

**Tropical Journal of Natural Product Research**Available online at <https://www.tjnp.org>**Original Research Article****Antifungal Activity of Nutmeg (*Myristica fragrans*) Flesh Extract from South Aceh Against *Candida albicans* on Denture Base**Diana Setya Ningsih<sup>1\*</sup>, Subhaini Jakfar<sup>1</sup>, Shahida Mohd-Said<sup>2</sup>, Sri Fitriyani<sup>1</sup>, Liana Rahmayani<sup>3</sup>, Evinalis<sup>4</sup>, Rawdlatul Jannah<sup>4</sup><sup>1</sup> Department of Dental Materials, Faculty of Dentistry, Syiah Kuala University, Banda Aceh, 23111 Indonesia<sup>2</sup> Department of Family Oral Health, Faculty of Dentistry, Universiti Kebangsaan Malaysia, Jalan Raja Muda Abdul Aziz, 50300 Kuala Lumpur, Malaysia<sup>3</sup> Department of Prosthodontics, Faculty of Dentistry, Universitas Syiah Kuala, Banda Aceh, 23111 Indonesia<sup>4</sup> Student of Faculty of Dentistry, Syiah Kuala University, Banda Aceh, 23111 Indonesia**ARTICLE INFO****ABSTRACT****Article history:**

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Nutmeg (*Myristica fragrans*) contains several antimicrobial and anticandidal metabolites, yet the bioactivity of its fruit flesh has received limited attention, particularly for managing denture-associated fungal infections. *Candida albicans* (*C. Albicans*) frequently colonizes acrylic denture materials and contributes to denture stomatitis, emphasizing the need for natural antifungal alternatives. This study investigated the chemical composition and antifungal potential of *M. fragrans* fruit flesh extract against *C. albicans* on acrylic denture surfaces. The extract was prepared and subjected to phytochemical screening and GC-MS profiling to identify its secondary metabolites. Antifungal activity was assessed using acrylic specimens inoculated with *C. albicans* for 24, 48, and 72 hours. Minimum fungicidal concentration (MFC) was determined via liquid and solid dilution assays at concentrations of 25%, 50%, 75%, and 100%. Biofilm inhibition was further evaluated using the crystal violet assay. GC-MS analysis revealed 9-octadecenoic acid E (25.9%) as the predominant compound. All extract concentrations (25–100%) resulted in weak biofilm formation, classified as Weak Biofilm Producer (WBP). Solid dilution tests showed complete absence of fungal colonies across all concentrations, indicating fungicidal activity with  $MFC \leq 25\%$ . At 24 hours, biofilm inhibition increased from  $25.00 \pm 5.00\%$  (25%) to  $42.00 \pm 5.00\%$  (100%), with significant differences between these concentrations ( $p = 0.007$ ), while no differences were noted at later times. The 75% and 100% concentrations demonstrated inhibitory effects comparable to the positive control ( $p = 0.156$  and  $p = 0.714$ ). In conclusion, *M. fragrans* fruit flesh extract effectively suppresses *C. albicans* biofilm formation on acrylic denture surfaces.

**Keywords:** *Myristica fragrans*, 9-Octadecenoic, *Candida albicans*, Denture Base, Biofilm.

**Introduction**

Acrylic resin is widely used as denture bases due to its non-irritating, compatible, dimensionally stable, and easy-to-repair or clean nature.<sup>1</sup> However, the material's high water absorption facilitates biofilm formation, particularly by *C. albicans*.<sup>2,3</sup> As reported previously, *C. albicans* can readily grow on denture surfaces, especially in the presence of poor oral hygiene, systemic disease, or surface irregularities.<sup>4</sup> Although *C. albicans* can also be present on the oral mucosa, studies show that the denture surface serves as a major reservoir, as suggested in the previous findings.<sup>5</sup>

Persistent colonization by the *C. albicans* may lead to denture stomatitis, which presents with erythema, edema, and discomfort.<sup>6</sup> The condition is associated with factors such as low salivary flow, poorly fitting dentures, material irritation, and *Candida* presence on the mucosa.<sup>6</sup> To reduce *C. albicans* accumulation on dentures, cleaning solutions are commonly used, with sodium hypochlorite being the most frequent choice due to its disinfectant properties.<sup>7,8</sup>

