Antifungal Effect and Selected properties of Acrylic Resin Space Maintainer Modified with Castor Oil

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Submit: 13/3/2024 | **Accepted**: 23/5/2025 | **Published**: 24/5/2025

Abstract

Background: For children using PMMA space maintainer device, it is necessary to implement modifications, such as incorporation of oils, that exhibit antifungal activity and influenced acrylic resin materials properties.

Aim: To assess the incorporation influence of different concentration of purify Castor oil on selected properties of acrylic heat cured materials (surface hardness and color test) and on the sensitivity and adherence of *C. albicans*

Materials and methods: eighty specimens number were constructed from heat resin acrylic according to ADA specification, divided into ;40 specimens for hardness test and 40 specimens for color change test .these 40 specimens were subdivided into four groups according to Castor oil addition, Ten specimens had prepared which was control group without additive and thirty specimens were prepared within corporation of Castor oil in these different concentrations (1%, 2% and 2.5% Castor oil) hardness test were measured by shore D device and color test were measured by spectrophotometer. Antifungal sensitivity test was tested by agar well techniques and biofilm formation by using the 96-well plates.

Result and Conclusion: these results revealed the addition of Castor oil increased the hardness of acrylic resin materials the difference was significant with control ($P \leq 0.05$) group, and the differences was non-significant on the color test of acrylic materials with control and among all groups. The oil inhibits the growth of *Candida albicans* the inhibition zone was increased when concentration of oil increased and had effect in reducing biofilm formation. The additives materials of pure natural Castor oil by different concentration improved hardness property and had no effect on color property of heat resin material. It had antifungal effect against candida albicans inhibit their growth and inhibit their biofilm formation.

Keywords: Castor oil , space maintainer, acrylic resin ,antifungal.

التأثير المضاد للفطريات وبعض الخصائص المختارة لمادة الأكريليك في جهاز الحفاظ على المسافة المُعدل بزيت الخروع

المقدمة: بالنسبة للأطفال الذين يستخدمون جهاز الحفاظ على المسافة المصنوع من مادة الاكريلك من الضروري إجراء تعديلات مثل دمج بعض الزيوت التي تمتلك نشاطًا مضادًا للفطريات، والتي تؤثر أيضًا على خصائص مادة الراتنج الأكريليكي

الهدف: تقييم تأثير دمج تركيزات مختلفة من زيت الخروع النقي على بعض الخصائص المختارة لمادة الأكريليك المعالجة حراريًا (اختبار الصلابة السطحية واختبار اللون)، بالإضافة إلى تقييم تأثيرها على حساسية والتصاق فطر المبيضة البيضاء

المواد والطرق: تم تصنيع ثمانين عينة من الأكريليك الحراري وفقًا لمواصفات جمعية طب الأسنان الأمريكية 40 عينة لاختبار الصلابة و40 عينة لاختبار التغير في اللون. تم تقسيم هذه الأربعين عينة إلى أربع مجموعات وفقًا لتركيز زيت الخروع المضاف؛ حيث تم تحضير عشر عينات كمجموعة ضابطة بدون أي إضافات، وثلاثين عينة تم تحضيرها مع دمج زيت الخروع بتركيزات مختلفة (1%، 2%، و2.5%). تم قياس اختبار الصلابة باستخدام جهاز شور دي وتم قياس اختبار اللون باستخدام جهاز الطيف الضوئي واختبار الحساسية ضد الفطريات فتم باستخدام تقنية الحفر في الأكار وتم اختبار تكوين الغشاء الحيوي باستخدام أطباق ابار 96.

