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Preparation of an Antibacterial Herbal Mouthwash with *Maerua Crassifolia Forsk* against Oral Pathogens in Patients Wearing an ODF (Dentofacial Orthopedics) Appliance

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ABSTRACT

Antibacterial agents are inherently present in flora. They are more manageable, less detrimental, more efficient, and exhibit low toxicity to mammals. *Maerua crassifolia Forsk* is a plant indigenous to Algeria. People use the leaves in beverages to treat various diseases. Our pilot clinical investigation examined the therapeutic efficacy of a mouthwash produced *by M. crassifolia Forsk in patients undergoing therapy for dental caries while utilizing* orthodontic gear. This study sought to evaluate the present condition of caries treatment and prevention while identifying viable techniques to mitigate this issue. Subsequent to sampling, the stems were severed, desiccated, and thereafter conveyed to the laboratory for examination. The secondary chemicals in the extracts were identified using the resultant chemical colorimetric reactions. *We examined the impact of Maerua crassifolia plant toxins using the brine shrimp assay.* We subsequently isolated oral bacteria from ODF carriers and evaluated the antibacterial and anti-inflammatory properties of these carriers. The crude yield of the hot-soaked *Maerua crassifolia* stem extract was recorded at 7.93%. All in vitro reactions demonstrated the presence of polyphenols, flavonoids, reducing sugars, alkaloids, and saponins. This result signifies that certain types of chemicals demonstrate significant biological activity. Extracts of *Maerua crassifolia* are deemed non-toxic to HaCaT cells. A consortium of bacteria was identified, comprising *E. coli, P. aeruginosa, Staphylococcus aureus*, and *Candida albicans*. The findings of a study on the inhibitory action of the crude extract of *M. crassifolia Forsk* indicated that the extracts had an inhibitory impact on all isolated strains. The diminished oral hygiene index indicates that our mouthwash incorporates antibacterial agents. This investigation exhibited favorable outcomes, with subsequent clinical findings for our patient indicating a distinct enhancement in the following references: The gingival pigmentation changes, making the gums appear biologically red; the gingival edema decreases; the gingival hemorrhage decreases or completely stops; and the gingival inflammatory response (gingivitis) lessens.
Keywords: *Maerua crassifolia Forsk*, Antibacterial agents, Non-toxic, Isolated strains, Gingivitis

INTRODUCTION

Antibacterial agents occur naturally in flora. They are more manageable, less hazardous, more efficient, and exhibit low toxicity levels for mammals [1]. Many studies have shown that essential oils and their components can be very effective as antibacterial agents in various industries and medicine. *M. crassifolia Forsk* is a plant that thrives in the arid desert savannah of Mauritania, Senegal, the eastern Sahel region of northern West Africa, and particularly Algeria. This plant is typically located in arid regions of the Sahelian thorny shrub on sandy soils, often in isolated patches [2]. The Tuaregs use this species as a "toothbrush" and believe it to have medicinal properties.

The leaves are utilized in an infusion to alleviate stomachaches [3]. In Mali, the leaves are used by soaking them in water, mixing them into milk, or chewing them to help with digestion and to relieve constipation. Ibraheim, 2008 recommends incorporating leaf powder into milk as an antipyretic. The aerial portions of *M. crassifolia* are utilized in Egypt for the treatment of headaches, toothaches, skin infections, digestive disorders, and mental ailments [4]. *Maerua crassifolia Forsk* undergoes several traditional therapies [5].

Orthodontic devices, through their mere presence (brackets, arches, retention wires, ligatures, and other auxiliaries) or their irritating effects, can contribute to the establishment of an adverse periodontal condition (inflammatory diseases of bacterial origin). Conversely, orthodontic treatments enhance periodontal health by establishing a suitable dental environment. They can also assist, alongside periodontology, in rectifying certain cosmetic and functional deficiencies related or unrelated to periodontitis [6]. This clinical experimental investigation examined the therapeutic efficacy of mouthwash derived from the medicinal plant *Maerua Crassifolia* in patients undergoing ODF treatment with orthodontic devices. The aim is to assess the current status of the dental caries treatment and prevention issue and suggest potential solutions.