However, sodium hypochlorite can increase acrylic surface roughness, promoting microbial adhesion and biofilm formation.<sup>8</sup> It may also cause allergic reactions, metal corrosion, and denture discoloration, limiting its long-term use.<sup>9</sup>

Given the limitations of chemical cleansers, natural plant-derived antifungal agents are being explored as safer alternatives. Nutmeg (*Myristica fragrans*) has demonstrated anticandidal activity against *C. albicans*.<sup>10</sup> With Indonesia, particularly South Aceh, producing most of the world's nutmeg, the locally abundant fruit represents a feasible natural alternative for antifungal applications.<sup>11</sup> The fruit of *M. fragrans* comprises the pericarp (skin and flesh), the aril (mace), and the seed. While research typically centers on the seed, the flesh accounts for nearly 78% of the fruit and remains largely unexplored.<sup>12</sup>

According to a previous study, *M. fragrans* flesh extract has a total phenolic content of 50.09  $\mu$ g/mL.<sup>13</sup> The *M. fragrans* flesh contains phenolic compounds, including both flavonoid and non-flavonoid constituents, which are known to contribute to antimicrobial activity.<sup>14,15</sup> The flesh extract also contains saponins, tannins, and alkaloids, which are known for their anticandidal properties.<sup>16,17</sup> A previous work showed that a 20% *M. fragrans* flesh extract inhibited *C. albicans*, although not specifically on denture acrylic.<sup>18</sup>

These compounds have anticandidal activity that can inhibit the development of *C. albicans* colonies and reduce biofilm formation.<sup>16</sup> Previously, 20% *M. fragrans* flesh extract inhibited *C. albicans*,<sup>18</sup> but this is not on denture material. However, despite evidence of its anticandidal properties, the activity of *M. fragrans* flesh extract on acrylic denture surfaces has not been examined. Thus, this study aimed to determine the effect of *M. fragrans* flesh extract on the growth of *C. albicans* on acrylic denture material.

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## Materials and Methods

### Plant Material Preparation

Nutmeg samples were collected between 1 - 5 September 2023 from Batu Itam Village, Tapaktuan Subdistrict, South Aceh District, Aceh, Indonesia ( $3^{\circ}26'90.44''$  N,  $97^{\circ}17'91.26''$  E). A total of 10 kg of *M. fragrans* flesh was washed with running deionized water and oven-dried at 40 °C for 72 h. The dried material was subsequently ground into a fine powder and stored in an airtight container at 4 °C until further use. The preparation steps followed procedures previously described in published work.<sup>19</sup>

### Determination of Nutmeg Plants

The plant specimen was taxonomically identified by Dr. Budi Irawan, M.Si, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran. A voucher specimen (Voucher No. 233/LBM/IT/X/2023) was prepared and deposited at the Jatinangor Herbarium, Laboratory of Biosystematics and Molecular Biology, Universitas Padjadjaran, Bandung, Indonesia, for future reference.

### Preparation of *M. fragrans* Flesh Extract

The complete extraction procedure followed the method described by a previous study.<sup>20</sup> Approximately 200 g of powdered *M. fragrans* flesh was sequentially extracted using solvents of increasing polarity (n-hexane, ethyl acetate, and methanol) using a Soxhlet apparatus. Extraction was performed for 24 h with n-hexane, 15 h with ethyl acetate, and 14 h with methanol at a controlled temperature of 70 °C. Each solvent extract was then concentrated to approximately 100 mL and further reduced under vacuum using a rotary evaporator at 50 °C.

### Gas Chromatography–Mass Spectrometry (GC-MS) Analysis

GC-MS analysis was performed according to the operating conditions described previously.<sup>21</sup> The system was equipped with an AOC-20I autosampler and an Elite-1 fused silica capillary column (30 m × 0.25 mm internal diameter, 1 μm film thickness; 100% dimethylpolysiloxane). Helium (99.999%) was used as the carrier gas at a flow rate of 1 mL/min, and 0.5 μL of sample was injected in split mode (10:1). The injector and ion source temperatures were set at 250 °C and 280 °C, respectively. The oven temperature program was initiated at 110 °C for 2 min, increased to 200 °C at 10 °C/min, and then to 280 °C at 5 °C/min with a final hold of 9 min. Mass spectra were acquired in electron impact mode (70 eV) over a scan range of 45–450 Da. Compound identification was performed by comparing the obtained mass spectra with entries in the NIST library.