النتائج والاستنتاج: أظهرت النتائج أن إضافة زيت الخروع زادت من صلابة مادة الأكريليك وكان الفرق ذا دلالة إحصائية مقارنة بالمجموعة السيطرة بينما لم يكن هناك فرق معنوي في اختبار اللون بين المجموعة السيطرة وباقي المجموعات حيث زادت منطقة التثبيط بزيادة تركيز الزيت، وكان له تأثير في تقليل تكوين الغشاء الحيوي. وبالتالي، فإن المواد المضافة من زيت الخروع الطبيعي النقي بتركيزات مختلفة حسّنت من خاصية الصلابة ولم تؤثر على خاصية اللون لمادة الأكريليك الحرارية، وأظهرت تأثيراً مضاداً للفطريات ضد المبيضات وكان له تأثير في تقليل تكوين الغشاء الحيوي فإن المواد المضافة

من زيت الخروع الطبيعي النقي بتركيزات مختلفة حسّنت من خاصية الصلابة ولم تؤثر على خاصية اللون لمادة الأكريليك الحرارية، وأظهرت تأثيراً مضاداً للفطريات خلال تثبيط نموها وتكوينها للغشاء الحيوي.

Introduction

Common problem for primary teeth was the premature losing that resulting in disruption of permanent teeth alignment, so that immediate using for a space maintainer was the safer way to reduce this problem. The use of space maintainers also prevents a complicated orthodontic treatment in future [1,2].

polymethyl methacrylate was still the first material for choosing and playing an important role in dentistry field [3] , Although it had very good biological and mechanical properties make it optimal for different dental applications [4,5] and regardless its popularity which satisfy more aesthetically , easy processing and simple in repairing , the major problems associated with acrylic material as space maintainer were lower strength especially for scratching by cleaning process and under fatigue failure inside the mouth in addition adherence of microorganism such as fungi that considered as the causative of oral candidiasis . Several attempts were made in order to overcome these problems, also to improve and modify the thermal properties, hardness and strength of the acrylic resin material. Possibility of adding some additives (reinforcing different materials) to improve properties as well as enhance optical properties and esthetic [6,7]which also considered as advanced therapeutic way towards acrylic stomatitis [8].

Castor oil

Dental researches had been conducted from the past decades up to recent time to enhanced the properties of PMMA and/or alter properties to reach the requirements of a suitable acrylic resin space maintainer materials [9]

Many of the additives from different sources have been used with acrylic resin to improve one or more properties in this study castor oil have been added successfully in different concentrations due to its well-known ability to inhibit the biofilm formation of the candida albicans there by preventing its growth on acrylic resin.

Castor oil was multipurpose oil (vegetable oil). It's made from extracting oil from of the Ricinus communis seeds plant, the oil be used safely so that people have habituated for thousands of years for medicinal and pharmaceutical purpose [10] essential oils adding to the polymer matrix had shown to change how working the material. The changes related on the types and amounts of essential oils [11,12,13]. Several endeavors had been made for improving the properties of acrylic space maintainer material by addition of medicinal plant extracts.

Hardness was important key that attribute in dentistry via it indicates how resistant it is to service scratching [13,14]. There were many methods to assess restorative materials hardness but the most common methods were shore D hardness tests which was the most perfect methods. although tests little differs from one to the others, but they all related on the penetration of symmetrically small, sharped indenter into the materials surface under test. The selection deepened on the interest of material and the anticipated hardness extent [14]. Study by Taqa *et al* [15] related to effect of Castor oil on properties of cold cured acrylic but there were a lack in studies concerning the influence of Castor oil on the heat cured acrylic properties

Color of prosthesis

Color of prosthesis and gloss were the most important factors for the appearance, esthetic and acceptance of prostheses, furthermore changes in color would be an index of degradation of the prosthesis. This changes in color could be described as visually perceptible or it was

clinically acceptable [16]. The most important property for the esthetic assessment of acrylic resin material was the color stability. It was crucial for the acrylic resin to keep its color despite the discoloration of everyday colorants. The change of appearance was indicated as a reduction to long term quality of the denture [17] For children using space maintainer device, it necessary for making modifications to the removable space maintainer this modification such as added additive oil was affect the acrylic resin material properties.

