

# Antifungal Effect of Some Medicinal Plant Extracts On Candida Albicans Adherence on Acrylic Resin Denture Base Material. An In Vitro Study

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## الخلاصة

**الأهداف:** تقييم مختبري للتأثير المضاد لبعض مستخلصات النباتات الطبية (النعناع والسذاب والرمان والثوم) على خلايا المبيضات البيضاء وتقييم التأثير المضاد لالتصاق خلايا المبيضات البيضاء على السطح الداخلي لقاعدة الطقم من الراتنج الاكريلي. **المواد وطرائق العمل:** تقييم التأثير المضاد لبعض المستخلصات النباتية على خلايا المبيضات البيضاء باستخدام طريقة التخفيف الدقيقة في الوسط السائل وبواقع تركيزين لكل مستخلص. لقد تم مشاهدة ، فحص وعدّ الخلايا الملتصقة باستخدام تقنية المجهز الوامض وكذلك تم من خلاله ملاحظة التأثير المضاد للالتصاق للمستخلصات النباتية المراد اختبارها أيضا فقد استُخدم تركيزين لكل مستخلص نباتي وعند ثلاث فترات تعرّض مختلفة وهي (ساعة واحدة، ٢٤ ساعة، ٤٨ ساعة) على التوالي. **النتائج:** تم استخدام جهاز المطياف الضوئي لتقييم التأثير المضاد للمستخلصات النباتية على عزلة المبيضات البيضاء حيث أظهرت النتائج ان مستخلصي السذاب والرمان عند التركيز الأول (الأعلى) أفضلها تأثيراً قاتلاً على عزلة المبيضات البيضاء في حين أعطى مستخلصي الرمان والثوم تأثيراً جيداً عند التركيز الثاني (الأوطأ). لقد كان معدل عدد الخلايا الملتصقة بعد غمر النماذج في التركيز الأول للمستخلصات النباتية لمدة ساعة واحدة هو [ (٣.١)، (٣.٧)، (٣.٧)، (٤.٧) ] على التوالي خلية في المليمتر المربع الواحد. وأما في التركيز الثاني لهذه المستخلصات فقد كان معدل عدد الخلايا الملتصقة [ (٤)، (٤.٤)، (٤.٤)، (٤) ] على التوالي خلية في المليمتر المربع الواحد ، للمستخلصات النباتية السابقة الذكر. **الاستنتاجات:** استنتجت الدراسة أن جميع المحاليل التي تم اختبار تأثيرها المضاد للالتصاق، كانت مؤثرة وبشكل معنوي عالي في إزالة خلايا المبيضات البيضاء من على سطوح نماذج الراتنج الأكريلي وبكلا التركيزين وبدون فروق إحصائية على مستوى زمن التعرّض.

## ABSTRACT

**Aims:** Evaluate the antifungal effect of some medicinal plant extracts (peppermint, rue, pomegranate and garlic) and their anti-adherent effect on *C. albicans* cells that attached on the fitting surface of acrylic resin denture base. **Materials and Methods:** This study evaluated the antifungal effect of plant extracts, using broth micro dilution method at two concentrations for each one. The visualization, inspection and enumeration of adherent *C. albicans* cells and the detection of the anti-adherent effect of these plant extracts was achieved by using fluorescent microscope. For each plant extract, two concentrations in addition to the time of immersion at three different intervals (1 h, 24 hrs and 48 hrs) were evaluated for the anti-adherent effect. **Result:** Antifungal effect of plant extracts on *candida albicans* cells, was measured by spectrophotometer which showed that both rue and pomegranate extracts had the best antifungal effect at first concentration, while in the second concentration, pomegranate and garlic extracts had the best antifungal effect . The mean number of remaining *C. albicans* cells which adhered on acrylic resin samples after treatment by immersion in the first concentration of plant extracts for one hour was (3.1), (3.7), (3.7) and (4.7) cells /mm<sup>2</sup> respectively; while after treatment with second concentration of plant extracts for one hour was [ (4), (4.4), (4.4) and (4) ] cells / mm<sup>2</sup> respectively for the above mentioned plant **Conclusion:** All examined medicinal plant extracts were significantly effective in dislodging *C. albicans* cells from acrylic resin samples at their different concentrations and statistically there was no significant difference among the different times of immersion.