MATERIALS AND METHODS

Sampling and Preparation of Plant Extract

The plant stems, measuring barely more than half a centimeter in diameter, are manually harvested at the conclusion of spring and the onset of summer 2024 in Tindouf (28.861327, -8.248580), Algeria.
The stems are desiccated and subsequently transported to the "Microbiology and Plant" laboratory at the Faculty of Sciences, University Center of El-Bayadh, Algeria, for classification and analysis.

Fig. 1: *Maerua crassifolia Forssk*. a. Habit. b. Clustered leaves on the branches. c. Flowers, represented by 30-40 stamens. d. Mature fruit. e. Fruits bagged to prevent insect attack. f. Seeds [7].

Screening Phytochimique

Phytochemical screening facilitated the identification of secondary metabolites in the extracts based on the colors produced during chemical reactions.

Table 1 Characterization the main chemical components in the plant by coloured reactions [8]

Chemical components	Coloured reactions
Alkaloids	Reagents of Dragendroff and Mayer
Flavonoids	Reaction of cyanidine
Tannins	Ferric chlorides
Sterols and terpens	Reagents of Liebermann Buchard
Saponosids	Foam index
Reducing compounds	Reagent of Fehling

Cytotoxicity assay of *Maerua Crassifolia*

The cytotoxic activity of aqueous extracts from *Maerua Crassifolia* samples was assessed using the brine shrimp assay [9].
Following incubation, active nauplii were chosen to evaluate the cytotoxic activity of *Maerua Crassifolia* extracts. Four distinct concentrations of the extract (stock solution, 10^{-1}, 10^{-2} and 10^{-3}) were prepared utilizing distilled water.
1 mL of each test solution was introduced into pre-labeled test tubes containing 5 mL of seawater and 10 nauplii. After 24 hours, the quantity of surviving nauplii in each test tube was enumerated using a magnifying lens and documented.

Extraction and Preparation of Mouthwash

Mouthwash made experimentally using our herb "Al Atil" 1 kg of air-dried and powdered stems of *Maerua Crassifolia* was sequentially percolated with water at ambient temperature. The aqueous extracts were filtered and evaporated under vacuum at low temperature, resulting in a dry residue of 79.3 g, which was then suspended in water. Consequently, 1L of the concentrated liquid extract (the mouthwash containing "Al Atil") was procured [10].

Preparation of Concentrations

To obtain the three concentrations of extracts 10^{-1}, 10^{-2} and 10^{-3}, a classic dilution is made by adding sterile distilled water to adjust the concentration [11].

Yield of Crude Extracts

The yield of the crude extract is defined as the ratio between the mass of the dry extract obtained and the mass of the plant material treated [12].

Isolation of Bacteria from the Mouths of ODF

Isolation involves distributing microorganisms across the surface of a glossy media within a Petri dish, facilitating the formation of different colonies.

Dental specimens need meticulous attention. The relevant region must be separated by positioning cotton rollers around the tooth to be tested, following the cleaning of the area. In practice, the swab is the predominant sample method utilized. It is simple to manage. It necessitates enhanced safeguards for its transportation. Post-sampling, meticulous attention is required to [13]:

- inhibit the desiccation of the pathogenic product,
- shield the bacteria from atmospheric oxygen.
- maintain the ensuing proliferation of aerobic and anaerobic microorganisms.