Although some studies showed the antifungal effect of Castor oil[18,19] in this study asses the antifungal effect in different concentration .The null hypothesis was that Castor oil had antifungal effect and would improve the microhardness as well as color stability of the acrylic space maintainer material so this study conducted to assess the influence of incorporation of various concentration of purify Castor oil on surface hardness ,color test of denture base acrylic resin materials and on sensitivity and adherence of *candida albicans.*

Materials and Methodologies

Specimen's preparation

Metal patterns design by (CNC) for color change the sample as disc their dimensions equal to50 ± 1mm in diameter and 0.5 ± 0.05 mm in thickness (Fig 1A),for surface hardness test ,their dimensions was (65mm x 10mm x 2.5mm) length ,width and thickness accordingly , (Fig 1B), accordingly to ADA specification [20], were prepared to obtain samples from heat cure acrylic resin.

Figure (1) **):** Metal Patterns(A) Color change tests (B) Surface Hardness

Sample grouping

Eighty specimens total number were prepared from heat cure acrylic according to ADA specification, divided into ;40 specimens for hardness test and 40 specimens for color change test .these 40 specimens were subdivided into four groups according to Castor oil addition, Ten specimens (control) had prepared without any additive and thirty specimens were prepared by incorporation of Castor oil in these various concentrations :1%, 2% and 2.5% Castro oil, each group consists of (10) samples respective to different ratio of Castor oil as shown in Table1.

Method of incorporation Castor oil into acrylic resin material

Acrylic resin material mixing procedure was according to manufacture instruction the control group (P/L ratio 2g : 1ml) Mixing procedure done in clean glass jar by weighting 20 g of acrylic powder and mixed with 10 ml of monomer liquid for control group [21],and for experiment specimens the percentage value of the oil extracts will be incorporated into acrylic, these percentages were decrease from the volume of the monomer liquid to obtain right P/L ratio as mention in Table (1), the oil was mixed with the acrylic liquid material about 20 seconds [22] via used of probe sonication device about 120 W with 60 KHz to ensure adequate homogeneity, after that the powder adding then mixed mixture till reached the dough stage was mixed according to manufacture instruction (P/L ratio: 2g of powder: 1ml of monomer liquid)

Table (1): Volume of castro oil mixed with soft liner monomer

Castor oil concentrations	Powder of acrylic	Monomer of acrylic	Amount of oil mixed with monomer
Control	20 gm	10 ml	0
Castor oil 1%	20 gm	9.9 ml	0.1 ml
Castor oil 2%	20 gm	9.8 ml	0.2 ml
Castor oil2.5%	20 gm	9.75 ml	0.25 ml

Powder and liquid for heat-cure acrylic used in this study (Maarc dental). Mixed together following the instruction of manufacturer's using a container with spatula. The container left till it reached the dough stage, (since the mixture separates from container wall) . Then was packed into stone mould, this done for the acrylic material arriving the dough stage, packing fasking, finishing and polishing all these step were done according to conventional methods [20,21].

Measurement of surface hardness

Shore D hardness device tester its accuracy 0-100HD (Time group Inc, China) using to measure the surface hardness of heat cure specimens according to ISO7619(ASTMD 2240) .The device was placed vertically on the flat specimen which supported by rigid and flat base as seen in Figure (2).The distance between the indenter of hardness tester and the surface of specimen was around (5- 12mm). The period of contact between the indenter and the specimen was equal to 6 seconds. Five hardness measurements for each specimen were obtained and take from the scale reading then the average of these reading was calculated [23,24]

Figure (2): Shore D hardness tester

Color change test

Method using for evaluation the color change by measure the amount of percentage of absorbed light was achieved by using UV –visible spectrophotometer [25]. Sample was placed on the outlet light device to expose to light, percentage of absorbing light was obtained by device screen. The instrument then auto zeroed with nothing in the sample across the working range to establish the baseline point. Spectrophotometer Shimadzu used in the study measures the wavelength ranging from 190 – 1100 nm; by measuring the intensity of light a crossing the sample (I), and comparing with intensity of the light before passes through the sample (Io). The absorbance (A)was obtained in a percentage (%) [26].

