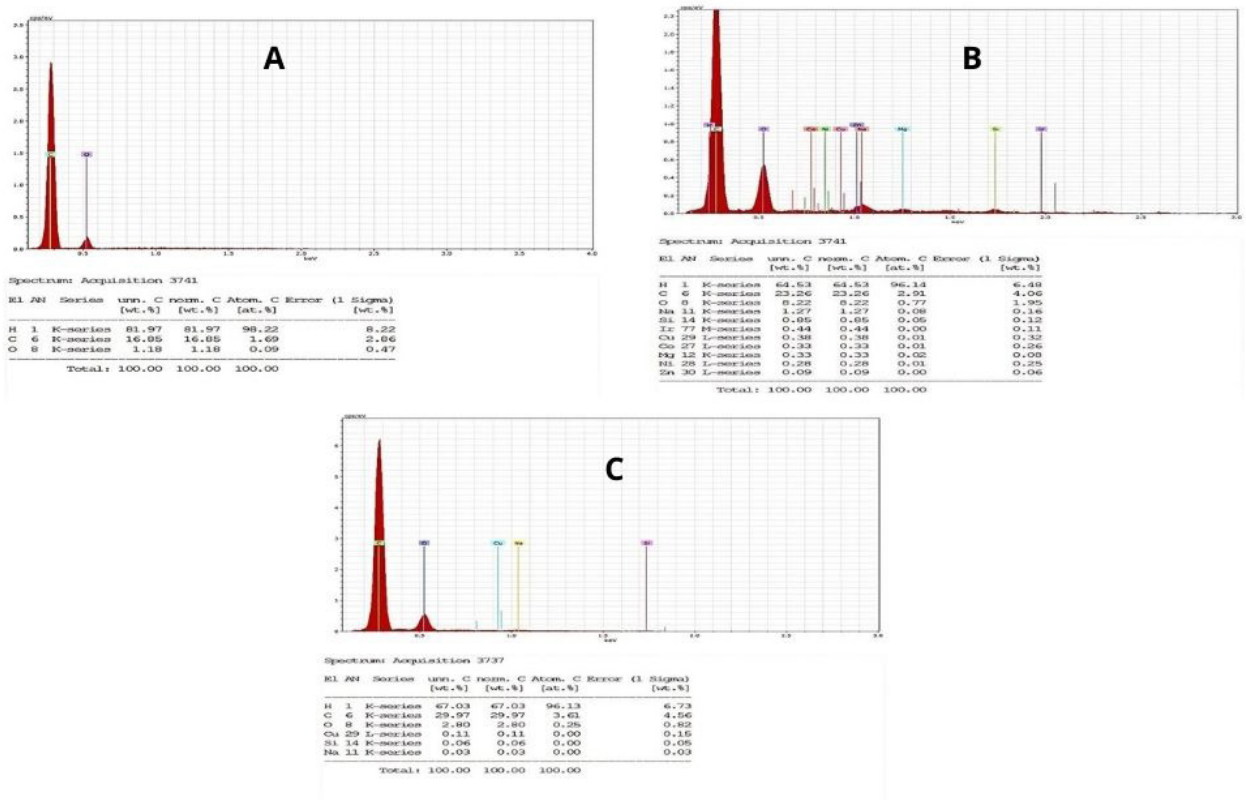

Figure 5 - Energy dispersive spectroscopy analysis for: (A) pure soft denture liner, (B) *cumin* oil, (C) *cumin* ethanol [33].

growth by *cumin* seed oil, as well as with Arici et al. (2005) [20], and Jabbar (2013) [40], who demonstrated the antifungal effects of *cumin* oil against various fungal species including *C. albicans*, *Cryptococcus,* and *Aspergillus flavus*.

Similarly, ethanol extracts of *cumin* seeds showed antifungal activity with an MIC of 60%, indicating that chemical components present in ethanol extracts also possess antifungal properties. Active components such as thymol and carvacrol in ethanol *cumin* extract are responsible for inhibiting *C. albicans* activity [15,42,43]. These results are aligned with studies [18] that have documented the antifungal action of ethanol extracts versus *C. albicans*.

To assess the safety of *cumin* extracts for oral use, toxicity tests were conducted on rabbits. These tests, which evaluated the effects of *cumin* extracts incorporated into lining materials for oral tissues, revealed no toxic effects after a 28-day study period. This finding corroborates previous studies [23,43] that found no adverse effects from *cumin* seed oil extracts when tested orally on rats for similar durations.

Shear bond strength remains a critical measure for evaluating the durability of bonds between lining materials and acrylic denture bases. Our results may indicate that *cumin* ethanol extracts significantly increased bond strength in comparison to the control group. This improvement can be assign to factors such as the uniform distribution of *cumin* extracts within the lining material [44], as observed in SEM (scanning electron microscope) images (Figure 4 A, B, and C). Higher concentrations of *cumin* extract may also reduce the plasticizer content in the lining material, thereby increasing resin hardness [45]. Conversely, *cumin* oil extracts did not significantly affect bond strength in comparison to the control group. This outcome may be due to the short 24-hour storage period in distilled water used in our study, which may not have allowed sufficient time for the oil to leach out and form a protective layer, potentially minimizing bond strength [46]. Additionally, the flowability of the lining material used in our study could have influenced bond strength, facilitating better adaptation to the bonding surfaces of heat-cured acrylic resin denture bases [28,47].

To the best knowledge, this study is the first study to investigate the incorporation of ethanol and/or oil *cumin* extracts into lining materials, making direct comparisons with previous studies challenging.

This study had several limitations. First of them, the research was conducted *in vitro*, and the results may not fully replicate the conditions within the human oral cavity. Additionally, the long-term effects of incorporating *cumin* extracts into soft lining materials were not evaluated. The study also did not explore the potential interactions between *cumin* extracts and other components of the oral environment, such as saliva and dietary substances. Furthermore, the sample size for the toxicity test was relatively small, which may limit the generalizability of the findings. Future studies should address these limitations by conducting *in vivo* experiments, assessing long-term effects, and using larger sample sizes to validate the results.

CONCLUSION

Both oil and ethanol *cumin* seeds extracts demonstrated antifungal action against *C. albicans.* The incorporation of ethanol *cumin* extracts significantly improved the shear bond strength of the soft liner material, while the oil *cumin* extract had non-significant impact on bond strength.

Author's Contributions

ANAN, AALR, ASA: Conceptualization. ANAN, ASA: Data Curation. ANAN, ASA: Formal Analysis. ANAN: Funding Acquisition. ANAN, ASA: Investigation. ANAN, AALR, ASA: Methodology. AALR: Project Administration. ANAN, AALR, ASA: Resources. ANAN, ASA: Software. AALR: Supervision. ANAN, AALR, ASA: Validation. ASA: Visualization. AALR: Writing – Original Draft Preparation. AALR: Writing – Review & Editing.

Conflict of Interest

No conflicts of interest declared concerning the publication of this article.

Funding

The authors declare that no financial support was received.

Regulatory Statement

This study was conducted in consistent with all the provisions of the Ethical Commission of Ministry of Industry and Minerals / Corporation for Research and Industrial Development / Center of Al-Razi for Research and Medical Diagnostic Kits Production/Baghdad /Iraq, under letter number No.110/228 in 22/11/2020.

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Editor: Bruno Mello de Matos

Date submitted: 2025 Mar 28
Accept submission: 2025 Jul 29