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IN VITRO STUDY OF POLYMETHYL METHACRYLATE COMBINED WITH SEED OIL OF *NIGELLA SATIVA* L. AS AN ANTIMICROBIAL BIO-BASED POLYMER FOR DENTAL APPLICATIONS

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ABSTRACT

There is a constant need for a biocompatible ingredient that can give restorative materials antimicrobial action without compromising their physical and mechanical characteristics. Although polymethyl methacrylate (PMMA) is the most well-known and contemporary material to make orthodontic retainers and dentures, and for repair, its exceptional properties, including its antimicrobial activity are lacking. The concentration-dependent cytotoxic effects of oil on normal dental oral cells were assessed using the SRB assay. *Nigella sativa* L. has demonstrated antimicrobial, anti-inflammatory, and antioxidant activities. The current study highlighted the preparation of PMMA, which combined with natural seed oil extract of *N. sativa* L. as bio-based filler to improve its antimicrobial, mechanical and physical properties. The extract of *N. sativa* L. seed oil (NSO) was prepared using cold press and Soxhlet extraction methods. Fourier transform infrared spectroscopy (FTIR) and gas chromatography-mass spectrometry (GC–MS) studies were used to implement precise qualitative analysis of the contents of the obtained essential oil and compounds. FTIR confirmed the presence of the O-H functional group that is related to phenols and alcohols, C-H stretching of an aliphatic group and denotes the presence of methyl and isopropyl components, C=O stretching of oxygen-containing groups of ester and carbonyl group, as well as C-O, and =C-H stretching bands. The GC–MS results indicated the presence of 23 different compounds. Amongst these, octadecadienoic acid (27.27%), monolinolein (18.64%), thymoquinone (11.71%), and (9.18%) of D-glucopyranose and D-(-)-fructopyranose were the most abundant compounds. Heat-curing acrylic resin polymer and monomer in the ratio 3:1 was prepared and mixed manually with different volumes (0.5 ml, 1 ml, and 1.5 ml) from NSO. Biosafe dose analysis showed the need for careful evaluation of oil biocompatibility in dental applications, as excessive concentrations may compromise cell membrane integrity, induce oxidative stress, and trigger inflammatory responses. The impact strength and flexural strength of the prepared modifier were tested and indicated to show a non-significant decrease in the impact strength values of each denture base and denture lining for the tested samples compared to the control groups after the addition of NSO with higher volumes than 1 ml. Similarly, a significant increase in flexural strength occurred after the addition of NSO to PMMA with NSO volume lower than 1 ml. The antimicrobial activity of the prepared modifier was also tested against *Candida albicans*, methicillin-resistant *S. aureus* (MRSA), and *Enterobacter aerogenes*. Agar well and minimum inhibition concentration (MIC) were investigated and confirmed the potent antimicrobial action of the prepared modifier with MIC values of 55 µg/ml, 90 µg/ml, and 50 µg/ml against C. albicans, MRSA, and E. aerogenes, respectively. As indicated by the obtained data, NSO appears to be a very promising supplementary additive to PMMA, capable of improving specific mechanical properties and producing significant antimicrobial activity.

Keywords: *Nigella sativa*, PMMA, Dental, Cytotoxicity, GC–MS, Antimicrobial

INTRODUCTION

Replacing missing teeth with removable dentures helps patients improve mastication, enhancing the patient's ability to speak, better pronounce words, and give self-confidence, enabling patients to enjoy the life quality (Chakaipa et al., 2022). Utilization of polymethyl methacrylate (PMMA, acrylic) based resins in dentistry has become very varied, including denture bases manufacture, temporary crowns, denture relining, and orthodontic removable appliances **(Alqutaibi et al., 2023)**. Though multiple material such as light-activated urethane dimethacrylate and polystyrene were inserted, PMMA still the better material of choice in dental laboratories (to make orthodontic retainers and dentures and for repair), dental clinics (for relining dentures and temporary crowns), and industry (such as fabrication of artificial teeth) **(Alsharif et al., 2024)**. Nevertheless, PMMA is not ideal on some side, because it has a shortage of mechanical properties **(Mousa et al., 2000)**. Fracture of the denture occurs due to either fatigue, masticatory force, or impact dropping **(Abobakr, 2020; Zafar, 2020)**. Denture liners are often used in conditions of hardly resorbed and wavy ridges to help in the regular allocation of mastication forces and supply a supporting effect. Although these materials are readily degraded with time, making them vulnerable to microbial colonization **(Kaur et al., 2022)**.

Essential oils (EOs) are colorless liquids that consist of aromatic and volatile molecules found in each part of plants **(Butnariu, 2021)**. They are most used as medication, fragrances, cosmetics, and food preservatives in many countries **(Sharmeen et al., 2021)**. EOs are complex mixtures of many various molecules, and the main component is monoterpenes, which have the highest number of hydrocarbons **(Dehsheikh et al., 2020)**. Because of the varied structure of bioactive compounds in essential oils such as thymol and carvacrol in oregano, eugenol in cloves, and so on, each essential oil has its special functional characteristic (Adorjan & Buchbauer, 2010).

Nigella sativa, also known as black cumin or black caraway seeds, is rich in nutritious substances and has high medical value **(Dubey et al., 2016)**. Also, these seeds are used in conventional medicine by many countries like Asia, the Middle East, and Egypt to treat many types of diseases such as rheumatism, coughs, abdominal pain, diarrhea, asthma, and headache **(Gurib-Fakim, 2006)**. The extracted oil from *N. sativa* (NSO) is rich in thymoquinone and has antimicrobial, anti-inflammatory, anticancer, antioxidant, immune booster, and analgesic actions **(Davies, 2021)**. *N. Sativa* extracts were reported as a good antibacterial agent against different pathogens such as *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Enterobacter aerogenes* **(Rafati et al., 2014; Al-Ameedy & Omran, 2019)**. Additionally, several studies documented the antifungal action of NSO against different pathogenic fungi such as Candida albicans, Aspergillus niger, and Microsporum canis (Khan et al., 2003; Mahmoudvand et al., 2014; Haitamy et al., 2024). Black seed oil production was used and applied as a bio-based filler material in ethylene-propylene diene rubber, and the results showed that the mechanical properties, including tensile, ductility, and hardness of the material, were increased as reported by **Güngör (2023)**.

Similarly, Gad et al. (2020) showed that the PMMA acrylic resin reinforced with the addition of natural essential oil, containing thymoquinone which showed a decrease in flexural strength. Thus, this study aimed to extract the EOs from seeds of *N. Sativa* L. The essential oil emulsion (ECEO) exhibited a dose-dependent cytotoxic effect on both healthy and tumor cells. The orange essential oil emulsion (EOEO) exhibited antiproliferative effects on both healthy oral and tumor cells, with minimal impact on skin cells. Non-invasive skin tests indicated that these emulsions did not significantly alter physiological skin parameters, suggesting their safety for human skin applications (Alexa et al., 2022). and to evaluate the cytotoxic effects of oil on normal oral cells to determine its biocompatibility and safety for potential dental applications, in addition to apply in combination with PMMA to assess and compare the impact and flexural strength of acrylic denture base and acrylic denture lining materials incorporated with NSO as new alternative antimicrobial bio-based modifier for dental applications.