Scanning Electron Microscopic (SEM)

The changes of surface morphological of specimens that randomly selected for each studied group examined by using a scanning electron microscope (SEM) (England) Figure (3). Specimens from each group were placed on the SEM table to examine. The photomicrographs of Scanning Electron Microscopic were done in various power magnification [27]

Figure (3): SEM device

Isolation of Candida albicans

A swab was taken from the mouths of patient wearing complete denture .Samples were collected from 5 women and 8 men by swab and cultured on BHI (Brain Heart Infusion) medium about (48 hours) at 37°C in an incubator Fig(4), then germ tube formation, grams stain, examination under microscopic, in addition API Candida systems was done to diagnosis the *Candida albicans* [28,29]

Fig.(4)*Candida albicans* species

Agar Disk Diffusion Method:

Disk diffusion test were prepared by using Mueller Hinton Agar media (Mueller-Hinton, France), colonies of C.aliban's was taken from pure culture using loop. Colonies were diluted by five ml of sterilized normal saline. Then the fungal suspension was putting in 0.9% sodium chloride solution the density of it equivalent to 0.5 McFarland barium sulphate standard (1*105 CFU/ml) of C.albican isolates. Mueller-Hinton agar media was inoculated by this suspension, by using forceps disks was placed on the agar and pressed down. Then incubated at (20 h. 37°C), inhibition zone diameter was appeared around the disk and measure by ruler [30,31]

Assessment of Antibiofilm Activity

The antibiofilm test was done by using the 96-well plate. Each well contains 99 µL of brain heart infusion broth, 1 µL glucose and 100 µL Castor oil. Firstly, the well 25.6 µgmL−1 of Castor oil from this well doing dilution process until 0.05 µg mL−1 started. Then, 10 µL of inoculum added to each well and in the next rows, samples containing the castor oil were prepared 1%,2% and 2.5% Then, the microplate incubated in 37 °C for 24 h. After time of incubation, the wells were emptied and rinsed 3 times with phosphate buffer saline (PBS), then dried at 37 °C for 24 each well staining with crystal violet dye (0.1%) for 15 min. The wells were rinsed 3times with PBS solution. After that, glacial acetic acid was added for 10 min for resuspension of biofilm. The intensity of the colored suspension was assessed by measuring the absorbance at 630 nm by ELIZA Tester Fig(5) The process was repeated for each MRSA strain for 3 times[32]

Fig(5) ELIZA Tester

Results

Surface Hardness

Descriptive Tables of surface hardness of tested groups showed in Table(2) and Fig(6), the maximum mean value of hardness recorded by group IV (2.5% castor oil), while the minimum mean value of hardness recorded by group I (control).the hardness value increased when the concentration of castor oil increased too**.**

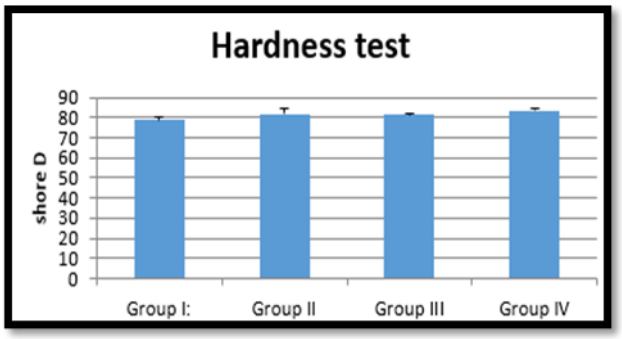

Figure (6): Bar chart of Descriptive data of all groups

Levene's test for surface hardness showed the differences was highly significant among these groups(P=0.002)(F=7.438)(df1=3,df2=20) Table(3) showed post-hoc-LSD test of hardness test, there were significant between all groups except between group II(1%Castor oil)with group III (2% Castor oil)and with group IV (2.5%castro oil)there were no significant differences between them.

