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The efficacy of *Acacia nilotica* extract as a disinfectant on addition silicone impressions: An *in-vitro* study

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Abstract
Acacia nilotica is a widely spread plant native to the Nile basin in central and north Sudan. Because of its proven antimicrobial activity it has been extensively used as a traditional and organic substitute for modern medicine. This study aimed to evaluate the efficacy of *Acacia nilotica* extract as a disinfectant on addition silicone dental impression material and its effect on the dimensional stability of the material.
A quasi experimental pre-test post-test design was applied through microbiological testing upon 30 sectional addition silicone impressions. Each sectional impression was immersed in an ethanolic extract of *Acacia nilotica* with a concentration of 75mg/ml for 10 minutes. The colony forming units (CFU) were recorded before and after the procedure. The dimensional stability was also recorded before and after the procedure using a highly sensitive CAD/CAM machine.
Extract of *Acacia nilotica* showed a reduction in (CFU) from an initial count of 227colonies of Gram positive and Gram negative bacteria to a mean (CFU) of zero when compared to the control group. No statistical difference was found when comparing the mean change of dimensional stability before 6.73± 1.74 and after 6.73±1.71disinfection of addition silicone impressions (p<0.982).
A bactericidal effect of *Acacia nilotica* against Gram positive and Gram negative bacteria occurred with an optimum concentration of 75mg/ml, and the change in dimensional stability was insignificant.

Keywords: *Acacia nilotica* extract, addition silicone, dimensional stability, disinfection

1. Introduction
Acacia nilotica is a genus of shrubs and trees that belong to the subfamily Mimosoideae of the family Fabaceae or Leguminosae. It is found in the eastern and western parts of Sudan along with Saudi Arabia, Egypt, Myanmar, Sri Lanka and India. *A. nilotica* is a multi-purposeful plant used for agro-forestry systems to increase the soil's nitrogen and is also widely used for treatment of various diseases, as it accommodates a number of groups among which are alkaloids, volatile essential oils, phenols and phenolic glycosides, resins, oleosins, steroids, tannins and terpenes [1]. More specifically the aqueous extract of *A. nilotica* is used for treating diarrhea and dysentery. In Nigeria it is also used for covering and cleaning infected wounds [2].
Different parts of this plant such as the leaves, roots, seeds, bark, fruits, flowers, gum and immature pods are engaged in the treatment of different ailments in the indigenous system of medicine, as they perform anti-fungal, anti-bacterial, anti-plasmodial, anti-platelet aggregatory functions and more [3].
Dental impression is a negative print of the oral cavity made by using an impression material loaded on an impression tray, where this impression is cast to an analogue fabricating the oral cavity structures. As the impression material inevitably contacts the patient's mouth, it gets contaminated by oral fluids such as saliva and blood.
The Dental British Association recommended the decontamination and disinfection of dental impressions before sending it to the laboratory. The guidelines published by the Dental British Association state the responsibilities for insuring impressions have been cleaned and disinfected before dispatch to the laboratory lies solely with the dentist [4].
Dental impressions are categorized as semi-critical objects in dental practice and require high level disinfection, which can only be achieved by 2% glutaraldehyde. Although glutaraldehyde is considered the best disinfectant, it is highly irritating to the skin and causes other health hazards to the eyes and the respiratory tract, making sodium hydrochloride the disinfectant of choice despite the fact that it only provides an intermediate level of disinfection [5].
Addition silicone is an elastomeric impression material used extensively by dental practitioners with different specialties for impression making due to its excellent surface reproductions and minimal shrinkage in addition to its easy handling.
Traditional medicine is the knowledge, skills and practices based on the theories, beliefs and experiences indigenous to different cultures, used in the maintenance of health and in the

prevention, diagnosis, improvement or treatment of physical and mental illness [6].