MATERIALS AND METHODS

Extraction of essential oils from *Nigella sativa* L. seeds

The seeds of *Nigella sativa* were purchased from the herbal plant and powder store in Damietta, Egypt. The seeds were sieved, and false and small seeds and inert material were removed. The *N. sativa* L. seeds were pressed at room temperature (25°C) by mechanical pressing without any heating treatment. The crushed seeds were stored for one night at room temperature to separate the oil phase from the fibres, and then the oil was filtered using Whatman #4 filter paper and a glass funnel. Essential oil extraction from *N. sativa* L. seed was performed using the soaking method for 3 weeks in three mixed solvents: methyl alcohol: chloroform: petroleum ether (1:1:1 v/v%). The extracted oils were placed in a rotary evaporator for 1 hr at a temperature of 55°C until the solvents evaporated from the oils **(Mohammed et al., 2016)**.

Chemical profile and constituents' identification of the extracted EOs

The identification of phytochemicals in the extracted EOs was studied using Fourier transform infrared spectroscopy (FTIR, FT/IR-4100typeA) and Gas chromatography-mass spectrometry (GC–MS, TSQ 9000 triple quadrupole mass spectrometer was coupled to a Thermo Scientific™ TRACE™ 1310 Gas Chromatograph) as described by **Fayed et al. (2025)**. The oven temperature was programmed at 70°C for 5 min, then raised to 230°C at 2°C / min, and held for 10 min at 230°C. EI mass spectra were recorded at 70 eV ionization voltage over the mass range 40–400 u.

Biosafety assessment dose oral epithelial cells

Cell culture

OEC: Oral epithelial cells were obtained from Nawah Scientific Inc. (Mokatam, Cairo, Egypt). Cells were maintained in DMEM media supplemented with 100 mg / mL streptomycin, 100 units / mL penicillin, and 10% heat-inactivated fetal bovine serum in a humidified, 5% (v/v) CO_2 atmosphere at 37°C.

Cytotoxicity Assay

Cell viability was assessed by the SRB assay. Aliquots of 100 µl cell suspension (5x10^3 cells) were in 96-well plates and incubated in complete media for 24 hr. Cells were treated with another aliquot of 100 µl media containing drugs at various concentrations. After 72 hr of drug exposure, cells were fixed by replacing media with 150 µl of 10% TCA and incubated at 4°C for 1 h. The TCA solution was removed, and the cells were washed 5 times with distilled water. Aliquots of 70 µl SRB solution (0.4%w/v) were added and incubated in a dark place at room temperature for 10 min. Plates were washed 3 times with 1% acetic acid and allowed to air-dry overnight. Then, 150 µl of TRIS (10 mM) was added to dissolve protein-bound SRB stain; the absorbance was measured at 540 nm using a BMGLABTECH®- FLUO star Omega microplate reader (Ortenberg, Germany) (**Youssef et al., 2025**).

Application of *N. sativa* L. seed oils as bio-based filler with PMMA

96 specimens (48 specimens of PMMA denture base, and 48 specimens of PMMA denture lining) was manufactured using metal molds measuring 75 mm x 10 mm x 10 mm to test impact strength and 65 mm x 10 mm x 2.5 mm to test flexural strength in accordance with ADA specification no 12 for denture base polymers **(ADA, 1975)**. Specimens were divided according to the ratios of the NSO in each test (impact and flexural) for PMMA denture base into group A1 (pure PMMA), group B1 (PMMA mixed with 0.5 ml NSO), group C1 (PMMA mixed with 1 ml NSO) and group D1 (PMMA mixed with 1.5 ml NSO). For PMMA denture lining, groups A2 (pure PMMA), group B2 (PMMA mixed with 0.5 ml NSO), group C2 (PMMA mixed with 1 ml NSO), and group D2 (PMMA mixed with 1.5 ml NSO) were prepared and tested. Each group includes 6 specimens.

Fabrication of wax patterns by using the metal molds and invested with dental plaster (type I), then dewaxed in the common method (Figure 1A). Heat curing acrylic resin polymer and monomer (Acrostone Dental Manufacture, Egypt) in the ratio 3:1. 0.5 ml, 1 ml, and 1.5 ml of NSO were added to the monomer and were mixed manually. Packing of acrylic resin and kept for 30 min in a hydromatic dental press to eliminate excess resin. Conventional curing of flasks was processed with a short curing cycle of 73°C for 90 min and then an increase in the temperature to 100°C for 30 min (long curing cycle) in a thermal polymerization unit. Then, removing of the flasks were removed from the water bath and kept at room temperature prior to deflasking. Trimming of the excessive resin from the specimen by tungsten carbide burs. Then, the specimens were finished using 200, 400, 800-grit sandpaper mounted on a spindle of sandpaper and polished using a rough brush mixed with soft pumice and water. The polished specimens were stored in distilled water at room temperature for 48 hr before testing (Figure 1B & 1C).

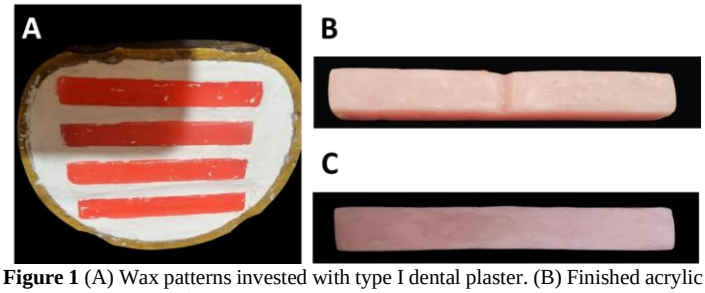

Figure 1 (A) Wax patterns invested with type I dental plaster. (B) Finished acrylic resin specimen used for impact strength test. (C) Finished acrylic resin specimen used for flexural strength test.

Evaluation of impact strength

Impact strength test was performed using Charpy's type impact tester in accordance with the MT 220 test specimen requirement (Figure 2A). The specimens were horizontally placed at their ends, and by a freely swinging pendulum which was released from a specific height, struck the span center on the unnotched specimen side. The absorbed impact energy by the specimen to fracture it in joules was measured when struck by a sudden shock (a pendulum of 15 J testing capacity was used). The impact strength of the specimen was recorded in KJ/mm² using the equation; IS= E/bd x 10^3, where IS = impact strength (KJ/mm^2), E = impact absorbed energy in joules, b = specimen width, and d = specimen thickness **(Hufenbach et al., 2008)**.