Table 2 : Descriptive statistics of surface hardness of all groups

Group	No.	mean	Std. error	Std. Deviation	95%Confidence Interval	
					Lower Bound	Upper Bound
Group I: Control (PMMA+0%of Castor oil	10	78.694	.809	1.71405	77.008	80.381
Group II (PMMA+1% of Castor oil)	10	81.861	.809	3.24793	80.174	83.548
Group III (PMMA+2% of Castor oil)	10	81.361	.809	0.66705	79.675	83.048
Group IV(PMMA+2.5% of Castor oil)	10	83.278	.809	1.32777	81.591	84.965

Table 3 (Post-hoc- LSD) of Surface Hardness between groups

(I) Groups	(J) Groups	Mean Difference (I-J)	P-Value	Sig.
GroupI: Control (PMMA+0%Castor Oil)	Group II(PMMA+1% Castor Oil)	-3.1667*	.012	S
	Group III (PMMA+2% Castor Oil)	-2.6669*	.030	S
	Group IV (PMMA+2.5% Castor Oil)	-4.5833*	.001	S
Group II (PMMA+1%Castor Oil)	Group III (PMMA+2% of Castor Oil)	.4997	.667	NS
	Group IV (PMMA+2.5% Castor Oil)	-1.4167	.230	NS
Group III (PMMA+2%Castor Oil)	Group IV (PMMA+2.5% Castor Oil)	-1.9164	.109	NS

Color Test:

Table(4)showed descriptive statistics of color, group I control recorded minimum mean value while Group IV (PMMA+2.5% of Castor oil) recorded the maximum mean value for color test Table (5) showed Levenes test showed no significant differences among all groups (p=0.053) (F=3.046)

Table *(4)* : Descriptive statistics of color test of all groups

Group	No.	Mean	Std. Deviation	Std. Error	95%Confidence Interval	
					Lower Bound	Upper Bound
Group I:Control (PMMA+0% of Castor oil	10	2.8	2.60502	2.503	0.3	2
Group II (PMMA+1% of Castor oil	10	2.81	2.62241	2.503	0.9	2.3
Group III(PMMA+2% of Castor oil	10	2.84	2.68782	2.503	0.92	2.8
Group IV (PMMA+2.5% of Castor oil	10	2.85	2.20256	2.503	0.94	2.8

Table (5); Post_hoc Tukey HSD

Groups	Group II (1%Castro oil)	Group III (2%Castor oil)	Group IV (2.5% Castor oil)
Group I: Control	P=.537 NS	P=.521 NS	P=.584 NS
Group II (1%Castor oil)	------	P=1.000 NS	P=1.000 NS
Group III (2%Castor oil)	------	------	P=1.000 NS

Scanning Electron Microscopic (SEM)

Figure (7) showed the surface of acrylic resin heat cured acrylic in control group

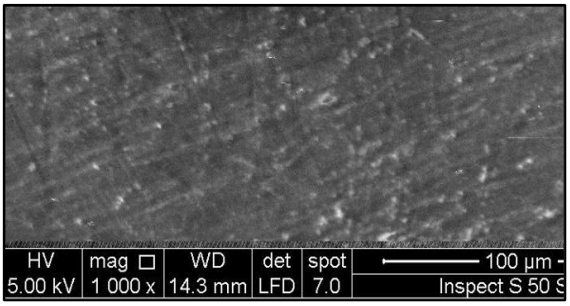

Figure (7) "SEM micrograph of unmodified heat-cured acrylic resin (control group) showing baseline surface morphology."

Figure (8) showed the surface of acrylic denture with incorporated with Castor oil at a concentration 1% the same of control and more regular surface in compared with control specimen

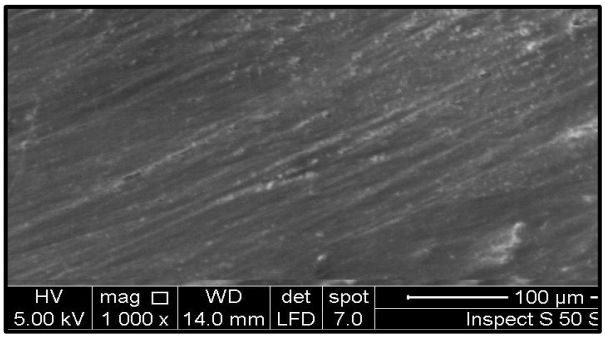

Figure (8); "SEM micrograph of heat-cured acrylic resin modified with 1% castor oil, demonstrating a more regular surface compared to control."