Despite the sky rocketing development and mass production of chemically synthesized drugs worldwide, a large section of the developing countries still depend on traditional medicine as their primary care. For example, the World Health Organization has stated approximately 80% of Africa's population still relies on traditional medicine. However, the use of traditional medicine is not limited to developing countries. In the past two decades there has been an increase in the use of traditional medicine in developed countries as well [7].

A. nilotica and its extract have been used as a natural organic substitute for modern medicine. It has a significant amount of health benefits and its medicinal uses have proven to be effective in combating illness and diseases through its antimicrobial activity. Nevertheless, its use in dentistry is minimal. The main objective of the study was to evaluate the efficacy of *A. nilotica* extract as a disinfectant on addition silicone dental impression material and its effect on the dimensional stability of the material. The specific objectives were to assess the efficacy of *A. nilotica* extract as a disinfectant for Gram positive and Gram-negative bacteria found on sectional addition silicone dental impressions, and to measure the effect of *A. nilotica* extracts on the dimensional stability of the sectional addition silicone dental impressions.

The null hypothesis (H_0) was that the ethanolic extract of *A. nilotica* will have no antibacterial efficacy against different pathogens found on addition silicone impressions. Another null hypothesis was that the treatment of addition silicone impressions with the ethanolic extract of *A. nilotica* will affect its dimensional stability. The alternative hypothesis (H_1) was that the decontamination of addition silicone impressions with the ethanolic extract of *A. nilotica* will have an antibacterial effect and the dimensional stability will stay unchanged.

2. Materials and Methods

2.1 Study design and duration

Quasi experimental, pretest posttest design. The course of the study was made between August 2019 and November 2019.

2.2 Study Population

A total of 30 addition silicone sectional impressions were taken from volunteering dentate patients attending University of Khartoum Restorative Clinic with no fixed or removable prosthesis aged between 18-40 years.

2.3 Methodology

2.3.1 Plant Material

The *A. nilotica*-fruits-were collected from a forest in Al Obeid, the capital of North Kurdufan, Sudan, between August 2019 and September 2019. They were about 1-2cm in diameter and 5-7.5cm in length; they were identified and authenticated by the taxonomists of Medicinal and Aromatic Plants and Traditional Medicine Research Institute (MAPTMRI). The fruits were air-dried under the shadow with good ventilation for 3 days.

2.3.2 Extract Preparation

Extraction was carried out according to the method described by Sukhdev *et al*. (2008) [8].

100 g of the dried powder of *A. nilotica* pods was weighted and put in a sterile glass container. Then, 500 ml of absolute ethanol (Duksan pure chemicals) was slowly added, tightly closed, and shook vigorously two or three times a day. This action was repeated for up to 3 days. The macerate was then filtered using Whatman filter papers No.1 and daily filtration and evaporation of the solvent under reduced pressure using rotary evaporator apparatus (Buchi Switzerland) took place. The filtrate was separated and stored in an incubator in 45 ℃ for up to 7 days to get a dry extract. At the time of the antibacterial experiment, a concentration of 75 mg/ml was prepared by reconstituting the crude extract in distilled water.

Fig 1: Crude ethanolic extract of *Acacia nilotica*

The yield percentage was calculated as follows

Weight of extract obtained/weight of plant sample X 100.

Table 1: Yield percentage calculation

Weight of plant in gm	Weight of extract in gm	Yield %
100 g	24 g	24

2.3.3 Impression Making

In room temperature, addition silicone impression material (PERFIT HUGE Dental Material Corporation) was mixed according to the manufacturing instructions and loaded in a stock sectional impression tray by a trained general dentist, followed by sectional impression making of the maxillary arch using a monophase impression making technique.

2.3.4 Disinfection of Addition Silicon Impressions

Two swabs were taken from each sectional impression, one before and another one after the disinfection. After taking the first swab, impressions were held under running tab water for 30 seconds, and then each sectional impression was fully immersed in a separate sterile container filled with the ethanolic extract of *A. nilotica* for 10 minutes.