Evaluation of flexural strength

Flexural strength test was measured using a three-point bending Universal testing machine (5ST, Tinius Olsen, England) (Figure 2B). The specimens were mounted on the apparatus, the central loading plunger was applied at the midpoint of the specimen with a 1 mm/min speed until fracture of the specimen. The maximum fracture load of each specimen was measured. The flexural strength of every specimen was calculated using the formula (FS=3WL/$2bd^2$), where FS= flexural strength in (Mpa), W= fracture load (N), L= distance between the two supports, b= specimen width, and d= specimen thickness **(Soygun et al., 2013)**.

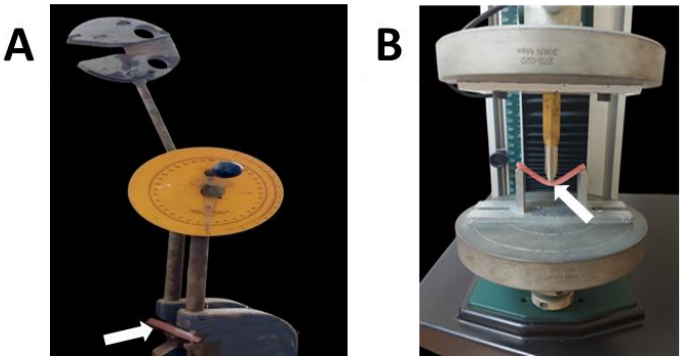

Figure 2 (A) specimen placed on Charpy's impact tester. **(B)** Specimen mounted on the Universal testing machine.

Antimicrobial activity agar well diffusion method

The antimicrobial potential of the prepared modifier against *C. albicans,* methicillin-resistant *S. aureus* (MRSA), *and E. aerogenes* was demonstrated *in vitro* using agar well diffusion test according to the guidelines of the Clinical and Laboratory Standards Institute **(CLSI, 2017)**. 5 mm discs from PMMA, PMMA combined with NSO, and penicillin (standard antibacterial agent) or miconazole (standard anticandidal agent) were prepared and added separately under aseptic

conditions on the surface of Muller-Hinton agar plates prior to inoculation with 0.5 MacFarland ($1\text{-}2\times10^8$) from each microbe. The inoculated plates were incubated at 37°C for 48 hr for bacterial strains or at 28°C for 48 hr for yeast. The inhibition zones were calculated in millimetres (mm).

Minimum inhibition concentration (MIC)

The MICs of PMMA, PMMA combined with NSO, and penicillin were studied according to the broth microdilution method **(CLSI, 2000; 2012)**. Serial dilutions (0–100 µg/ml) of the tested materials were prepared and tested against a 0.5 McFarland standard of each microbial strain that was sub-cultured in Mueller-Hinton broth medium. After the incubation at 37°C for 48 hr, microbial growth was evaluated spectrophotometrically at 630 nm.

Statistical analysis

Using SPSS software version 18, the results were analysed using the ANOVA test at a significance threshold of 0.05. Three iterations of the experiments were conducted. The mean and standard deviation (SD) for each outcome were given (**O'Connor, 2000**).

RESULTS AND DISCUSSION

PMMA acquired popularity for diverse dental applications due to its unique properties, including its aesthetics, low density, ease of manipulation, cost efficiency, etc. Although there are many concerns related to using PMMA, such as denture fracture due to poor impact and flexural strength and water sorption, the ongoing studies have introduced a different modification to overcome and enhance its properties **(Zafar, 2020)**. For example, various studies reported the reinforcement of PMMA materials using a variety of fibers **(Nejatian et al., 2019)**, nanotubes **(Wang et al., 2014)**, nanoparticles **(Alamgir et al., 2019)**, and antimicrobial agents to improve its biological properties **(Kaur et al., 2022)**. Another study documented that the addition of chitosan to PMMA denture base enhanced and improved in the flexural study groups when compared with the flexural control group **(Abobakr, 2020)**. *N. sativa*, commonly known as thymoquinone, that is incorporated into acrylic denture base to evaluate and compare of flexural strength, surface roughness, and porosity percentage of denture base resins incorporated with thymoquinone, shows that this addition significantly reduced flexural strength and increased the porosity percentage **(Kaur et al., 2022)**. In addition, **Gad et al. (2020)** reported that the addition of thymoquinone to PMMA denture base material significantly decreased flexural strength and elastic modulus at high concentrations, while no significant differences were observed at low concentrations. **Güngör (2023)** showed that the mechanical properties (tensile, ductility, and hardness) of the material increased with the use of cumin black, which is used as a filling material, and decreased with the increase in the amount of it.

Chemical composition of the extract EOs using FTIR

FTIR spectra of the NSO samples, including crude oil and fractions A, B, and C, are shown in Figure 3. Abroad absorption bands at 3377.71 cm^{-1}, 3408.57 cm^{-1}, 3425.92 $cm^{-1,}$ and 3427.85 cm^{-1} confirmed the presence of the (O-H) functional group that is related to phenols and alcohols. The bands at 2940.91 cm^{-1}, 2955.38 cm^{-1}, 2965.05 cm^{-1}, 2835.81 cm^{-1}, 2843.52 cm^{-1}, and 2842.56 cm^{-1} are assigned to (C-H) stretching of an aliphatic group and denote the presence of methyl and isopropyl components. The weak band at 1717.3 cm^{-1} is due to (C=O) stretching of oxygen-containing groups such as ester. The strong peaks 1656.55 cm^{-1}, 1646.91 cm^{-1}, 1644.02 cm^{-1},1642.09 cm^{-1} imply the presence of (C=O) stretching of oxygen-containing constituents of the carbonyl group. Other successive bands were observed at 1453.1 cm^{-1}, 1409.71 cm^{-1}, 1456.96 cm^{-1},1388.5 cm^{-1}, 1461.78 cm^{-1}, 1396.21 cm^{-1}, 1395.25 cm^{-1} indicates the presence of methyl rock and (C-H) bending (scissoring) that also matched with the obtained results of **Shafodino et al. (2022)** and **Mohammed et al. (2019)**. The peaks appeared in fraction samples (A, B, and C) at 1250.61 cm^{-1}, 1268.93 $cm^{-1,}$ and 1247.72 cm^{-1}, respectively are due to (C-O) stretching. These peaks were also recorded by **Olaniyan & Sarah (2024)**. The peaks at 1124.3 $cm^{-1,}$106.94 cm^{-1}, 1107.9 cm^{-1}, 1105 cm^{-1} correspond to the (C-O) group, and bands were observed at 1055.84 cm^{-1}, 1022.09, 1027.87 cm^{-1}, 1021.12 cm^{-1}, 1018.23 $cm^{-1,}$ indicating the presence of (=C-H) bending **(Shafodino et al., 2022)**.