### Preparation of Acrylic Resin Specimens

Acrylic resin specimens measuring 5 × 5 × 1 mm were fabricated using a standard flasking technique. Baseplate wax patterns were invested in type III dental stone within a cuvette coated with Vaseline. After 15 min, the wax was eliminated by immersing the mold in boiling water for 5 min, and the mold cavity was coated with CMS. Heat-cured acrylic resin was packed at the dough stage, pressed, and polymerized in boiling water at 100 °C for 30 min. The cured specimens were retrieved and immersed in 30 mL of distilled water at room temperature for 24 h to remove residual monomers. This fabrication procedure followed a previously published standard protocol.<sup>22</sup>

### Adhesion and Biofilm Formation of *C. albicans* on Acrylic Specimens

Each acrylic specimen was vertically positioned in a 96-well flat-bottom microplate and inoculated with 200 μL of *C. albicans* suspension. The plates were incubated at 37 °C for 90 min to allow the adhesion phase. Non-adherent cells were removed by rinsing with phosphate-buffered saline (PBS), after which the specimens were transferred to fresh wells containing 200 μL of peptone water. Biofilm formation was induced by incubation at 37 °C for 24, 48, and 72 h, with the culture medium replaced every 24 h.<sup>23</sup>

### Determination of Minimum Fungicidal Concentration (MFC)

A total of six acrylic specimens were immersed in a *C. albicans* suspension adjusted to the McFarland standard ( $1.5 \times 10^8$  CFU/mL) and incubated at 37 °C for 48 h. Following incubation, each specimen was

transferred into test tubes containing the respective extract concentrations, along with positive and negative controls, for 20 min. The specimens were then rinsed twice with PBS, placed in 1 mL of 0.9% NaCl solution, and vortexed for 30 s. From each suspension, 0.1 mL was plated onto Sabouraud dextrose agar using a bent rod and incubated at 37 °C for 48 h. Colony counts were determined using a colony counter and expressed as CFU/mL, as previously recommended.<sup>24,25</sup>

### Biofilm Inhibition Assay

The Soxhlet extract was diluted with distilled water to concentrations of 25%, 50%, 75%, and 100%. Acrylic specimens pre-exposed to *C. albicans* biofilms were rinsed with PBS and vertically positioned in 96-well plates containing 135 μL of the extract.<sup>24</sup> PBS served as the negative control, while 0.5% sodium hypochlorite served as the positive control (K+). After a 20-min immersion, the specimens were washed with PBS, transferred to vials containing 1 mL of 0.9% NaCl, and vortexed for 30 s to detach adherent cells.<sup>26-28</sup> A 100 μL aliquot was inoculated into 96-well plates containing 100 μL of Sabouraud dextrose broth and incubated at 37 °C for 24, 48, and 72 h. Subsequently, the media were discarded, and the wells were washed with PBS, air-dried, stained with 200 μL of crystal violet for 15 min, rinsed, and decolorized with 200 μL of 96% ethanol. Biofilm quantification was performed spectrophotometrically at 625 nm, following previously published recommendations.<sup>29</sup>

Optical density (OD) measurements were used to determine biofilm strength, following the previous work.<sup>30</sup> The OD<sub>test</sub> value for each sample was compared with the optical density cut-off (OD<sub>cut</sub>) to classify the biofilm. The OD<sub>cut</sub> value was determined using the formula:

$$OD_{cut} = OD_c + (3 \times SD_{OD_c})$$

where OD<sub>c</sub> represents the mean optical density of the negative control and SD<sub>ODc</sub> denotes the standard deviation of the control OD. Samples with OD<sub>test</sub> values relative to OD<sub>cut</sub> were then classified according to standard biofilm strength criteria. Biofilm inhibition was further calculated using:

$$\text{Inhibition (\%)} = \frac{OD_{\text{negative control}} - OD_{\text{test}}}{OD_{\text{negative control}}} \times 100\%$$