2.3.5 Incubation

The two swabs taken before and after the disinfection were planted into blood and MacConkey agar plates. These plates were placed in an incubator for two days (48 hours) at 37 ˚C. The colony forming units were counted using the naked eye method and recorded. Comparing the number of colonies formed before and after immersion determined the antimicrobial efficacy of the ethanolic extract. Gram stain was used to differentiate Gram positive from Gram negative colonies.

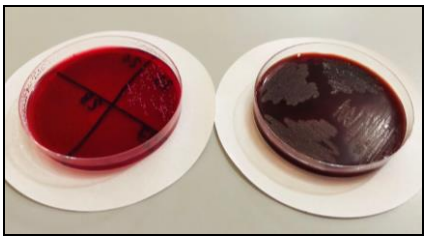

Fig 2: Bacterial colonies formed in blood and MacConkey agar media before the immersion process

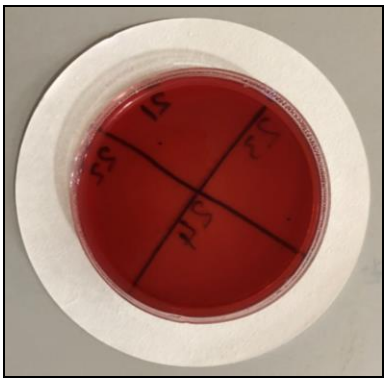

Fig 3: MacConkey agar plate showing no bacterial colonies formed after the immersion process

2.3.6 Dimensional stability recordings

The effect of the *A. nilotica* extract on the dimensional stability of addition silicone sectional impressions was measured by recording the distance between the two fixed points. Three readings of the same fixed points were measured before and after the immersion treatment and the mean change in difference was recorded using a highly accurate CAD/CAM system (Zirkonzahn), with a sensitivity of 0.001mm.

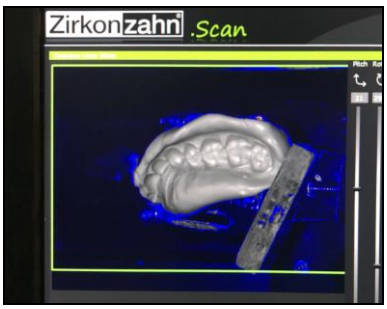

Fig 4: Scanning sectional addition silicone impressions by Zirkonzahn scan software

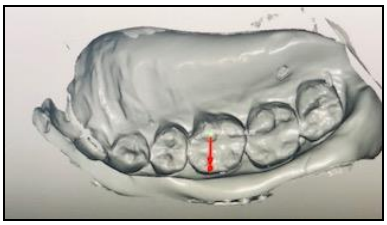

Fig 5: Measurement of the distance between two fixed points on a scanned sectional addition silicone impression

2.4 Statistical analysis

Statistical procedures performed using SPSS 23.0 Statistical Software Program (SPSS, Inc., USA). T test was used to compare mean colony forming units between groups. Significance level was set at 0.05.

2.5 Ethical considerations

The study was approved by the ethical committee of the University of Khartoum. Patients whose their impressions were used in the study signed an informed written consent to leave their impressions voluntarily for the purpose of this study.

3. Results and discussion

The ethanolic extract of *A. nilotica* fruits was screened for its antimicrobial activity against Gram negative and Gram-positive bacteria planted into blood and MacConkey agar plates, by counting the colonies formed per unit area (CFU) before and after the immersion process. The extract obtained from the fruits exerted a broad antimicrobial activity against all types of bacteria tested as indicated by the zero number of colonies formed after the immersion of addition silicone impressions in the ethanolic extract of *A. nilotica.*

Out of thirty cultures tested, five types of Gram positive (cocci in chains 86%,cocci in clusters 3%, cocci in groups 3%, bacilli 3% and micrococcus 5%) and two types of Gram negative (cocci 92% and bacilli 8%) bacteria grew on blood and MacConkey agar plates before immersion took place.

By contrast, blood and MacConkey agar plates showed zero bacterial growth after immersion in the *A. nilotica* extract of a concentration of 75mg/ml, indicating that the ethanolic extract of *A. nilotica* exerted a strong bactericidal activity on the different types of pathogens found before immersion. (Figure 6, 7).