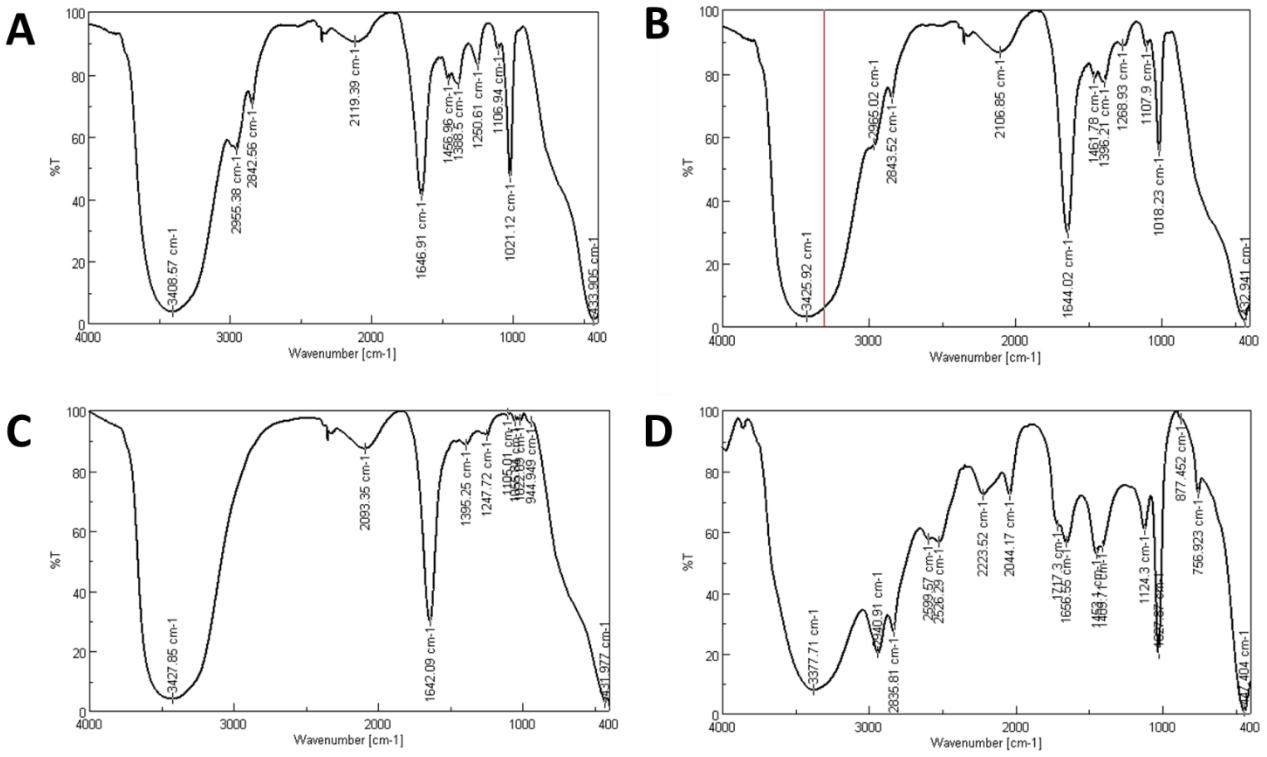

Figure 3 FTIR spectra of NSO crude oil; (D) and its fraction samples; (A, B & C).

GC–MS analysis

Table 1 and Figure 4 show 23 different compounds of NSO, including octadecadienoic acid (27.27%), monolinolein (18.64%), thymoquinone (11.71%), and (9.18%) of D-glucopyranose and D-(-)-fructopyranose as main constituents. The obtained results showed that NSO contains different bioactive compounds such as thymoquinone and palmitic acid that could be attributed to the antimicrobial activities of the oil **(Baka et al., 2024)**. **Saleh et al. (2018)** documented the presence of several peaks corresponding to Beta-pinene (10.0%), thymoquinone (8.6%), palmitic acid (5.6%), oleic acid (8.1%), and thymol (10.3%) during the study of the GC-MS chromatogram of *N. sativa* oil. While the GC–MS analysis of *N. sativa* revealed the existence of D-Glucose, O-Cymene, 6-O-α-Dgalactopyranosyl, α- D-Glucopyranoside, DL-Arabinose, 2-Propyl-tetrahydropyran-3-ol, Terpinen-4-ol, Thymoquinone, Limonen-6-ol, Phenol, 4-methoxy-2,3,6-trimethyl, l-(+)-Ascorbic acid 2,6-dihexadecanoate, 9,12-Octadecadienoic acid (Z,Z)-, and Stiqmasterol **(Hadi et al., 2016)**.

Table 1 Chemical composition of NSO constituents

Rt	Component	Content (%)	Molecular formula
8.739	Glycerol, 3TMS derivative	9.10	$C_{12}H_{32}O_3Si_3$
10.342	Erythritol, 4TMS derivative	0.64	$C_{16}H_{42}O_4Si_4$
10.582	2,3-Butanediol, 2TMS derivative	0.11	$C_{10}H_{26}O_2Si_2$
10.714	tert-Butylhydroquinone, 2TMS derivative	0.06	$C_{16}H_{30}O_2Si_2$
10.891	D-Erythro-Pentitol, 2-deoxy-1,3,4,5-tetrakis-O-(trimethylsilyl)-5	0.32	$C_{17}H_{44}O_4Si_4$
11.086	L-Rhamnose, 4TMS derivative	0.80	$C_{18}H_{44}O_5Si_4$
11.463	D-Glucopyranose, 5TMS derivative	0.26	$C_{21}H_{52}O_6Si_5$
11.560	9(1H)-Phenanthrone, 2,3,4,4a,4b,5,6,7,8,8a-decahydro-	0.55	$C_{14}H_{20}O$
11.852	Phosphoric acid, bis(trimethylsilyl) 2,3-bis[(trimethylsilyl)oxy]propyl ester	0.78	$C_{15}H_{41}O_6PSi_4$
12.156	Methyl galactoside, 4TMS derivative	3.18	$C_{19}H_{46}O_6Si_4$
12.505	D-Glucopyranose, 5TMS derivative	4.02	$C_{21}H_{52}O_6Si_5$
12.659	Hexadecanoic acid, methyl ester	2.31	$C_{17}H_{34}O_2$
12.928	β-D-Glucopyranose, 5TMS derivative	3.92	$C_{21}H_{52}O_6Si_5$
13.157	Palmitic Acid, TMS derivative	3.48	$C_{19}H_{40}O_2Si$
13.466	Thymoquinone	11.71	$C_{10}H_{12}O_2$
13.946	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	27.37	$C_{19}H_{34}O_2$
14.324	2-Amino-4-chloro-5-formyl-thiophene-3-carboxylic acid, amide	1.18	$C_6H_5ClN_2O_2S$
14.730	11,14-Eicosadienoic acid, (Z)-, TMS derivative	1.92	$C_{23}H_{44}O_2Si$
15.011	Geranylgeraniol, TBDMS derivative	0.52	$C_{26}H_{48}OSi$
15.160	Deoxyglucose, 4TMS derivative	0.45	$C_{18}H_{44}O_5Si$
15.394	Monopalmitin, 2TMS derivative	2.24	$C_{25}H_{54}O_4Si$
15.566	beta-D-Lactose, (isomer 2), 8TMS derivative	1.02	$C_{36}H_{86}O_{11}Si_8$
16.024	1-Monolinolein, 2TMS derivative	18.64	$C_{27}H_{54}O_4Si_2$
16.544	beta-Gentiobiose, octakis(trimethylsilyl) ether	1.76	$C_{36}H_{86}O_{11}Si_8$
16.710	D-(-)-Fructopyranose, 5TMS derivative (isomer 2)	0.98	$C_{21}H_{52}O_6Si_5$
17.002	α-D-Xylopyranose, 4TMS derivative	0.99	$C_{17}H_{42}O_5Si_4$
18.204	β-D-Xylopyranose, 4TMS derivative	0.16	$C_{17}H_{42}O_5Si_4$
18.438	2-(2-Bromo-4-methylphenoxy)-N'-([1-(4-nitrophenyl)-2-pyrrolidinyl]methylene)acethydrazide	0.68	$C_{15}H_{13}BrN_2O_4$
18.633	threo-2,5-Hexodiulose, 1,3,4,6-tetrakis-O-(trimethylsilyl)-	0.37	$C_{18}H_{42}O_6Si_4$
18.970	β- Sitosterol, TMS derivative	0.35	$C_{32}H_{58}OSi$
19.319	α-D-Xylopyranose, 4TMS derivative	0.12	$C_{17}H_{42}O_5Si_4$