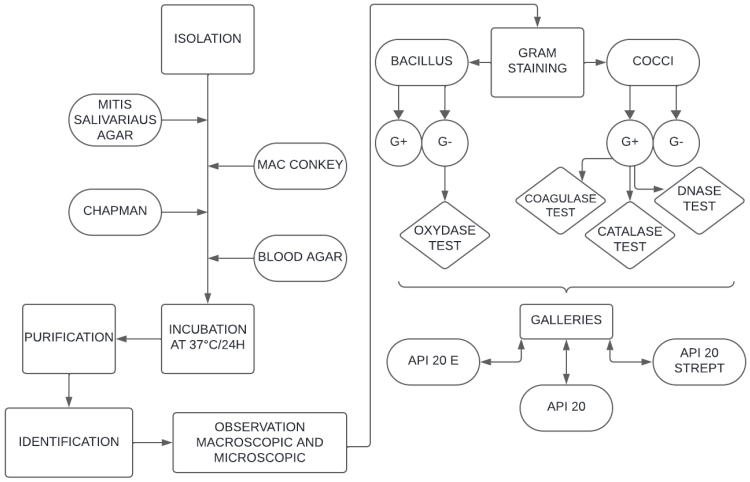

Fig. 2 method of isolation and identification of oral bacteria

As for *Candida albicans* was isolated from clinical specimens with Sabouraud Dextrose Agar (SDA) supplemented with chloramphenicol to suppress bacterial proliferation. Following incubation at 37°C for 24–48 hours, colonies exhibiting a creamy, smooth appearance were chosen. Microscopic analysis and Gram staining verified the existence of oval, Gram-positive budding yeast cells. [14-15-16]. The germ tube test, an essential diagnostic instrument, was used to verify *C. albicans*, with positive germ tube production indicating its existence. Additional verification was conducted using chromogenic agar and sugar assimilation assays, guaranteeing precise species-level identification

Antimicrobial Activity Test

The techniques employed to assess the antibacterial efficacy of the extracts are derived from those outlined by the Clinical and Laboratory Standards Institute [17-18]. The disk diffusion method was used for the determination of antibacterial activity [19]. Sterile disks, 6 mm in diameter (Wattman paper No. 1 - SELECTA, Germany), impregnated with 5 and 10 µL of each dilution (10^{-1}, 10^{-2} and 10^{-3}). The disks were placed in Petri dishes on Mueller-Hinton agar, which had been surface-spread with 1 ml of isolated bacteria; in logarithmic phase fusion at 108 CFU/ml fixed by optical density (OD = 0.08 and 0.1) [20]. The Petri dishes were then incubated for 18 h at 37 °C. The diameter of the inhibition zone was measured to compare the in vitro antibacterial activity.

Anti-inflammatory Test on WOA Carriers

The diagnostic results classify the clinical form of periodontal disease. Initially, it is essential to understand a straightforward, comprehensive classification that is widely acknowledged as an international consensus reference. [21-22-23]

The study was performed at the dental clinic of Dr. Amran Abdelaziz in El Bayadh from March to June 2024. The objective of this clinical investigation was to assess the therapeutic efficacy of a mouthwash formulated from the medicinal plant *Maerua Crassifolia*. We enlisted eleven participants who had orthodontic devices (ODF). (9 females and 3 males; mean age 25 ± 7.35 years).

Initially, we conducted a diagnostic assessment before administering the mouthwash, thereafter prescribing it after confirming that the individuals were not on any medications, particularly antibiotics.

RESULTS AND DISCUSSION

Extraction Yield

The crude yield of the extract obtained from the stems of *Maerua Crassifolia* by hot maceration in water (7.93%) is lower than that of Christian and his colleagues in 2017; they found a high yield of about 10.35% (methanolic extract) [24].

Screening Phytochimique

Table 2 Phytochemical Screening of *Maerua Crassifolia*

Chemical components

Alkaloids	+++
Flavonoids	+++
Tannins	++
Sterols and terpens	-
Saponosids	+
Reducing compounds	++

(+: traces ++: abondant +++: très abondant - : absent)

All the tube reactions obtained show that polyphenols, flavonoids, reducing sugars, alkaloids, and saponins. On the other hand, steroids and polyterpenes are absent. Phenolic compounds are substances recognized for their antioxidant properties; the presence of tannins gives the plant astringent and antiseptic properties.

These classes of compounds are reported to show important biological activities [25- 26] and their presence may be responsible for the anti-diarrheal properties observed in the extract. Earlier studies indicated that the anti-diarrheal properties of medicinal plants were due to tannins, alkaloids, saponins, flavonoids, steroids, and terpenoids [27- 28- 29].