The classification criteria for bacterial biofilm strength were shown in Table 1

**Table 1: Biofilm Strength Classification**

Cut-off value calculation	Mean of OD value results	Biofilm producing power
OD <sub>test</sub> ≤ OD <sub>cut</sub>	OD ≤ 0.05	Non-biofilm producers
OD <sub>cut</sub> < OD <sub>test</sub> ≤ 2 × OD <sub>cut</sub>	0.05 < OD ≤ 0.1	Weak biofilm producer
2 × OD <sub>cut</sub> < OD <sub>test</sub> ≤ 4 × OD <sub>cut</sub>	0.1 < OD ≤ 0.2	Moderate biofilm producer
4 × OD <sub>cut</sub> < OD <sub>test</sub>	OD > 0.2	Strong biofilm producer

### Data analysis

All experiments were performed in triplicate, and results are expressed as mean ± standard deviation (SD). Statistical analysis was conducted using the Kruskal–Wallis test to evaluate differences among treatment and control groups, using IBM SPSS Statistics version 26 (IBM Corp., Armonk, NY, USA). A significance level of  $p < 0.05$  was applied for all statistical tests, including Dunn's post hoc analysis.

## Results and Discussion

### Phytochemical Profile of *Myristica fragrans* Flesh Extract

Phytochemical screening of the methanolic extract of *M. fragrans* flesh collected from the Tapaktuan area, South Aceh, confirmed the presence of several bioactive secondary metabolites, including terpenoids, saponins, flavonoids, phenolics, and tannins (Table 2). Stronger color

intensities observed in the qualitative assays indicated higher concentrations of these metabolite groups compared with reactions showing only slight color changes. The presence of these compounds supports the antifungal potential of the extract, as they are widely reported to possess antimicrobial and antibiofilm activities.

**Table 2:** Results of Phytochemical Analysis of Methanol Extract of *M. fragrans* flesh

Compound name	RT	Molecular Formula	Pharmacological Effects	Content (%) of compounds in the sample
4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	6.42	C <sub>6</sub> H <sub>8</sub> O <sub>4</sub>	Antimicrobial, antioxidant, insecticide. <sup>40</sup>	1.45
5-Hydroxymethylfurfural	9.55	C <sub>6</sub> H <sub>6</sub> O <sub>3</sub>	Antioxidant, anti-inflammatory, anti-hyperuremic effects. <sup>41</sup>	6.02
Trans-Isoeugenol	12.9	C <sub>10</sub> H <sub>12</sub> O <sub>2</sub>	Antibacterial, antifungal, and analgesic. <sup>42</sup>	1.76
Myristicin	18.9	C <sub>11</sub> H <sub>12</sub> O <sub>3</sub>	Antioxidants. <sup>43</sup>	1.44
Phenol, 2,6-dimethoxy-4-(2-propenyl)-	22.1	C <sub>11</sub> H <sub>14</sub> O <sub>3</sub>	Antioxidant, anti-inflammatory, antimicrobial, and anticancer. <sup>44</sup>	4.66
6-Pentadecenoic acid, 13-methyl-, (6Z)-, O-methyl	30.1	C <sub>16</sub> H <sub>30</sub> O <sub>2</sub>	Antiangiogenic, antibacterial. <sup>45</sup>	6.22
Hexadecanoic acid, methyl ester	30.3	C <sub>17</sub> H <sub>34</sub> O <sub>2</sub>	Antimicrobial, antioxidant, Analgesic. <sup>46</sup>	3.82
n-Hexadecanoic acid	30.9	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	Antimicrobial, antioxidant, Analgesic. <sup>46</sup>	9.25
Methyl 9-cis,11-trans-octadecadienoate	31.6	C <sub>19</sub> H <sub>34</sub> O <sub>2</sub>	Antiangiogenic, antibacterial. <sup>37</sup>	4.52
cis-13-Octadecenoic acid, methyl ester	31.8	C <sub>19</sub> H <sub>36</sub> O <sub>2</sub>	Antiangiogenic, antioxidant, antibacterial. <sup>47</sup>	6.31
9-Octadecenoic acid, (E)-	32.1	C <sub>18</sub> H <sub>34</sub> O <sub>2</sub>	Antiangiogenic, antioxidant, antibacterial. <sup>48</sup>	25.23

**Table 3:** Secondary metabolites found in *M. fragrans* flesh

Metabolite Content	Result
Alkaloid	-
Steroid	-
Terpenoid	+
Saponins	+
Flavonoid	+
Phenolic	+
Tannin	+

Note: (+) Presence of secondary metabolite, (-) Absence of secondary metabolite

GC-MS analysis further revealed a diverse secondary metabolite profile in the *M. fragrans* flesh extract (Table 3).