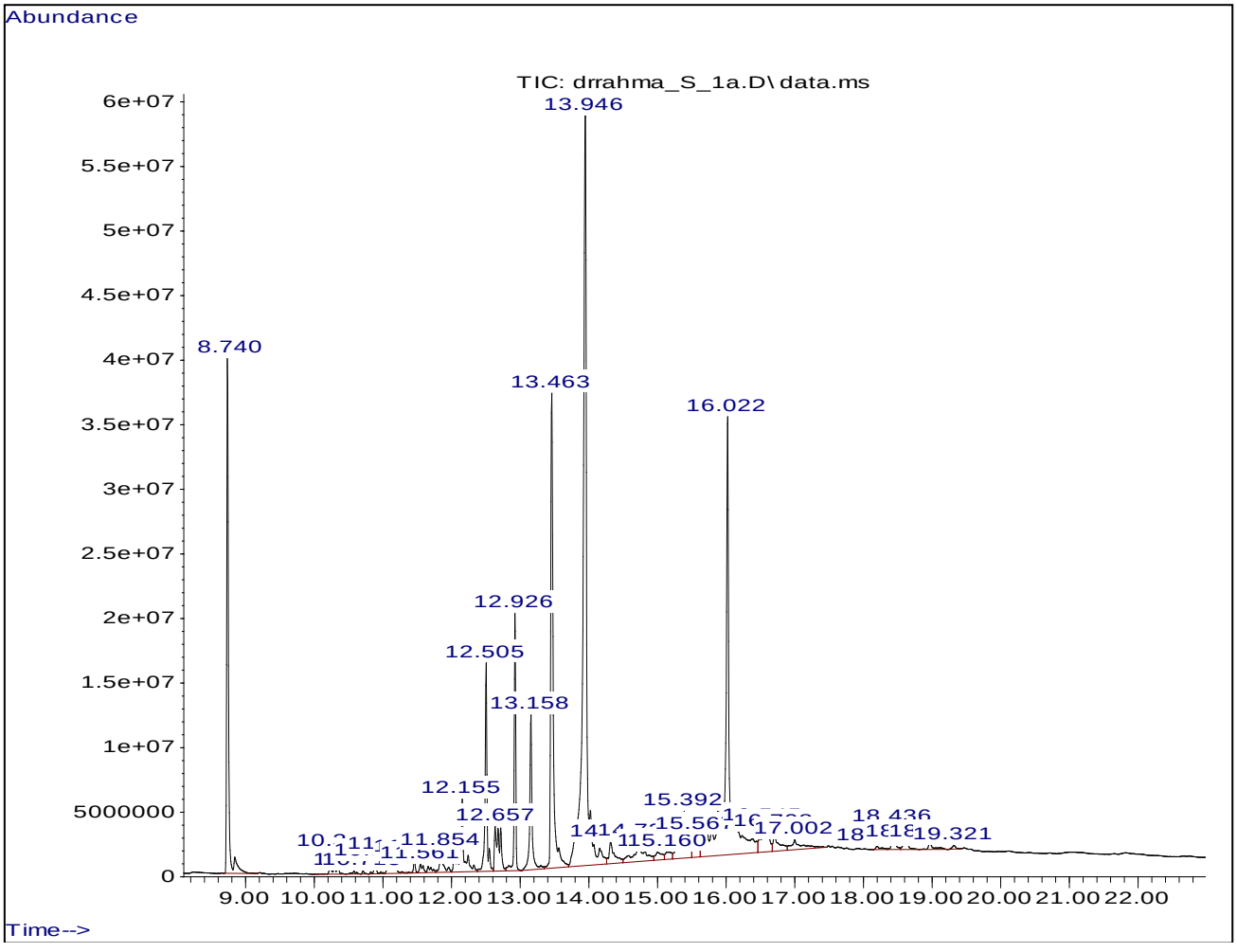

Figure 4 GC-MS chromatography analysis of NSO.

Biosafety dose of NSO for oral cells

Figure 5 shows evaluating the effect of NSO on normal epithelial oral cells; in control (No oil), the cells appear densely packed with a uniform, healthy structure, and vigorous staining intensity indicates high cell viability with minimal damage or morphological changes. At **low oil concentration (0.01** µg/ml**),** some structural changes are observed compared to the control, with no alterations in cell density or integrity; staining remains relatively strong, suggesting cell viability. However, at **higher oil concentration (100** µg/ml**),** significant changes in cell morphology are evident, including possible gaps and reduced cell density. The staining intensity is weaker, indicating a decrease in viable cells and increased cytotoxic effects compared with low concentration, likely due to the higher oil concentration. Figure 6 shows that the IC_{50} of NSO is > 100 µg\ml. This analysis suggests that while lower concentrations of oil have minimal impact on normal dental cells, higher concentrations may cause oral cell irritation and inflammation. These findings are consistent with previous studies on the biocompatibility and cytotoxicity of various oils for oral cells. Cytotoxicity of oils in oral tissues has been widely investigated, particularly regarding their use in dental treatments, medicated mouth rinses, and essential oil-based formulations. Some studies have shown that certain plant-based oils, such as coconut oil and tea tree oil, can exhibit antimicrobial properties while maintaining biocompatibility with oral cells at low concentrations **(Shekar et al., 2015)**. However, at higher concentrations, certain oils may disrupt cell membranes, induce oxidative stress, or interfere with metabolic activity, leading to reduced cell viability **(Mohammed et al., 2023)**. One possible mechanism behind the cytotoxic effects of high oil concentrations is the disruption of the cell membrane integrity and mitochondrial function. Lipid-based compounds have been shown to interact with cellular lipids, leading to changes in permeability, increased reactive oxygen species (ROS) production, and eventual apoptosis or necrosis **(Mohammed et al., 2023)**. Additionally, oil-induced cytotoxicity could also result from the presence of certain bioactive compounds that trigger inflammatory responses or enzyme inhibition **(Radu et al., 2023)**.