Table 3 Cytotoxicity experiment

Concentrations	Dead	Alive
stock solution	0	10
10^{-1}	0	10
10^{-2}	0	10
10^{-3}	0	10

There were no lethal or toxic reactions observed at any of the doses of methanolic leaf extract from *Maerua crassifolia* used in the study. All animals were alive, healthy, and active during the observation period. Yonbawi and his colleagues in 2021 reported in their research that *Maerua crassifolia* extracts can be considered non-toxic to HaCaT cells [30].

Isolated Bacteria and Antimicrobial Effect

These studies identified *E. coli, P. aeruginosa, Staphylococcus aureus*and *Candidas albicans* as associated with gingivitis [31].

Table 4 antimicrobial activity test results for *Maerua Crassifolia* plant extracts on isolated bacterial strains

Isolated bacteria	stock solution	10^{-1}	10^{-2}	10^{-3}
E.coli	11,6	9,69	6,66	5,66
P. aeruginosa	8,33	8	7	7
Staphylococcus aureus	13,3	10	8	7,8
Candidas albicans	11,5	10,3	6	5

The results of the study of the inhibitory activity of the crude extract of *Maerua Crassifolia* show that the extracts obtained have an inhibitory effect on all the strains isolated. The inhibition may be due to the various chemicals present in the extract, such as saponins, tannins, flavonoids, and alkaloids… Sensitivity is marked in *Escherichia coli*, *Candida albicans,* and *Staphylococcus aureus*, but less so in *Pseudomonas aeruginosa*. This sensitivity may be due to strain morphology, physiology, or type. These results are similar to those of Kingsley Chimsorom Ckilaka et al., 2015 [32].

And the work of Chaib *et al*. (2005) shows that *Maerua Crassifolia* essential oils have an inhibitory effect on *E. coli*, *P. aeruginosa*, and *S. aureus* strains; this activity is due to the terpene compounds in the oil. (1,8-cinéole,α- *caryophyllene*, βpinène,et 9-*epinE*)-*caryophyllene*) [33].

According to Ali *et al*. (2002), crude water extracts generally act on bacterial growth, particularly of *Streptococcus mutans* and *Streptococcus faecalis*, at extraction rates of 5 g/100 ml of plant matter from "Alatill *(Maerua Crassifolia*) [34].

In 2015, Ckilaka and his colleagues assessed the antibacterial properties of the methanol leaf extract of *M. crassifolia* against *Staphylococcus aureus*, *Shigella spp*., *Salmonella typhi, Bacillus subtilis, and Escherichia coli* utilizing disc diffusion and broth microdilution techniques. The extract demonstrated efficacy against the examined pathogens, with inhibition zones measuring between 7.8 mm and 29.6 mm, while the lowest inhibitory concentration and minimum bactericidal concentration values varied from 7.0 µg/ml to 24.0 µg/ml [35]. No bacterial species associated with gingivitis was distinct; hence, their presence cannot be deemed pathognomonic [36].

Anti-inflammatory Test on ODF Carriers

The experimental study concerning the therapeutic effect of our lotion prepared with *Maerua Crassifolia*; on 11 patients mainly wearing Dental braces, favoring nutritional stagnation and consequently plaque formation, which provokes an inflammatory reaction at the level of the superficial periodontium (gingivitis).

Table 5 Clinical signs before/after use of *Maerua Crassifolia* mouthwash ***(****B° : before using mouthwash. A°: after using the mouthwash)*

Patient number	bucco-dental hygiene		Gingiva color		Gingival bleeding		Gingival swelling		Gingivitis		Periodontitis		Tooth color		Mouth odor	
	B°	A°	B°	A°	B°	A°	B°	A°	B°	A°	B°	A°	B°	A°	B°	A°
N° :01	2	1	3	1	2	1	3	1	2	1	0	0	3	1	1	0
N° :02	2	1	3	1	1	1	3	1	3	1	0	0	3	1	1	0
N° :03	2	1	3	1	1	0	1	1	3	1	0	0	3	2	2	1