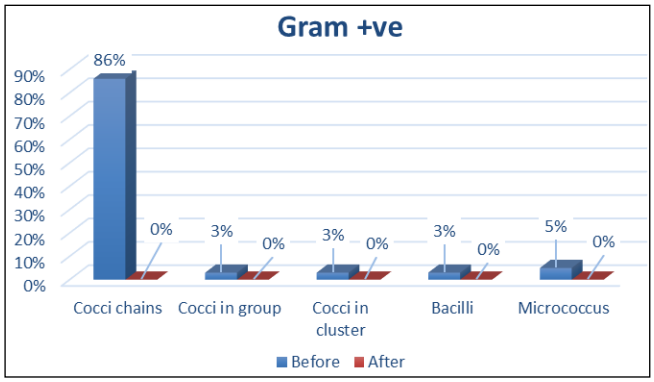

Fig 6: A bar chart demonstrating the percentages of Gram positive bacteria before and after the use of *Acacia nilotica* extract

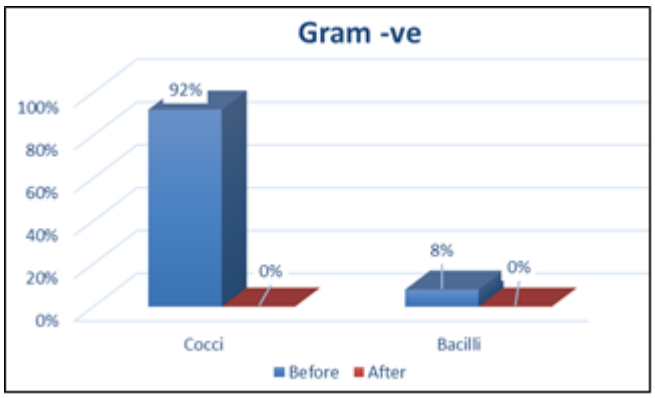

Fig 7: A bar chart demonstrating the percentages of Gram negative bacteria before and after the use of *Acacia nilotica* extract

Comparing the mean dimensional change before and after the use of the extract, statistical analysis concluded that there is no significant difference ($P > 0.05$) in the dimensional stability of the addition silicon impression material. (Table 2).

Table 2: Mean dimensional change in the dimensional stability of addition silicon impression material before and after the use of *Acacia nilotica* extract

Before (Mean ± SD)	After (Mean ± SD)	P value
6.73±1.74	6.73±1.71	<0.982

The study demonstrated the presence or absence of antimicrobial activity of the ethanolic extract of *A. nilotica* fruits as well as its efficacy on oral pathogens, and its effect

on the dimensional stability of addition silicone impression material. In accordance to the results obtained, disinfection of addition silicone impressions using *A. nilotica* extract has shown bactericidal activity at a concentration of 75mg/ml, while the change in the dimensional stability was insignificant ($P > 0.05$).

In this study the anti-bacterial activity of the ethanolic extract of *A. nilotica* was evaluated and the results indicate that *A. nilotica* ethanolic extract has a bactericidal activity against the bacterial strains tested.

Gmaraldeen SM *et al*. found that the inhibition zones created by the methanolic extract of *A. nilotica* is of a greater diameter (11-39mm) when compared to gentamicin antibiotic discs (10 µg/disc) as a control (20-32mm), against five Gram negative bacteria (*Escherichia coli*, *Shigella flexneri*, *Salmonella typhi*, *Klebsiella pneumonia and Pseudomonas aeruginosa*) and two Gram positive bacteria (*Listeria monocytogenes* and *Bacillus cereus*) indicating a higher antibacterial activity of the methanolic extract over the gentamicin antibiotic discs [9].