**Keywords:** *Candida Albicans*, Medicinal Plant Extracts

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## INTRODUCTION

The increased incidence of oral candidiasis among patients wearing dentures

and other removable appliances is associated with a great increase in number of *Candida* that can be cultivated from the

mouth<sup>(1)</sup>, mostly in patients who wear complete dentures and have less than ideal mouth care.<sup>(2)</sup> Recently, there has been an increased interest in antimicrobial agents from medicinal plants which have been used in folk medicine.<sup>(3,4)</sup> Antibiotics are produced either synthetically or through microbial fermentation, plants, however, may provide additional source for antimicrobial substances.<sup>(5,6)</sup> Much of work has been performed to screen the antimicrobial effect of many plants<sup>(7)</sup> and for the isolation of their active ingredients<sup>(8)</sup>:

1-Peppermint: The antimicrobial activity of volatile oil isolated from peppermint has an inhibitory effect on the growth of large numbers of bacteria, yeasts including *Candida*<sup>(9)</sup>. In addition to anticaries effect of its watery extract<sup>(10)</sup>

2-Rue: The active ingredient is the volatile oil which contain rutin. It has antispasmodic and antirheumatoid effects which can be used in the treatment of gingivitis and tonsillitis<sup>(11)</sup> in addition to antibacterial, anticaries effects of rue leaves extract when used as a mouth rinse.<sup>(12)</sup>

3-Pomegranate: its water extract has an inhibitory effect on *C. albicans*<sup>(13)</sup> and pathogenic bacteria.<sup>(14,15)</sup>

4-Garlic (*Allium sativum*): Garlic has antibacterial, antifungal, antiviral properties<sup>(12,16)</sup>; its inhibitory effect against *C. albicans* by inhibiting its adhesion to buccal epithelial cells and reduces germ tube formation. For this reason, it is effective in the treatment of chronic atrophic candidiasis.<sup>(17)</sup> The aim of this study is to evaluate the antifungal anti-adherent effect of medicinal plant extracts (peppermint, rue, pomegranate and garlic) on the colonization of *C. albicans* on acrylic resin denture base surface (An in vitro study).

## MATERIALS AND METHODS

The methodology in this study consists of two parts; prosthetic and mycological methods. In the first part, a total of (75) specimens from heat cured acrylic resin (Major Prodotti Dentari S.P.A Italy) were fabricated in dimensions (10X10X3) (0.5) mm<sup>(18)</sup>, the acrylic resin samples were conditioned and disinfected before adhesion assay.<sup>(19)</sup> The mycological methods were involved isolation of *C. albicans* by taking swabs from of inner surface of up-

per complete denture for (8) patients selected randomly, those were wearing their dentures for more than one year<sup>(20)</sup> and they were attending prosthodontic department/College of Dentistry/Mosul University. These swabs were cultured on Sabouraud's dextrose agar medium<sup>(21)</sup> and identified according to culture characteristic, microscopic appearance and germ tube formation. The unstimulated whole saliva was collected from (30) volunteers, (healthy students about 22 years old) using spitting method, each student was instructed to rinse his / her mouth, gargling with distilled water several times for seconds to remove any remnant of food particles and debris between teeth, then, a stop watch was started. The oral cavity was closed by lips; saliva was collected in the oral cavity expelled into a graduated tube which was immediately closed with a plastic stopper at the end of collection to minimize the contact of saliva, then saliva pooled, clarified using refrigerated centrifuge.<sup>(22)</sup> The medicinal plants extracts were used in this study includes: garlic, peppermint, rue and pomegranate. The fresh leaves of rue and peppermint were collected, washed gently with water, then mixed with distilled water, ground or milled with blender. The extracts were filtered with clean gauze to separate the solid particles from liquid extracts. The same procedure was used with bulb of garlic and fruit pericarp of pomegranate. The extract powder was prepared from aqueous extract of leaves, bulb and fruit pericarp of previously mentioned plants using the freeze-dried method (Lyophilization) by using lyophilizer machine.<sup>(23)</sup> From this powder of fine particles, 1 g from each plant extract was used to prepare dilution at 1:10, 1:100 according to Abdul-Rahman (2001). All the prepared M.P.E.s were kept at (25 ± 1) °C in a clean, dry containers.<sup>(24)</sup> Antifungal assay was carried out according to Mustafa (1995) Abdul-Rahman (2001) and Abdul-Rahman and Kalaf (2002). For adherence experiment, the acrylic resin disks were coated with saliva and divided into four groups according to the number of M.P.E.s. For each group, the disks were divided according to plant extract's conc.s as two conc.s for each plant extract and at different times of

immersion (1 hr, 24 hrs and 48 hrs) respectively. For this experiment the total number of acrylic resin disks used were (75). A standardized *C. albicans* suspension (2ml) was added to each specimen, incubated for one hour at 37 °C<sup>(19,25)</sup>, then washed with phosphate buffer saline to remove the loosely adherent cells. Fixation and staining of the remaining adherent cells to the surface was achieved.<sup>(26)</sup> The number of adherent cells was examined using fluorescent microscope (Olympus, Japan) for at least three replicates for each variable was taken.<sup>(19)</sup> Statistical analysis

was carried out using One Way Analysis of Variance (ANOVA) and Duncan's Multiple Range Test at levels of significance 0.01 and 0.05. to determine the difference between the studied factors.