The predominant compound was 9-octadecenoic acid (E) (25.23%), followed by n-hexadecanoic acid (9.25%), cis-13-octadecenoic acid methyl ester (6.31%), 6-pentadecenoic acid, 13-methyl-, (6Z)-, O-methyl (6.22%), and 5-hydroxymethylfurfural (6.02%). Myristicin was detected at the lowest relative abundance (1.44%).

The predominance of the monounsaturated trans-fatty acid 9-octadecenoic acid (E) is noteworthy, as this compound has been reported to modify microbial membrane permeability by reducing surface hydrophobicity and interfering with metabolic or signaling pathways involved in microbial growth and biofilm development.<sup>31,32</sup> The phytochemical profile observed in this study is generally consistent with previous reports on *M. fragrans* flesh, although slight differences in GC-MS composition have been documented. These variations may be attributed to geographical factors such as altitude, temperature, and soil chemistry, as well as differences in extraction methods.<sup>33,34</sup>

#### Anticandidal Effect of *M. fragrans* Flesh Extract

The anticandidal evaluation demonstrated that *M. fragrans* flesh extract exhibited potent fungicidal activity against *C. albicans*. The minimum fungicidal concentration (MFC) was determined to be  $\leq 25\%$ , as no fungal growth was observed at this concentration in the liquid dilution assay (Table 4). Turbidity and fungal growth, with an average of 34 CFU/mL, were observed only in the negative control. Subsequent subculturing onto agar media confirmed the absence of viable fungal colonies in all extract-treated groups (25–100%) as well as in the positive control, indicating a fungicidal rather than fungistatic effect. These findings are consistent with previous studies reporting the antifungal activity of nutmeg extracts against *C. albicans*.<sup>35,36</sup>

#### Inhibition of *C. albicans* Biofilm

Biofilm assessment showed that *C. albicans* exhibited weak biofilm-producing capacity across all treatment groups and incubation periods (24, 48, and 72 h), as indicated by optical density measurements (Table 5). Despite this classification, treatment with *M. fragrans* flesh extract resulted in a measurable reduction in biofilm biomass. The percentage of biofilm inhibition increased with increasing extract concentration (Table 6), indicating a concentration-dependent effect. Statistical analysis revealed no significant differences in inhibition among incubation times (Kruskal-Wallis,  $p = 0.826$ ), whereas significant differences were observed among extract concentrations ( $p = 0.011$ ). At 24 h, biofilm inhibition increased from  $25.00 \pm 5.00\%$  at 25% concentration to  $42.00 \pm 5.00\%$  at 100%, with a statistically significant difference between these concentrations ( $p = 0.007$ ). At higher concentrations (75% and 100%), the inhibitory activity of the extract was comparable to that of the positive control, as indicated by non-significant  $p$ -values of 0.156 and 0.714, respectively (Table 7; Figure 1). These results are in agreement with previous reports demonstrating concentration-dependent anticandidal activity of *M. fragrans* extracts.<sup>37</sup>

**Table 4:** Minimum inhibitory concentration of *C. albicans* from *M. fragrans* flesh extract with liquid and solid dilution tests

Concentration	Dilution	
	Liquid	Solids (CFU/ml)
25%	Clear	0
50%	Clear	0
75%	Clear	0
100%	Clear	0
K+	Clear	0
K-	Cloudy	34

Note: K+: Positive Control (Sodium Hypochlorite); K-: Negative Control (PBS)

**Table 5:** Classification of Biofilm Formation (OD) in *M. fragrans* Flesh Extract

Concentration	Biofilm Formation Activity of <i>C. Albicans</i>		
	24 hours	48 hours	72 hours
25%	WBP	WBP	WBP
50%	WBP	WBP	WBP
75%	WBP	WBP	WBP
100%	WBP	WBP	WBP
K+	WBP	WBP	WBP
K-	WBP	WBP	WBP

Note: WBP = Weak Biofilm Producer. All groups consistently demonstrated weak biofilm formation across concentrations and incubation times.