N° :04	2	1	3	2	2	1	2	1	3	1	0	0	3	1	1	0
N° :05	2	1	3	1	2	0	2	0	3	1	0	0	3	2	2	0
N° :06	2	1	3	2	2	1	2	1	3	1	0	0	3	2	2	1
N° :07	2	1	3	2	3	1	1	0	3	1	0	0	3	2	1	0
N° :08	2	1	3	1	2	0	2	1	3	1	0	0	3	2	0	0
N° :09	2	1	3	1	2	0	0	0	3	2	0	0	3	2	2	1
N° :10	2	1	3	2	2	0	0	0	3	1	0	0	3	2	2	1
N° :11	2	1	3	1	0	0	2	0	3	1	0	0	2	2	1	0

0: absence of plaque, Normal gum, no inflammation, no discoloration, no bleeding; 1: Thin plaque film along the gingival margin, only recognizable by the passage of the probe, Mild inflammation, slight color change, slight surface change, no bleeding; 2: moderate plaque along the gingival margin, visible to the naked eye, interdental spaces free, Moderate inflammation, redness, swelling, bleeding on probing and pressure; 3: Significant plaque along the gingival margin, interdental spaces filled with plaque, Severe inflammation, significant redness and swelling, tendency to spontaneous bleeding, possibly ulceration.

The values in this table are numerical expressions of defined diagnostic criteria (expressing an alteration, a disease, or the severity of a disease). When we compared the patients' clinical scores, we noted a reduction in the evaluation scores, meaning that the effect of the mouthwash is evident in the table. As far as periodontitis is concerned, we have not recorded any signs before or after the use of our plant-based mouthwash in patients [37]. The decrease in the bucco-dental hygiene index means that our mouthwash contains antibacterial agents, where Moctar *et al. (2020)* found in their research that this plant treats mouth ulcers [38].

Paradental indices monitor the frequency of various pathological processes, the role of different etiological agents, and the effectiveness of prophylactic, preventive, and therapeutic methods. Current epidemiology measures periodontal disease, including periodontal indices, pocket measurements, and radiographic recording of bone level loss [39].

The bacteria isolated in this study that are not associated with gingivitis are not unique, and their presence cannot therefore be considered pathological. Fortunately, this explains why no infections were recorded in the patients we studied. This is what Trombelli and his colleagues in 2018 point out in his article: if the presence of inflammatory bacteria does not mean that there is inflammation, it means that there is inflammation [40].

The accumulation of bacteria responsible for bleeding gums can cause gingivitis. It is known that healthy gums appear as a pale pink structure; when inflamed, they can become red and swollen. While in many cases they can be asymptomatic, they can cause pain and bleeding, and if not treated properly, they can become complicated by periodontitis (inflammation of the supporting tissues of the tooth). So the main cause of the problems is bacteria [41].

In our research, we noticed that almost all values of gingiva color, gingival bleeding, gingival swelling, and gingivitis decreased by one or two points for different patients. This is due to the effect of the "Atill" mouthwash, as the antibacterial substances significantly affected the bacteria causing the symptoms in patients. The improvement of the plaque index and bleeding index after the use of plant mouthwash is consistent with previous reports by Pistorios and co-workers in 2003 and Hamilton and others in 2001 [42-43].

As they showed, this mouthwash had significant effects on plaque accumulation and gingival inflammation [44]. We noticed through the works of Yaghini et al. (2019) that *Aloe vera* also contains compounds with confirmed antibacterial effects, and the positive effect of this plant on the plaque index can be attributed to these compounds [45].

The odor and color of the teeth varied across patients, influenced by individual physiology, adherence to prescribed dosages, and the uniformity and concentration of antibacterial agents in the mouthwash [46].