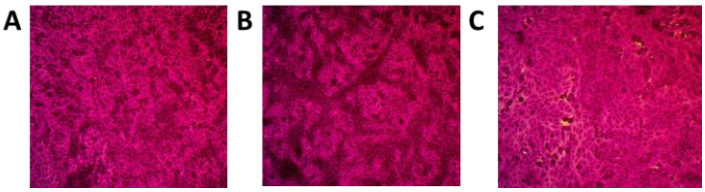

Figure 5 Effect of crude essential oils of *N. sativa* L. seeds on normal dental oral cells: Morphological Changes at Different Concentrations using SRB stain. (A) Oral epithelial cells (control). (B) Oral epithelial cells at low concentration (0.1 µg/ml). (C) Oral epithelial cells at high concentration (100 µg/ml).

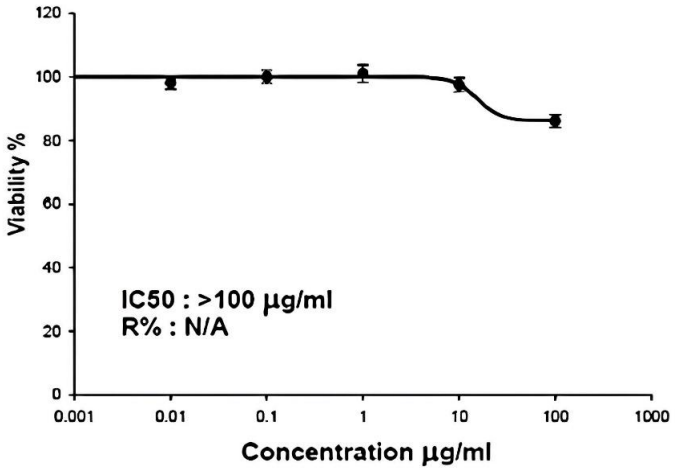

Figure 6 Dose Response curve for crude essential oils of *Nigella sativa* L. seeds on normal dental oral cells viability.

Evaluation studies of NSO/PMMA for dental application

Comparison of intergroups was done using One way ANOVA test, Post Hoc test (Tukey), and Kruskal-Wallis test to evaluate the mean difference between the groups. The mean values and SD of impact strength of denture base material for groups A1, B1, C1, and D1 were illustrated in Table 2 and Figure 7. There was non-statistically significant difference between all four test groups (p value = 1.000). Table 3 and Figure 8 represent mean values and SD of impact strength of denture lining material for groups A2, B2, C2, and D2, there was non-statistically significant difference between all four test groups (p value = 0.859). The results obtained from this study displayed that there was a non-significant decrease in the impact strength values of three study groups of each denture base and denture lining tested specimens compared with control groups after adding of NSO in each ratio (0.5 ml, 1ml and 1.5 ml) for each denture base and denture lining specimens. The explanation of this decrease is due to the low impact strength of PMMA by nature **(Darbar et al., 1994)**. Also, after mixing the PMMA with NSO, the impact strength of the composite still decreases. That was due to there was no chemical bond between NSO and PMMA **(Gad et al., 2020)**. In addition, the presence of the moisture content of the *N. sativa* seeds by nature leads to high porosity of the blend **(Kaur et al., 2022)**, and other materials like Sago starch film **(Ekramian et al., 2021)**. Also, the porosity of black cumin seed increased with the increase in moisture content. This could be attributed to the expansion and swelling of seeds that may have resulted in more void space between the seeds and increased the bulk volume **(Gharibzahedi et al., 2010)**.

Table 2 Denture base specimens in (KJ/mm²) for groups A1, B1, C1, and D1 (Mean values ± SD).

Impact strength values in (KJ/mm²)	Group A1 (n = 6)	Group B1 (n = 6)	Group C1 (n = 6)	Group D1 (n = 6)	H	*p*
Min. – Max.	0.0 – 1.20	0.0 – 1.50	0.0 – 1.50	0.0 – 1.0		
Mean ± SD.	0.62 ± 0.56	0.56 ± 0.66	0.60 ± 0.64	0.38 ± 0.40	0.00	1.000
Median	0.50	0.20	0.40	0.30		
% of Change	-	↓9.68	↓3.23	↓38.71	-	-

Table 3 Denture lining specimens in (KJ/mm²) for groups A2, B2, C2, and D2 (Mean values ± SD).

Impact strength values in (KJ/mm²)	Group A2 (n = 6)	Group B2 (n = 6)	Group C2 (n = 6)	Group D2 (n = 6)	H	*p*
Min. – Max.	0.0 – 1.20	0.0 – 1.10	0.0 – 1.20	0.10 – 0.70		
Mean ± SD.	0.64 ± 0.59	0.58 ± 0.43	0.61 ± 0.51	0.44 ± 0.24	0.759	0.859
Median	1.0	0.65	0.80	0.50		
% of Change	-	↓8.7	↓4.7	↓31.3	-	-

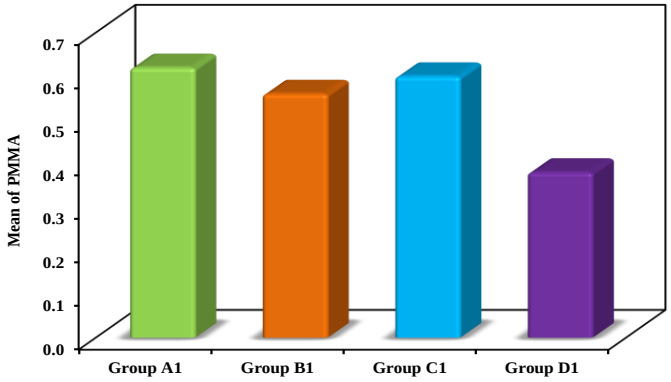

Figure 7 Impact strength values in KJ/mm² for groups A1, B1, C1, and D1.

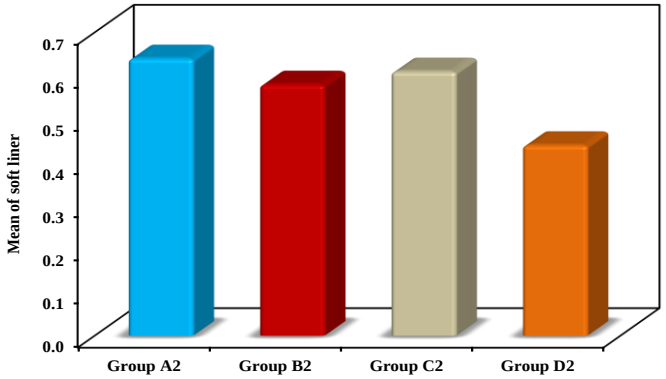

Figure 8 Impact strength values in KJ/mm² for groups A2, B2, C2, and D2.

