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# Evaluation of the Effect of Essential Oils on Microbial Biofilm on Denture Base Surface

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**ABSTRACT**

**Today's world is increasingly seeking ways to replace the synthetic drugs with the therapeutic power of natural products to decrease the percentage of many side effect which results from conventional treatment.**

**Aims of the study:** To investigate the antimicrobial action of essential oils against grown microorganisms on the surface of acrylic resin denture base materials.

**Materials and methods:** Four types of natural oils (Radish, Blackseed, Linseed, and Harmal) were used to investigate their antimicrobial effects against the biofilm formation of three types of bacteria and one fungus on the surface of denture base material. The total numbers of specimens were thirty, prepared from heat cured acrylic resin denture base materials, specimens were of (10mm×10mm×2mm).The collected data were analyzed using analysis of variance (one way ANOVA) at $P \leq 0.05$ or 0.01 and T test for independent samples.

**Results:** All the concentrations of radish oil and blackseed oil showed a significant effect on bacteria. In addition to its effect on bacteria only radish oil showed significant effect against *candida albicans*. Linseed oil was the less effective than other types of essential oils used in this study especially against *Streptococcus pyogenesa.*

**Conclusion:** All the tested natural oils showed antibacterial effect. While only radish oil showed significant effect against *candida albicans*.

**Keywords:** acrylic, antifungal, antibacterial, denture stomatitis, radish oil.

## 1. INTRODUCTION

Denture stomatitis is the most common infectious disease affecting the palatal mucosa and is highly prevalent in denture wearers, mainly characterized by the presence of *Candida albicans*. This condition is not a specific disease entity as other factors exist such as bacterial infection [1, 2]. According to several studies conducted in universities and hospitals, 65% of denture wearers suffer from problems caused by *Candida albicans*[3]. *Candida albicans* can actually be classified into Candida species, the most common and most widely found fungus in oral cavity. *C. albicans* can be found in the entire of oral mucosal surface, especially palatal mucosa and tongue [4].

In recent years, the uses of antibiotics are most important for the treatment of the various bacterial diseases and disorders. Microbial drug resistance is a difficult problem. Various side effects arise commonly after the antibiotic use and development of drug resistance also comes as a bigger problem [5,6]. To reduce dose dependent side effects and the development of drug-resistance in accordance with the maintenance of the effectiveness, the alternative approach nowadays is to go for natural plants for minimization of the above problems.

Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects [7].

Many studies have been conducted on the effect of blackseed extract in inhibiting the growth of *C. albicans* on heatcured acrylic resin. This is probably because blackseed extract contains active compounds that have antimicrobial power [4].In addition, some natural oils (sun flower oil, sesame oil, nigella sativa oil, flax oil and ginger oil) has been found to be effective antifungal agents of heat and cold cured acrylic resin denture base materials when immersed for 8hrs in these oils[8].Recently many researchers improve that the essential natural oils has antifungal, antiviral and antibacterial action, including blackseed, radish, linseed, and harmal [9,10,11,12].

In the present study, the effect of some essential oils was investigated against microbial biofilm found on denture surface.

## 2. MATERIALS AND METHODS

**Oil preparation:**

Four types of oils were used in this study Radish (*Raphanus sativus*), Blackseed (*Nigella sativa*), Linseed or flax (*Linum usitatissimum L*), and Harmal (*Peganum harmala L*)that are purchased from Hemani International KEPZ Karachi-Pakistan. These oils were diluted with ethanol to prepare different concentrations of oils (0.25%, 0.5%, 0.75, 1%, 5%, and 25%).

**Microorganisms:**

Test microorganisms used in this study are three types of bacteria and one fungus; *Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus pyogenes* and the fungus *Candida albicans* obtained from the central public health laboratory of Karbala. The test microorganisms were cultured on different media; bacteria were cultured on Muller Hinton Broth (MHB) and on Sabouraud Dextrose Broth (SDB) for *Candida albicans* at 37°C for 24 hr.