Figure (9) showed the surface of acrylic resin when incorporated with Castor oil at a concentration 2% had little micro changes but more regular surface in compared with control specimens.

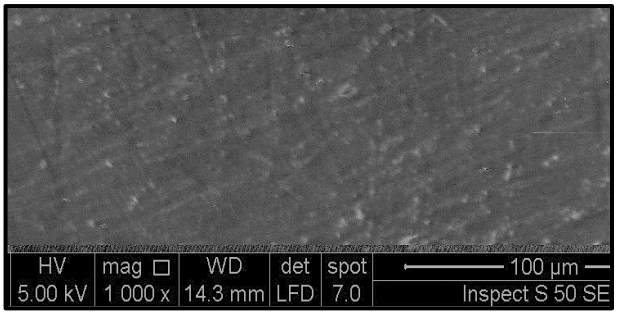

Figure (10) showed the surface of acrylic resin with incorporated with Castor oil at a concentration 2.5% showed some irregularity (blue arrow) on surface in compared with control specimen

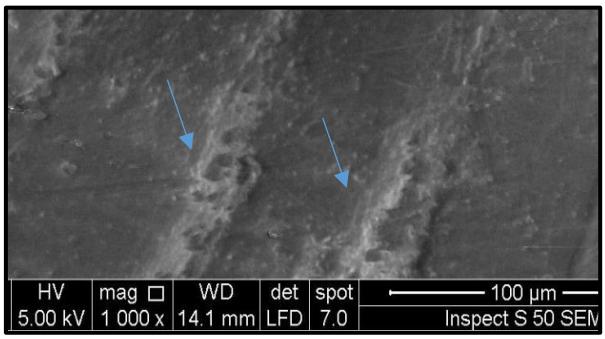

Figure (10): "SEM micrograph of heat-cured acrylic resin modified with 2.5% castor oil, highlighting localized surface irregularities (blue arrow)."

Result of Sensitivity test

Table 6 showed descriptive statistics of all groups; minimum, maximum, mean, S.D., S. E. Results showed the maximum mean value for sensitivity test was recorded by 2.5% Castor oil and lower mean value was reported by control group

Table 6: Descriptive tables of sensitivity of all groups.

	N	Min.	Max.	Mean	S.E	S.D	ANOVA P<0.05 S.
Control	10	1	2.5	2.5	.05375	.16997	
1%	10	17	17.5	17.5	0.4373	.13222	
2%	10	18	19	19	.04714	.14907	
2.5%	10	20	22	22	0.3333	.12329	

As shown in this Table the ANOVA test among groups there were significant differences p<0.05 Table(7) showed Pairwise comparison test revealed significant differences between tested groups

Table 7: Pairwise comparison of sensitivity test between groups.

Pairwise comparison	Control	2%Castor oil	2.5% Castor oil
1% Castor oil	0.01	0.04	
2% Castor oil	0.01		0.03
2.5%Castoroil	0.001		

Result of Antibiofilm Activity

Table 8 showed statistics descriptive of adherence test all groups; min., max., mean, S.D, S.E. Results showed the low mean value for adherence test was showed by 2.5% Castor oil and high mean value was showed in control group

Table 8: Statistical description of Adherence Test of Castor oil against Candida albicans

Groups	N	Mean	SD	SE	Min.	Max.	Anova P-value
Control 0%	5	.0110	.00022	.00007	.00	.012	≤ 0.0001 H.S
1%Castor oil	5	.0023	.00074	.00033	.00	.003	
2% Castor oil	5	.0022	.00182	.00081	.01	.002	
3% Castor oil	5	.0021	.00707	.00316	.01	.003	

Table(9) showed Multiple comparison analysis test of adherence test, there were significant

between control group with Castro oil groups but the differences were no significant between Castor oil groups with each others .