Groups (A1) and (A2) displayed the highest impact strength. Results of Tukey's post hoc test showed non statistically significant difference between the mean impact strength between control groups (A1) and (A2) within each study group. Table 4 and Figure 9 showed mean values and SD of flexural strength of denture base material for groups A1, B1, C1, and D1, there was a statistically significant difference between all four test groups (*p* value <0.001). Table 4 showed that group (C1) exhibited the highest flexural strength, with a statistically significant difference between the mean flexural strength of group (C1) and group (D1). According to **ADA (1975)** specification no. 12, the flexural strength of the acrylic denture base should be at least 65 MPa. The mean flexural strength of denture base increases significantly in the values by adding 1 ml of NSO to PMMA in group C1 (81.50 MPa) when compared with group A1 (control group) (73.87 MPa) by 9.97%. This could be due to the chemical composition of the major unsaturated fatty acids were linoleic acid, followed by oleic and lignoceric acids, and due to the water sorption phenomenon of methyl methacrylate **(Hatim et al., 2010)**. But there was a significant decrease in the values of the flexural strength by adding 0.5 ml and 1.5 ml of NSO to PMMA in group B1, D1) (67.30 MPa, 68.25 MPa) by 6.28% and 7.04%, respectively. The obtained result agrees with **Güngör (2023),** who concluded that the effect of *N. sativa* L. as bio-based filler improved the chemical, rheological and mechanical properties of EPDM composites and also reported that the PMMA acrylic resin reinforced with of thymoquinone showed a significantly decreases of the flexural strength in comparison with the control group. This decrease may be due to the agglomeration of thymoquinone, which resulted in the formation of loosely attached clusters that acted as a concentrating stress in the center of the matrix and adversely affected flexural strength. Also, due to there was no chemical bond between NSO and PMMA. In addition, there was a significant decrement in the values of the flexural strength by adding 1.5 ml of NSO in group D2 (0.23 MPa) by 8% when compared with group A2 (control group) (0.25 MPa). This result agrees with **Kaur et al. (2022)** as they reported that the Addition of 1% thymoquinone and 2.5% silver nanoparticles to acrylic denture base resin significantly reduced flexural strength due to thymoquinone and nanoparticles act as a contaminant when dispersed within the acrylic matrix and affect the degree of conversion of the chains of polymer that lead to a greater extent of residual monomer, which weakens the resin by acting as a plasticizer **(Al-Thobity et al., 2017)**.

Table 4 Denture base specimens in (Mpa) for groups A1, B1, C1, and D1 (Mean values ± SD).

Flexural strength values in (Mpa)	Group A1 (n = 6)	Group B1 (n = 6)	Group C1 (n = 6)	Group D1 (n = 6)	F	*P*
Min. – Max.	73.30 – 74.49	65.12 – 74.57	77.33 – 83.70	67.20 – 71.40		
Mean ± SD.	73.90 ± 0.54	69.26 ± 4.72	81.27 ± 2.39	68.70 ± 1.73	21.641*	<0.001*
Median	73.87	67.30	81.50	68.15		
p_0		0.078	0.004*	0.043*		
Sig. bet. grps	-	p_1<0.001*, p_2=0.989, p_3<0.001*			-	-
% of Change		↓6.28	↑9.97	↓7.04		

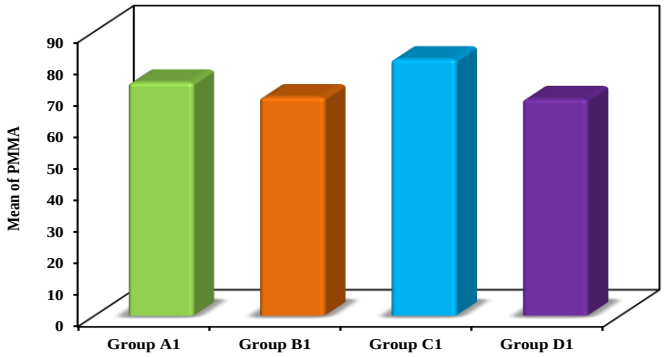

Figure 9 Flexural strength values in (Mpa) for groups A1, B1, C1, and D1.

Table 5 and Figure 10 displayed mean values and SD of flexural strength of denture lining material for groups A2, B2, C2, and D2, there was a statistically significant difference between all four test groups (*p* value <0.001). Table 5 revealed that there is a significant increase in the flexural strength values of group (B2), followed by group (C2).

Table 5 Denture base specimens in (Mpa) for groups A2, B2, C2, and D2 (Mean values ± SD).

Flexural strength values in (Mpa)	Group A2 (n = 6)	Group B2 (n = 6)	Group C2 (n = 6)	Group D2 (n = 6)	F	*p*
Min. – Max.	0.23 – 0.28	0.42 – 0.48	0.30 – 0.37	0.19 – 0.25		
Mean ± SD.	0.25 ± 0.02	0.45 ± 0.02	0.33 ± 0.03	0.23 ± 0.02	99.725*	<0.001*
Median	0.25	0.46	0.33	0.23		
p_0		<0.001*	<0.001*	0.314		
Sig. bet. grps	-	p_1<0.001*, p_2<0.001*, p_3<0.001*			-	-
% of Change		↑80	↑32.0	↓8.0		

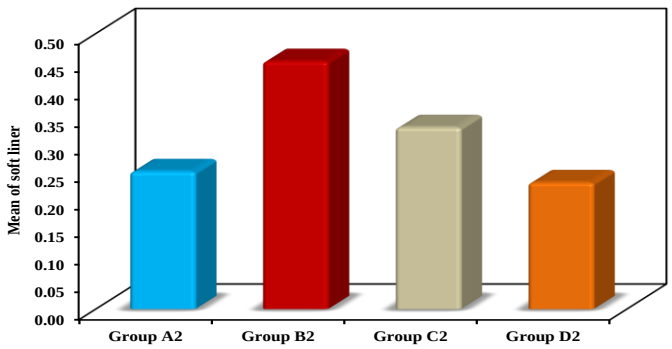

Figure 10 Flexural strength values in (Mpa) for groups A2, B2, C2, and D2.

Antimicrobial investigations

The current study examined the antimicrobial activity of the essential oil extracted from the medicinal plant black cumin (*N. sativa* L.) against 3 different pathogenic strains such as *C. albicans* as the most common human fungal pathogen causing diseases ranging from mucosal to systemic infections especially oral superficial infections, MRSA as a multidrug-resistant strain in dental infections which plays a major role in the etiology of primary and persistent endodontic infections, and *E. aerogenes* as a model for Enterobacteriaceae infection in the oral and maxillofacial region during hospitalization after oral and maxillofacial surgery. **Paul et al. (2024)** studied and recorded that the most effective antimicrobial action of denture base resin material specimens increased after incorporating natural oils. Agar well diffusion test indicated the strong antimicrobial action of NSO alone and combined with PMMA, as shown in Table 6. It was notable that the inhibition zones of NSO combined with PMMA were higher than the standard drugs (penicillin and miconazole), which highlights the good competence of the new modifier to the current and traditional drugs. NSO combined with PMMA has stronger antibacterial action against Gram-negative bacteria compared to Gram-positive bacteria. This difference might be due to the difference in cell wall structure between the types of bacteria **(Fayed et al., 2024)**. NSO caused inhibition zones of 3.3 ± 5.4, 18.3 ± 4.3 mm, and 19.3 ± 3.5 mm against *S. aurous*, *E. coli*, and *E. aerogenes* as reported by **Al-Ameedy & Omran (2019)**. Also, PMMA containing NSO showed anti-yeast action against *C. albicans* compared to PMMA, which matched with **Albarrag et al. (2017)**. Compared to the current study, **Nadaf et al. (2015)** documented the anti-yeast action of NSO against *C. albicans* with an inhibition zone of 12 ± 0.6 mm.