**Sample preparation:**

The total numbers of specimens were thirty, prepared from heat cured acrylic resin denture base materials; specimens were of (10mm×10mm×2mm) according to Agrawal [13]. Specimen preparation of heat cured acrylic denture base material, whereas the hard elastic foil of 2mm thickness were cut into plastic specimens of (10mm×10mm×2mm). Flasking was done by the conventional method. Packing accomplished according to the manufacture instruction. Then curing for the heat cured acrylic resin specimens were carried out by placing the clamped flask in the thermostatically controlled water bath for (1.5 hr. at 74°C then 1hr. at 100°C), according to the manufacture instruction. After the completion of curing flasks were allowed to bench cool. The acrylic specimens were removed from their stone moulds. Any flashes of excess resin material were removed from the specimens by using acrylic bur. The specimens were stored in distilled water at 37ºC in the incubator for 7 days for conditioning. According to AL- Sumaidae, [8] all specimens were sterilized by autoclave at 15 pound/inch$^2$/121˚C for 15 minutes.

**Antimicrobial assay:**

Standard microbial density of approximately $1.5\times10^8$ was prepared by matching with Macfrland 0.5. Antimicrobial assay was performed by disc diffusion method [14]. A 0.1ml of the standard microbial density was spread on SDA for bacteria and MHA for *candida albicans*. Whatman Filter paper No.1 was used to prepare discs (6mm). The discs were then sterilized by autoclaving. The discs were submerged into different concentrations of oils and added on the inoculated plates. After that plates were inoculated at 37°C for 24 hr. Inhibition zones were measured after incubation periods. Two types of controls were used; 0.2% chlorohexidene and ethanol as a positive control and discs with sterilized distal water as a negative control.

**Determination of MIC (Minimum Inhibition Concentration):**

The MIC of the active oils was determined against bacteria and *candida albicans*. Micro titration plate was used to determine MIC of the tested microorganism. A 100µl of SDB forfungi or MHB for bacteria in each well of the plate. Then 20µl of oil at different concentrations were added to well with media. After mixing, 20µl of microorganism was added for each well. The plate was inoculated at 37°C for 24hr. visual growth was recorded as the MIC values. Two types of controls were used; 0.2% chlorohexidene and ethanol as a positive control and sterilized distal water as a negative control.

**Biofilm forming assay:**

The PMMA resin samples were treated with MIC of each essential oil and other samples were treated with sterile distilled water according to Sookto with some modifications[15]. All PMMA resin samples were socked in 2ml of MIC of oils for 30 min. Then the samples were washed with phosphate buffer solution. Then put 20ml of the prepared standard microbial density in a screw capped bottle then immerse one acrylic samples in each one. The inoculated PMMA was agitated for 1 hr. at 37°C in a shaking incubator (130rpm). The non-adherent cells were removed from the resin samples by gently dipping 3 times with 2ml of phosphate buffer solution. The remaining adherent cells on the surface of the PMMA resin samples were stained by sufranin stain for 90sec. and then examined under optical microscope for biofilm counting.

**Statistical test:**

The collected data were analyzed using analysis of variance (one way ANOVA) at $P \leq 0.05$ or 0.01 and T test for independent samples.

## 3. RESULTS

All the concentrations of radish oil and blackseed oil showed a significant effect on bacteria *(Pseudomonas aeruginosa, Staphylococcus aureus*, and *Streptococcus pyogenesa*). In addition to its effect on bacteria only radish oil showed significant effect against *candida albicans*. There is no significant difference between the effects of radish oil on all the types of bacteria and candida used in this study. While a significant difference observed between the action of blackseed, linseed and harmal on all the types of bacteria and candida, as they are more effective against bacteria. The most effective concentrations of radish oil was (5% and 25%) while blackseed oil was most effective at (1% and 5%) against all the bacteria. The effective concentration of radish oil against *candida albicans* was 5%.

Linseed oil was the less effective than other types of essential oils used in this study especially against *Streptococcus pyogenesa.* While the most resistant microorganism to the action of the tested essential oil (blackseed, linseed, and harmal) was *candida albicans* (Table 1).