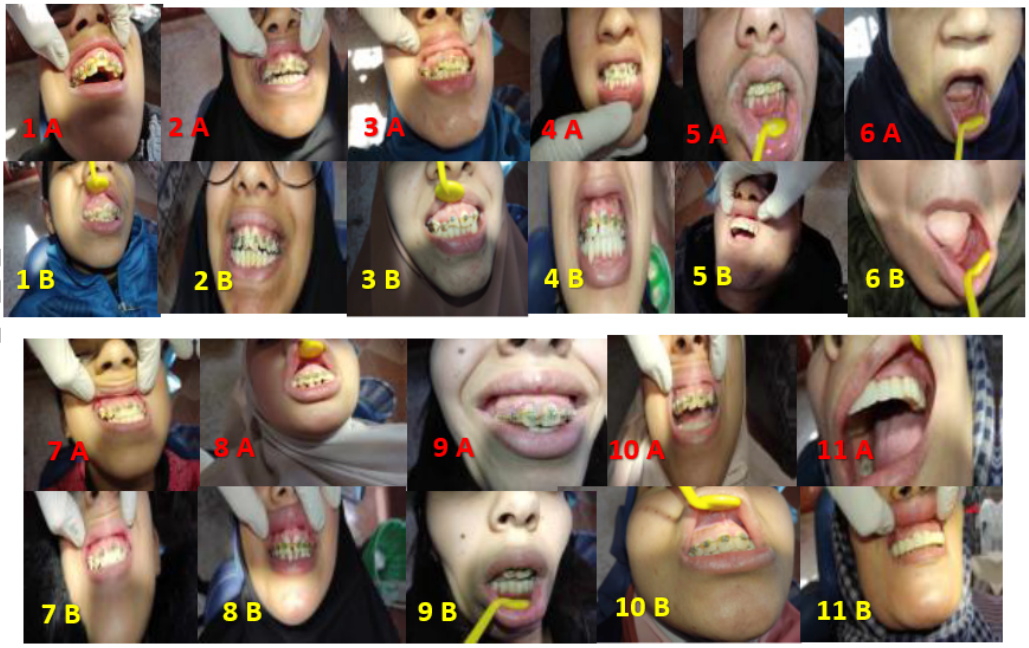

Fig. 3 Clinical signs before/after use of *Maerua Crassifolia* mouthwash *(B°: before using mouthwash. A°: after using the mouthwash)*

CONCLUSION

Oral health is a fundamental component of overall health and directly influences individuals and their lives. The incidence and severity of periodontal disease escalate with age and inadequate dental hygiene. The primary risk factor for these diseases is directly associated with their bacterial genesis.

This investigation demonstrated reasonably favorable outcomes, as the subsequent clinical findings of our patient indicate a distinct enhancement in clinical indications that can be listed:

- Change in gum color; the color of the gums has become a physiological reddish color.
- Regression of gingival swelling
- Reduction or complete disappearance of gingival bleeding
- Regression of the inflammatory reaction of the gums (gingivitis)
- Periodontitis is ruled out on the first day of the consultation, before the placement of the ODF appliance, thanks to radiological examinations
- No tooth mobility, which is a sign of destruction of the deep tissues of the periodontium
- During this follow-up period, I observed a positive effect on gum pain

Improve your mouthwash, particularly by incorporating additional herbs such as ginger, cloves, and mint leaves into our formulations. Mouthwash serves as an adjunct to tooth brushing, cleansing the entire oral cavity and aiding in the accessibility of difficult-to-reach regions of the mouth. Regardless of the significance of mouthwash quality, it cannot substitute for the mechanical action of a toothbrush, which remains the primary recommendation for optimal dental hygiene.

It has been observed that there is an absence of an adequate public health index that can furnish objective data regarding the distribution, prevalence, incidence, and treatment requirements of populations. The diversity of indexes complicates comparisons based on the available data.

Conflict of Interest

The authors declare no conflict of interest.

Human and Animal Rights

No animals were used in this research. All research procedures followed were in accordance with the ethical standards of the committee responsible for human experimentation (institutional and national), and with the Helsinki Declaration of 1975, as revised in 2013. (http://ethics.iit.edu/ecodes/node/3931)

Consent for Publication

Patients were briefed about the study and signed informed consent forms.

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