My study is compatible with many other studies that say: *"Acacia nilotica* is commonly used to treat eye conditions, open wounds and dermatological ailments. Acting much as antacid it can also treat digestive problems" [10]. *A. nilotica* has antibiotic activity and its aqueous extracts are antibacterial [11]. It has soothing, astringent, and antiseptic properties [10].

Previous studies that also compared different methods of extraction showed that the ethanolic extract exhibited a high antimicrobial activity. This may be due to a/the stronger extraction capacity of an/the active component responsible for antibacterial activity in comparison to its aqueous extract hence it was the method of choice for the extraction [9].

There can be several factors which can affect or reduce the efficacy of the medicinal plants in their antimicrobial activity. This begins from the time of plant collection (it is recommended that the collection of plant parts in most cases, but not always should be done after the flowering stage of the plant), to the state of plant processing, and the state of storage of plant [12]. The method of plant extraction is another factor which affects the antimicrobial activity of the medicinal plant.

Different concentrations of the extract were previously tested. In accordance with Hameed FR, the optimum concentration was found to be 75mg/ml after comparing three different concentrations, (50, 75 and 100mg/ml) on tested bacteria using the disc diffusion method [13].

However, *A. nilotica* extract exerts bacteriostatic activity against Gram positive bacteria at lower concentrations, as low as 12.5mg/ml according with Emad *et al*. [19]. Hence the concentration of 75mg/ml was the concentration of choice in the study.

Generally, the results reported from different studies are difficult to compare because of the use of different test methods, bacterial strains and sources of antimicrobial samples used. As a result, differences in the type and concentrations of the secondary metabolites across different plants, variation in antimicrobial activities are expected.

Dimensional stability of the material is a significant factor for accuracy of dental restoration. There are controversies concerning the disinfection impact on impression materials or stone casts. Some of the studies concluded a significant effect of disinfection solution not only depends on water absorption but also chemical interactions. However, some studies declared that there is no significant effect on impression dimensional accuracy. Walker *et al*. stated that sodium hypochlorite (0.5%) had an adverse effect on polyether impression surface. It was also noted that polyether significantly expanded when disinfected with sodium hypochlorite [15]. However, Amin WM *et al*. noted that there is not any significant change in addition silicone dimensional stability after disinfection [16].

Technical improvements in 3D imaging procedures enable a direct digitalization of impression negatives [17]. It is an advantageous method for assessing the dimensional accuracy and stability of impressions than other two-dimensional methods [17, 18].

Many of the studies measured the dimensional accuracy between selected points on the model especially at the canine and first molar regions. The length between selected points were compared among models in two dimensions and concluded as differences between distances or percentages [18, 19, 20, 21].

In this study, addition silicone dimensional change was measured with a 3D CAD/CAM scanner before and after being treated with *A. nilotica* extract and concluded as differences between distances.

Two fixed points were measured three times before and three times after the immersion treatment. Mean differences in distances were concluded and no statistical significant change was found as seen in the results.

An inconsistency in the measurements of the dimensional stability may have occurred, the use of the CAD/CAM software is subjective and the determination of the two fixed points before and after the disinfection is difficult to standardize. Therefore, a superior method for measuring the dimensional stability, such as scanning with electron microscope, is recommended.

Similarly, Gokce *et al*. used a 3D optic scanner to compare dimensional changes with 10 microns accuracy. In their method, four holes were created at the canine and first molar positions. The holes were used for superposing the whole impression surfaces in 3D software. Percentages of surface area deviation values were obtained using 3D scanning method with superposing the images [22].

Although, the application of computers and computer-guided systems in dentistry provides us with a new perspective and wider possibilities for intraoral tissue registration, the use of impression materials will remain a reliable method for years to come.

4. Conclusion

- The alternative hypothesis was correct; the extract has shown antibacterial effect against different oral pathogens.
- In terms of efficacy, *A. nilotica* ethanolic extract was found to produce bactericidal effect on all Gram positive and Gram negative species found.
- Regarding the dimensional stability, differences in the measurements before and after the use of the *A. nilotica* extract on addition silicon impressions were of no significance.

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