**Table 1: Antimicrobial effect of essential oils**

| Microorganisms | | *Pseud.* | *Staphylo* | *Strepto.* | *Candida* | F | Sig. |
|---|---|---|---|---|---|---|---|
| Oil | Conc. (%) | Inhibition zone diameter(mm) | | | | | |
| Radish | 1 | 10 | 10 | 12 | 11 | 0.952 | 0.427 |
| | 5 | 12 | 12 | 11 | 15 | | |
| | 25 | 15 | 12 | 12 | 12 | | |
| Blackseed | 1 | 14 | 13 | 12 | – | 6.970 | 0.002* |
| | 5 | 12 | 12 | 12 | – | | |
| | 25 | 10 | – | – | – | | |
| Linseed | 1 | 13 | 10 | – | – | 4.387 | 0.016* |
| | 5 | 10 | 11 | 10 | – | | |
| | 25 | – | – | 10 | – | | |
| Harmal | 1 | – | – | – | – | 4.499 | 0.014* |
| | 5 | 12 | 10 | 12 | – | | |
| | 25 | 15 | 12 | 13 | – | | |
| Chlorhexidine | 0.2 | 10 | 12 | 11 | 12 | 2.750 | 0.112 |
| F | | .589 | .624 | .679 | 107.276 | | |
| Sig. | | .673 | .649 | .613 | .000* | | |

(-) resistant to oil.*: significant differences between essential oils and chlorhexidine at P≤ 0.05(horizontal).
*: significant differences between microorganisms for each oil at P≤ 0.05(vertical).

The MIC of effective essential oils was determined against *Pseudomonas aeruginosa*. The value of the MIC showed as 1%, 1%, 5%, and 0.25% for blackseed oil, linseed oil, harmal oil, and radish oil respectively. While the MIC of radish oil on *candida albicans* was 0.25% as shown in Table (2).

**Table (2): MIC of effective essential oil on *Pseudomonas aeruginosa* and *Candida albicans***

| | *Pseudomonas aeruginosa* | | | | *Candida albicans* | | | |
|---|---|---|---|---|---|---|---|---|
| | Blackseed oil | Linseed oil | Harmal oil | Radish oil | Blackseed oil | Linseed oil | Harmal oil | Radish oil |
| 25% | – | – | – | – | + | + | + | – |
| 5% | – | – | – | – | + | + | + | – |
| 1% | – | – | + | – | + | + | + | – |
| 0.75% | + | + | + | – | + | + | + | – |
| 0.5% | + | + | + | – | + | + | + | – |
| 0.25% | + | + | + | – | + | + | + | – |

(–) No growth; (+) growth

The result of biofilm counting of *Pseudomonas aeruginosa* and *candida albicans* growing on denture surface shown that radish oil was more effective than other oils in reducing the biofilm formation of microorganism on the denture base surface. Blackseed oil, linseed oil, and harmal oilwere also showed significant affectivity against *Pseudomonas aeruginosa* (Table3).

**Table 3: Biofilm count of *Pseudomonas aeruginosa* and *Candida albicans* on denture base surfaces**

| Studied groups | *Pseudomonas aeruginosa* | | | | *Candida albicans* | | | |
|---|---|---|---|---|---|---|---|---|
| | Mean biofilm N0. | SD. | T | Sig. | Mean biofilmN0. | SD. | T | Sig. |
| Radish oil 0.25% | 1.4 | 1.76 | -13.196 | ** | 0.53 | 0.63 | -12.154 | ** |
| Blackseed oil 1% | 3.3 | 3.01 | -12.70 | ** | 13.2 | | | |
| Linseed oil 1% | 8.4 | 3.29 | -11.67 | ** | 13.9 | | | |
| Harmal oil 5% | 9.13 | 3.20 | -11.53 | ** | 12.8 | | | |
| Control(distilled water) | 67.33 | | | | 14.73 | | | |

SD: standard deviation **: significant differences between essential oils and control at P≤ 0.01

## 4. DISCUSSION

In recent years, an explosive spread of multidrug-resistant bacterial pathogens has become a serious concern worldwide in terms of public health and economic effects [16]. Interest in medicinal plants has burgeoned due to increased efficiency of the new plant-derived drugs and the growing interest in natural products. Because of the concerns about the side effects of conventional medicine, the use of natural products as an alternative to conventional treatment in healing and treatment of various diseases has been on the rise in the last few decades[17].