**Table 6:** Inhibition of *C. albicans* Biofilm

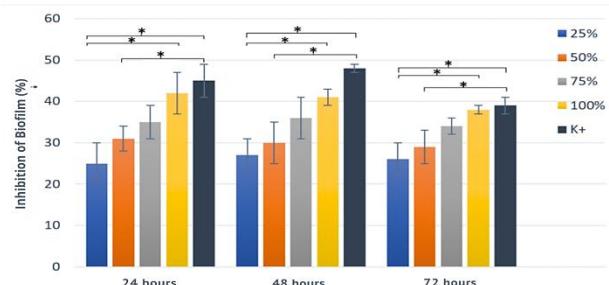
Concentration	Time $\pm$ SD			pValue
	24 hours	48 hours	72 hours	
25%	25.00 $\pm$ 5.00	27.00 $\pm$ 4.00	26.00 $\pm$ 0.04	
50%	31.00 $\pm$ 3.00	30.00 $\pm$ 5.00	29.00 $\pm$ 4.00	
75%	35.00 $\pm$ 4.00	36.00 $\pm$ 5.00	34.00 $\pm$ 2.00	0.011*
100%	42.00 $\pm$ 5.00	41.00 $\pm$ 2.00	38.00 $\pm$ 1.00	
K+	45.00 $\pm$ 4.00	48.00 $\pm$ 1.00	39.00 $\pm$ 2.00	
p time	0.826			

Note: Kruskal Wallis test ( $p < 0.000$ ), with confidence interval 95% (95% CI)

**Table 7:** Post hoc *C. albicans* Biofilm

Concentration	25%	50%	75%	100%	K+
25%		0.41	0.099	0.007*	0.002*
50%	0.41		0.41	0.061	0.025*
75%	0.099	0.41		0.293	0.156
100%	0.007*	0.061	0.293		0.714
K+	0.002*	0.025*	0.156	0.714	

Note: Post Hoc Dunn's Test. Significant difference at  $p < 0.05$ .

**Figure 1:** Percentage of *C. albicans* biofilm inhibition at different concentrations of *M. fragrans* flesh extract (25%, 50%, 75%, 100%) compared with positive control. Error bars represent mean  $\pm$  SD. Asterisks (\*) indicate statistically significant differences ( $p < 0.05$ ).

The observed anticandidal and antibiofilm activities can be attributed to the synergistic effects of multiple secondary metabolites present in the extract. Terpenoids have been reported to disrupt fungal membrane integrity and interfere with cell cycle progression, thereby inhibiting biofilm formation.<sup>38</sup> Flavonoids may damage the plasma membrane and mitochondria of *Candida*, disrupting membrane permeability and nutrient transport, ultimately leading to cellular toxicity. Saponins exert antifungal effects by forming pores in lipid bilayers, resulting in membrane destabilization,<sup>39</sup> while tannins inhibit extracellular enzymes and degrade substrates required for fungal growth and biofilm maturation.<sup>37</sup> The combined action of these compounds likely underlies the potent fungicidal and antibiofilm effects observed in this study.

Despite these promising results, the present study is limited by its in vitro design, which cannot fully replicate the complex biological interactions occurring in vivo. Factors such as metabolism, bioavailability, salivary flow, and host immune responses may influence the antifungal efficacy of *M. fragrans* flesh extract under physiological conditions. Therefore, these results should be interpreted with caution, and further in vivo studies are recommended to validate the efficacy and safety of nutmeg flesh extract for potential oral health applications, particularly on acrylic denture materials.

## Conclusion

The methanolic extract of *M. fragrans* flesh contained bioactive compounds, including terpenoids, flavonoids, phenolics, and fatty acids, which may contribute to its inhibitory effects on the growth and biofilm formation of *C. albicans*. At concentrations of 75% and 100%, the extract exhibited biofilm inhibition comparable to that of the positive control. The minimum fungicidal concentration (MFC) was estimated to be below 25%. These results indicate the potential of *M. fragrans* flesh extract as a natural anticandidal agent, particularly for the development of herbal denture cleansers; however, further in vivo and clinical studies are required to confirm its efficacy and safety.

## Conflict of Interest

The authors declare no conflict of interest.

## Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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