### RESULTS

In this study, the absorbance values in (nm) of replicates were measured and compared with control group by D.N.M.R. for determining the antifungal effect of the first and second conc.s of different plant extracts against *C. albicans* isolate. The results were listed in (Table1).

**Table (1):** Duncan's New Multiple Range Test for the antifungal Effect of the 1<sup>st</sup> and 2<sup>nd</sup> Conc. of Plant Extracts Against *C. albicans* Isolate

1 <sup>st</sup> and 2 <sup>nd</sup> Conc. of Plant Extracts	Absorbance Mean (nm) ± SD		Duncan's Grouping*	
	1 <sup>st</sup> conc	2 <sup>nd</sup> conc	1 <sup>st</sup> conc	2 <sup>nd</sup> conc
Control (Positive)	0.62 ± 0.2	0.62 ± 0.2	A	A
Peppermint	0.46 ± 0.02	0.56 ± 0.04	AB	A B
Rue	0.3 ± 0.08	0.4 ± 0.06	B	B C
Pomegranate	0.29 ± 0.05	0.2 ± 0.002	B	C
Garlic	0.49 ± 0.07	0.2 ± 0.04	A B	C

In this table the first conc.(1:10) of M.P.E had antifungal effect significantly different from control group and both rue and pomegranate extracts statistically had the same and the highest antifungal effect, while both garlic and peppermint extracts had the least values. For the second conc. (1:100) of plant extracts ,both garlic and

pomegranate had the highest antifungal effect which was significantly different from control group followed by rue extract .This study was also evaluated , the anti-adherent effect of 1st and 2nd conc.s of different plant extracts at different time of immersion for each one. From (Tables 2-5)

**Table (2):** Duncan's New Multiple Range Test for the Anti-adherent Effect the 1<sup>st</sup> and 2<sup>nd</sup> Conc. of Peppermint Extract on *C. albicans* Cells

Time of Immersion (h)	Number of Adhered <i>C. albicans</i> Cells / mm <sup>2</sup>	Number of Adhered <i>C. albicans</i> Cells / mm <sup>2</sup>	Duncan's Grouping*
	(Mean ± SD) 1 <sup>st</sup> Conc	(Mean ± SD) 2 <sup>nd</sup> Conc	
Control ●	29.1 ± 2.7	29.1 ± 2.7	A
Peppermint T <sub>1</sub> (1 h)	3.1 ± 0.3	4 ± 1.08	B
Peppermint T <sub>2</sub> (24 hrs)	2.9 ± 0.3	3.75 ± 1.08	B
Peppermint T <sub>3</sub> (48 hrs)	2.5 ± 0.3	3.48 ± 1.07	B

\* The different letters mean significant difference exists.,

● Saliva coated sample

**Table (3):** Duncan's New Multiple Range Test for the Anti-adherent Effect the 1<sup>st</sup> and 2<sup>nd</sup> Conc. of Rue Extract on *C. albicans* Cells

Time of Immersion (h)	Number of Adhered <i>C. albicans</i> Cells / mm <sup>2</sup> (Mean ± SD) 1 <sup>st</sup> conc	Number of Adhered <i>C. albicans</i> Cells/mm <sup>2</sup> (Mean±SD) 2 <sup>nd</sup> conc	Duncan's Grouping*
Control •	29.1 ± 2.7	29.1 ± 2.7	A
Rue T <sub>1</sub> (1 h)	3.7 ± 0.06	4.4 ± 0.06	B
Rue T <sub>2</sub> (24 hrs)	3.4 ± 0.05	4.2 ± 0.08	B
Rue T <sub>3</sub> (48 hrs)	3.1 ± 0.1	4 ± 0.2	B

\* The different letters mean significant difference exists.

• Saliva coated samples.