Nowadays, attention is focused on replace the synthetic drugs with the therapeutic power of natural products to decrease the percentage of many side effect which result from conventional treatment; one of these medicinal plants was blackseed (*Nigella sativa*) which was used so extensively that it became known as the seed of blessing "Habbatul Barakah" due to its powerful healing qualities for many ailments [4,18]. Many studies have been conducted on the effect of blackseed extracts on varies body systems in vitro or invivo. Blackseed has been used for medicinal purposes, both as medicinal herb and as medicinal oil. It contains saponin and atsiri oils that have antifungal, antimicrobial and antibacterial effects[19,20,21,22,23]. In fact, many believe that the blackseed is true potential is yet to be discovered; therefore it rightly deserves the name "Natures Miracle [24].

The results of this study showed that all the concentrations of blackseed oil showed a significant effect on bacteria *(Pseudomonas aeruginosa, Staphylococcus aureus,* and *Streptococcus pyogenesa*). While no effect has been shown against *candida albicans*.

Blackseed may represent as a promising prophylactic adjunct to conventional chemotherapy for reducing the severity of oral mucositis[25]. Blackseed oil demonstrated itself as a more efficient remedy against certain types of bacteria including those which most strongly resist antibiotic drugs. In addition it prevent undesirable inflammation and improve bone formation and maturation[26,27]. Antibacterial effect of blackseed (*Nigella sative*) oil might be due to the complex chemical structure of the seeds. These little seeds have over than one hundred different chemical components, including abundant sources of all the essential fatty acids, though that is most often used medically[28].

The blackseed oil extract has a bactericidal effect against *mutans streptococci* at a concentration of 10%, and can inhibit the adherence of these microorganisms to tooth surface[17]. Methanolic extracts of blackseed have the strongest antifungle effect followed by the chloroform extracts against different strains of *candida albicans*[29]. Blackseeds caused concentration-dependent inhibition of Gram-positive *Staphylococcus aureus* and Gram-negative *Pseudomonas aeruginosa* and *E. coli* and a pathogenic yeast *Candida albicans*[30].

Other study concluded that the additives materials of pure natural oil of 1.5% of blackseed and thyme were recommended to give acceptable properties, beside its antimicrobial effect after curing of the acrylic resin denture base, but thyme oil showed no effect on the color after curing in relation to blackseed [9].

Radishes are a crop grown in the Yuma area as part of the winter vegetable production programs. The scientific name of radish is *Raphanus Sativus*. Radishes can be white, red, purple or black, and in terms of shape, it can be long and cylindrical or round. The oil obtained from the seeds of radish is also used in a number of products and beneficial health applications[31].

In present study, radish oil possessed the highest antibacterial and antifungal effects against *Pseudomonas aeruginosa* and *candida albicans* in all the concentrations used in this study. These results were in accordance with Anonymous, [32] how mentioned that radish contain raphanin which is antibacterial and antifungal and inhibit the growth of *Staphylococcus aureus, Streptococci, pneumococci* and *E.coli*.

The cysteine-rich peptides (Rs-AFP1 and Rs-AFP2) isolated from radish showed substantial antifungal activity against several fungal species with minimal inhibitory concentration (MIC). Both Rs-AFPs are among the most potent antifungal proteins characterized[33].

Gutiérrez and Perez [10] reported that radish contained Caffeic acid which showed antifungal properties in vitro against *Helminthosporium maydis*. It has antibacterial, antifungal activities. Ferulic acid is active against *Sytaphylococcus aureus, Bacillus subtilis, Corynebacterium, Diphtheria, Aspergillus niger*, and *Candida albicans*. These acids displayed antibacterial activity against Gram-positive bacteria *Bacillus subtilis* and *Staphylococcus aureus*, and the Gram-negative *Escherichia coli* and *Kliebsiella pneumoniae.*Leaves and roots of Radish have been used in various parts of the world to treat cancer and as antimicrobial and antiviral agents.

The radish released biocidal compounds, mainly isothiocyanates, produced during the enzymic degradation of glucosinolates present in the plant cell. The highest fungicidal activity depended on concentration of isothiocyanates[34]. Study done by scientists confirmed the presence of Myrosinase in radish. The study also confirmed Myrosinase having antimicrobial and antimutagenic properties[35].