Table 6 Antimicrobial activity using agar well diffusion test.

Antimicrobial agent	Inhibition zones (mm)		
	C. albicans	MRSA	*E. aerogenes*
NSO	28 ± 0.06	25 ± 0.01	30 ± 0.03
PMMA	13 ± 0.23	-ve	16 ± 0.14
PMMA combined with NSO	25 ± 0.03	13 ± 0.03	27 ± 0.06
Penicillin	-	-ve	15 ± 0.14
Miconazole	18 ± 0.03	-	-

The minimal inhibitory concentration (MIC) was determined by the microdilution method (Figure 11). Antimicrobial experiments revealed that the extracted essential oils inhibited the growth of *C. albicans*, *S. aureus*, and *E. aerogenes* at MIC values ranging from 10-50 µg/ml, 5-50 µg/ml, and 15-50 µg/ml, respectively. **Kurnia et al. (2024)** reported MIC values of NSO ranged from 0.1 to 0.8 mg/ml against Gram-positive and Gram-negative oral cavity bacteria. **Hannan et al. (2008)** documented that MRSA strains were sensitive to NSO at a concentration of 4 mg/ml and an MIC range of 0.2–0.5 mg/ml. **Moghim et al. (2015)** recorded the measured values of MIC of *N. sativa* as 10 mg/ml against *C. albicans*. Other studies showed that *N. sativa* extracts have a range of MICs of 0.2–2048 mg/ml against different *Candida* sp. strains **(Kokoska et al., 2009; Karaman, 2020)**.

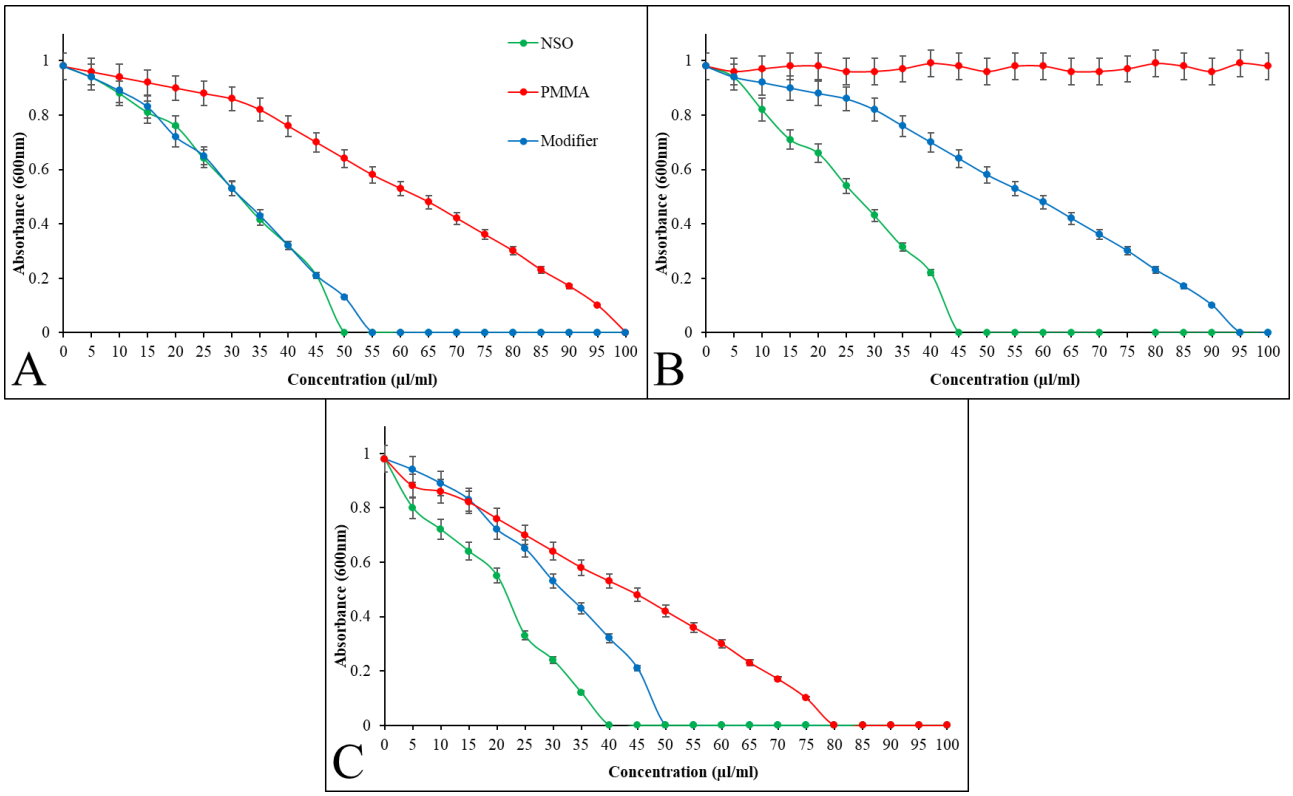

Figure 11 Minimum inhibition concentration test for crude essential oils of NSO, PMMA, and PMMA combined with NSO; (modifier), against *C. albicans*; (A), MRSA; (B), and *E. aerogenes*; (C).

CONCLUSIONS

Essential oils of *Nigella sativa* L. seeds were extracted, identified, and combined with PMMA to enhance their antimicrobial activity. Chemical composition of NSO constituents was investigated using GC-MS analysis and confirmed the presence of bioactive compounds, including thymoquinone and palmitic acid, that might contribute to the antimicrobial potential of NSO. The importance of carefully evaluating the biocompatibility of oils, especially when used in dental applications. The observed cytotoxic effects may be attributed to membrane disruption, oxidative stress, or inflammatory responses. Future research should focus on identifying the specific bioactive components responsible for cytotoxicity and exploring protective strategies to mitigate potential adverse effects. Understanding the safety and mechanisms of oil-induced cytotoxicity is essential for optimizing its application in oral healthcare. The mechanical properties and antimicrobial action of PMMA increased against Gram-positive and Gram-negative bacteria, as well as pathogenic yeasts, after its combination with NSO. It is anticipated that employing natural products as therapeutic agents won't likely cause microbes to develop resistance. Future work should be done to extract and purify the active ingredients of this natural plant and employ them in experimental animals.

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