**Table (4):** Duncan's New Multiple Range Test for the Anti-adherent Effect the 1<sup>st</sup> and 2<sup>nd</sup> Conc. of Pomegranate Extract on *C. albicans* Cells

Time of Immersion (h)	Number of Adhered <i>C. albicans</i> Cells / mm <sup>2</sup> (Mean ± SD) 1 <sup>st</sup> Conc	Number of Adhered <i>C. albicans</i> Cells / mm <sup>2</sup> (Mean ± SD) 2 <sup>nd</sup> Conc	Duncan's Grouping*
Control •	29.1 ± 2.7	29.1 ± 2.7	A
Pomegranate T <sub>1</sub> (1 h)	3.7 ± 0.4	4.48 ± 0.2	B
Pomegranate T <sub>2</sub> (24 hrs)	3.4 ± 0.4	4.2 ± 0.2	B
Pomegranate T <sub>3</sub> (48 hrs)	3.3 ± 0.4	4 ± 0.2	B

\* The different letters mean significant difference exists.

• Saliva coated sample

**Table (5):** Duncan's New Multiple Range Test for the Anti-adherent Effect the 1<sup>st</sup> and 2<sup>nd</sup> Conc. of Garlic Extract on *C. albicans* Cells

Time of Immersion (h)	Number of Adhered <i>C. albicans</i> Cells / mm <sup>2</sup> (Mean ± SD) 1 <sup>st</sup> Conc	Number of Adhered <i>C. albicans</i> Cells / mm <sup>2</sup> (Mean ± SD) 2 <sup>nd</sup> Conc	Duncan's Grouping*
Control •	29.1 ± 2.7	29.1 ± 2.7	A
Garlic T <sub>1</sub> (1 h)	4.7 ± 0.2	4 ± 0.15	B
Garlic T <sub>2</sub> (24 hrs)	4.5 ± 0.2	3.8 ± 0.1	B
Garlic T <sub>3</sub> (48 hrs)	4.2 ± 0.2	3.6 ± 0.14	B

\* The different letters mean significant difference exists.

• Saliva coated sample

The results indicated that all the different times of immersion for 1st and 2nd conc.s of plant extracts were significantly effective in reducing the number of *C. albicans* cells / mm<sup>2</sup> adhered to acrylic resin disks, although there was no significant difference among the different time of immersion for each conc.

### DISCUSSION

The fungicidal activity of plant extracts in this project was evaluated using broth microdilution method. This is because it gives more scientific result in comparison with the

disk diffusion method which has low credibility for samples of plants that are difficult to diffuse in the media and also there is no relationship between diffusion power and antimicrobial activity.<sup>(27)</sup>The inhibitory effect of watery extract of peppermint against *C. albicans* was due to their content of volatile oils which had a significant inhibitory effect against large numbers of yeasts including *C. albicans*<sup>(27)</sup> and the inhibitory effect of the two conc.s of watery extract of rue leaves against *C. albicans* were due to the presence of rutin (active ingredient) in volatile oils of

rue.<sup>(28)</sup> A high antifungal activity of pomegranate was evaluated which was agreed with (Mustafa 1995) result who reported that the fungicidal effect of pomegranate was significantly higher than CHX 0.2%. Lastly the antifungal activity of watery extract of garlic is agreed with (Marrino et al 1999, Ngane et al 2000)<sup>(29,30)</sup> results which reported that watery extract of garlic contain active substance in the volatile oils which had an inhibitory effect against *C. albicans* and also resulted from the conversion of naturally occurring allium to allicin in the presence of allinase enzyme. All the extracts of (peppermint, rue, pomegranate, and garlic) had a significant effect on the adherence of *C. albicans* on acrylic resin surface. This ability in removing the attached yeast cells indicated the effectiveness of these extracts as antiadherent agents. This result was obtained with even (high and low) conc.s of these extracts and at different times of immersion. The mechanism of antiadherent effect of watery extract of garlic on the adhesion of *C. albicans* on buccal epithelial cells, and the reduction of germ tube formation might be due to the interference of garlic with the synthesis of adhesins involved in the adhesion process or it may cause mechanical distortion of the adhesins already present on the outer envelope, thus blocking the adherence<sup>(17,31)</sup>. It was possible that garlic inhibits thiol enzyme involved in the synthesis of adhesins. Our results also agreed with (Leven et al 1979)<sup>(32)</sup> result who suggested that it would be useful to use garlic extracts as an effective measure in the treatment of (denture stomatitis) whether as a denture cleanser or mouth rinse.

### CONCLUSION

All plant extracts used in this study had antifungal and antiadherent effect on candidal cells on acrylic resin denture base material. For this reason can be used as disinfectants, mouth wash or denture soaking solutions and as an alternative to drug solutions in the treatment of denture stomatitis. The study of anti-adherent effect of medicinal plant extract on *C. albicans* colonization on acrylic resin denture base surface using fluorescent microscope represents the first one at the Prosthodontic Department / College of Dentistry in that approach.

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