In present study, the antibacterial activities of radish oil are not in correlation with a study carried out by saeed and tariq[14] they found that radish has no effect on bacterial growth.

Linseed or flax (*Linum usitatissimum L*, the Linaceae family), as a source of oil and fiber, is a widely used crop plant in food and textile industries [36]. Linseed oil has a favorable fatty acid composition with high linolenic acid content. Linseed oil contains nearly 60% α-linolenic acid, and omega-3 fatty acid is reported to be high in linseed oil[37]. Linseed shows evidence of digestibility, bioactive peptides, antimicrobial, anti-Parkinson's, anti-proliferative, antihypertensive, anticancer, immune enhancing, antiulcer and antioxidant activities [38]. Linseed oil was found to contain high levels of linolenic (53.21%) followed by oleic (18.51%), and linoleic (17.25%), while the dominant saturated acids were palmitic (6.58 %) and stearic (4.43%) [11]. Joshi[39] concluded that linseed oil was individually a good antimicrobial agent and also confirms its potency to synergize the antimicrobial effects when analyzed in combination with gemifloxacin. It was found in the present study that linseed oil is ineffective against *candida albicans*, these resistant patterns are likely to be related to differences in fungus cell wall structures and protein synthesis, and these results are in agreement with Gaafar [40].

Harmal (*Peganum harmala L.*) is known as Syrian rue, Wild rue and Harmal. *P.* Harmalaextracts are considered important for drug development, because they are reported to have numerous pharmacological activities in the Middle East, especially in Iran and Egypt. For a long time *P.* harmalahas been used in traditional medicines for the relief of pain and as an antiseptic agent. *P.* harmalaalso have antibacterial, antifungal, antiviral, antioxidant, antidiabetic, antitumor, antileishmanial, insecticidal and cytotoxic activities [16,41]. *P. harmala* can be assigned as a source of antibacterial compounds for treatment of infections caused by multi-drug resistant (MDR) bacterial pathogens [42].

Other study found that histological examination of organs and tissues of treated rats given intramuscularly aqueous extract of Iraqi Peganum harmala were normal indicating that the extract has a low level of toxicity[43].
*Peganum harmala L.* is the only species found growing wild in the middle and northern parts of Iraq. The plant is rich in alkaloids and contains up to 4% total alkaloids.Study done by scientists confirmed that P. harmala as a potential sourceof antimicrobial drug against the four urinary pathogens *Proteus mirabilis* (*P. mirabilis*), *E. coli*, *P. aeruginosa* and *S.aureus.* This is particularly important in the fight against the recent resistant organisms with multiple drugs[44]. There are several reports which indicate the variety of pharm zacological and biological activities of *Peganum harmala L*. such as antibacterial, antifungal and MAO inhibition (Monoamine oxidases). The smoke of its seeds is traditionally used as adisinfectant [12,45,46].

Results of this study found that *Peganum harmala L.* have the ability to inhibit the growth of *Pseudomonasaeruginosa, Staphylococcus aureus, and Streptococcus pyogenes* at both 5% and 25%concentrations. These findings were in coincidence with Al-Izzy [12], who discovered that *Peganum harmala L.* extract (aqueous and alcoholic) is very effective against *Lactobacilli*and Candida in vitro. This may be attributed to the presence of the principle alkaloids which have the ability to intercalate with DNA of the microorganisms including harmaline, harmine, harmalol and peganine [16].

It has been reported that harmane as a highly aromatic planar alkaloid exerts its antibacterial activity through intercalation with DNA [47], thus, this antibacterial mechanism must be considered for active extract of P. harmala seed

and root. The observed antibacterial activity of P. harmala might also be attributed to the high quantity of polyphenols, which are known to possess efficient antibacterial activity [16].

## CONCLUSION

The tested natural oils were effective antibacterial agent. In addition to its effect on bacteria only radish oil showed significant effect against *candida albicans***.** Radish oil was more effective than other oils in reducing the biofilm formation of microorganism on the denture base surface. Blackseed oil was also showed significant affectivity against *Pseudomonas aeruginosa*.

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