Table 9: Multiple comparison analysis of of Adherence Test by Castor oil on Candida albicans

(I) G	(J) G	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Control 0%Castor oil	**1%** Castor oil	-.01400*	.00144	.000	-.0170	-.0110
	2% Castor oil	-.05900*	.00144	.000	-.0620	-.0560
	2.5% Castor oil	-.02000*	.00144	.000	-.0230	-.0170
1% Castor oil	**2%** Castor oil	-.04500*	.00144	.065	-.0480	-.0420
	2.5% Castor oil	-.00600*	.00144	.066	-.0090	-.0030
2% Castor oil	**2.5%** Castor oil	.03900*	.00144	.068	.0360	.0420

*. The mean difference is significant at the 0.05 level

Discussion

Most commonly space maintainer materials used in prosthesis was heat cure acrylic resins. The crucial limitation represented by this material act as reservoir of microorganisms. Reducing of microorganisms adherence could be achieved by chemical modification on the surface charge of acrylic resin material composition as available.

Heat cured denture base material were mostly available as Powder / liquid, in the present study oil additives was done to monomer because it was better dissolved in it and to ensure its homogeneity. In the present study properties hardness and color test of acrylic denture base material was evaluated after incorporation the castor oil in different concentration, mixed different percentage of Castor Oil) with monomer.

For hardness the high effect of Castor oil additive on the hardness of acrylic recorded by Group IV(PMMA+2.5% of Castor oil) while the minimum value recorded by control group with significant differences between all group with control, the null hypothesis related to this study was accepted because when the addition of Castor oil was likely contributed to the increased hardness property by increased concentration this was due to structure of organic oil additive will entered between polymer matrix leading to change in its structure and converted to more stronger one that lead more resistance to penetration other explanation was the physical configuration will converted from irregular form into more regular and straight form after addition of Castor oil as seen in SEM scanning that illustrated that addition of 1%and 2% caused more regular than control but2.5% showed little change and irregular than control group this might be due to increasing of oil concentration leading to increase viscosity and hydrophobic reaction with matrix of acrylic as a result the oil tends in forming separate phases instead of mixing evenly , the limitation of this study ,there was no previous study in order to compare with it . Also the most important clinical properties for dental material was color stability which change in color give impression for aging destroying of material surface which had most important properties to assume that more hardness, more dense material, and more resistant to wear, additive of Castor oil showed the differences was no significant in color stability among the different groups this no effect on color stability of polymethyl methacrylate acrylic risen was due to properties of Castor oil such its transparent color, the oil solution was colourless no effect on color of acrylic and also don't have an unpleasant odour so these efficacy with antifungal action make its possible to use for space maintainer cleaning. This result the same of Taqa et al[15] that showed that the Castor oil additive(1,5%,2%,2.5%) on cold cured acrylic showed no significant difference in color test with control. Further studies were needed concerning the relation of Castor oil on the others acrylic resin properties.

Castor oil was a popular natural treatment for help treat denture stomatitis, a painful condition thought to be caused by an overgrowth of candida. This is a common problem in older people who wear dentures [33]. This study reveal that Castor oil inhibit growth of *C. albican* also inhibit their adherence this result agree with other studies that with dental-associated stomatitis that showed the treatment with castor oil improved clinical signs of stomatitis this indicate that this oil had antifungal effect against *C.albican* species [34,35,36]

This study was limited to *in vitro* conditions. Factors such as aging, salivary interaction, biofilm development, and long-term stability of the oil-polymer blend were not assessed. Further studies are recommended to evaluate the long-term clinical performance, biocompatibility, and aging behavior of castor oil-modified PMMA.

Conclusions

Within the limitations of this *in vitro* study, the incorporation of castor oil into heat-cured PMMA resin enhanced its antifungal activity against *Candida albicans* in a concentration-dependent manner. Additionally, the incorporation of pure natural castor oil at varying concentrations improved the surface hardness of the heat-cured resin material used for removable space maintainers, without affecting its color properties. These findings suggest that castor oil may serve as a promising natural additive to improve the hygienic properties of acrylic resin

materials, particularly for patients at risk of fungal infections. However, further *in vivo* investigations and long-term performance evaluations are needed before clinical application can be recommended.

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