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Antimicrobial Activity Of *Azadirachta Indica* (Neem) Of Jequitinhonha Valley (Brazil) Against Oral Pathogen Microorganisms.

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Abstract
Background: *Azadirachta indica (Neem) is a versatile tree of the family Meliaceae and exhibits antimicrobial, antipyretic, and anti-inflammatory properties.*
Aims: *To verify the in vitro antimicrobial activity of extracts and product content in a Brazilian neem against oral pathogen microorganisms.*
Material and Methods: *Crude extracts and oils obtained from Brazilian neem macerated leaves and fruits were isolated or associated with bacteria and levures. All microorganisms tested were ATCC-standard, and all the antimicrobial tests were made according to the CLSI. Inoculum were prepared from overnight cultures of each microorganism containing $1.0x10^8$ for bacteria and $1,0x10^6$ for yeasts CFU/mL of the standard 0.5 McFarland scale. Blanc disks were soaked with 20µL of each product and placed on the surface of the agar containing the microorganisms. For viscous or creamy consistency products, 5,0mm diameter x 5,0mm height cavities were made in agar, which were filled with 20mg of each product. Cultures were left for 24 hours at 37°C in the environment, according to the microbial requirement, and the diameters of the inhibition zones were measured and the standard deviations were taken.*
Results: *All microorganisms were sensitive to the tested controls, chlorhexidine (bacteria) and nystatin (yeasts). The MIC varied between 0.6mg /mL and 0.27 mg/mL for yeasts and 0.54mg /mL and 0.13 mg/mL for bacteria. N. gonorrhoeae and L. casei showed sensitivity to 5% neem extract, and to mouthwash containing 5% neem.*
Conclusion: *Neem cultivated in Jequitinhonha Valley has biological activities similar to those observed for Neem cultured in India.*

Keywords: *Azadirachta indica (Neem), Antimicrobial tests, Brazilian Neem, Jequitinhonha Valley, Oral microorganisms.*

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I. Introduction

Azadirachta indica, popularly called Neem, is a tropical plant of Indian origin from the family Meliaceae, known for its pesticidal and medicinal properties [1]. Several characteristics of neem make it an interesting plant, among them: the fact that the tree is not destroyed for the production of the extracts since the active substances are found in the seeds, leaves, fruits, and barks; the difficulty of insects becoming resistant because it has a great diversity of active chemical substances in high concentration and soluble in water; easy extraction and low cost; the high toxicity of substances to pests; and their harmlessness to man and the environment, as they are biodegradable and of low permanence [2]. In addition, Neem has been used therapeutically as an anti-inflammatory and healing agent in the treatment of psoriasis, malaria, skin ulcers, mycoses, and other diseases and can be inserted in various sectors of the economy [3].

The main active substance in Neem is azadiractin (AZA), which was first isolated from Neem seeds by J. H. Butterworth and E. D. Morgan [4,5]. In addition to AZA, several other active substances have been isolated from Neem: AZA-like substances (AZA A), namely, azadiractin B, C, D, E, F, G, and HI, have already been

elucidated, and spectral data from nuclear magnetic resonance (NMR) and masses exist for most of them. Nimbolinin, nimbin, nimbidin, nimbidol, sodium nimbinate, gedunin, azadiractol, azadirone, villusin, meliacarpine, and quercetin. Leaves contain ingredients such as nimbin, nimbanene, 6-desacetylnimbinene, nimbandiol, nimbolide, ascorbic acid, n-hexacosanol, and amino acids. 7-desacetyl-7-benzoylazadiradione, 7-desacetyl-7-benzoylgedunin, 14-epoxiazadiradione, 17-hydroxyazadiradione, nimbiol, meliantrol, melianone, and gedunine are described by other authors, and quercetin and ß-sitosterol, polyphenolic flavonoids, were purified from neem fresh leaves and were known to have antibacterial and antifungal properties, and seeds hold valuable constituents, including gedunin and azadirachtin [6, 7, 8, 9, 10, 11].

In India and Pakistan, neem is traditionally used as a medicine for preventing malaria, curing leprosy, causing disturbances in the production of bile, as a tonic, relieving cough, asthma, urinary problems, deworming, contraceptives, healing, soothing, painkillers, and febrifuges. Some of the pharmacological activities have already been scientifically proven to be antifungal and antibacterial, antiviral and antimalarial [12, 13, 14], antifertility, antipyretic, anti-inflammatory, analgesic, anticulcerogenic, antihyperglycemic, immunostimulant, antioxidant, and anticancer [15, 16, 17]. Neem derivatives are also used in pharmaceutical and toilet products such as toothpaste, soap, and dermatological ointments to control scabies and other parasites [18].

The oral microbiome is characterized by the complexity of ecological niches filled by microorganisms with peculiar characteristics and defined roles in the microbiome in relation to oral diseases. Important bacteria, whether aerobic, microaerophilic, or anaerobic, are involved with periodontal diseases: *Prevotella intermedia*, *Porphyromonas gingivalis*, *Fusobacterium nucleatum*, and *Aggregatibacter actinomycetemcomitans*. Associated with Carie Disease: *Streptococcus mutans* and *Lactobacillus casei*; root canal infections: *Enterococcus faecalis.* Candida species are involved with lesions of the oral mucosa in immunodepressed patients and patients with prostheses (prosthetic stomatitis), patients on chronic use of medications, and radiotherapy in the head and neck region [19, 20]. More recently, *Candida* spp. has been associated with refractory periodontitis due to its location in the periodontal pockets: *Candida albicans*, *Candida krusei*, and *Candida tropicalis* [21, 22–23]. *Lactobacillus casei* is the anaerobic bacterium most involved in the progression of dentin caries [24, 25], while *Neisseria gonorrhoeae* is related to sexually transmitted disease and may be associated with asymptomatic pharyngitis, thus contributing to the transmission of gonorrhea through oral sex [26, 27].

Given the high resistance of bacteria and fungi to antibiotics and antifungals in hospital environments, it is necessary to investigate natural products, including plants, in order to rescue the importance of phytotherapy in the public health complex, mainly in countries with a population least favored [28].

Neem is an exotic plant in Brazil. There are few neem plantations in Brazil and few studies on the antimicrobial activity of the Brazilian neem against oral microorganisms. The aim of this study was to verify the antimicrobial activity of ethanolic and hydroethanolic extracts and products of oral use with *Azadirachta indica* content against oral pathogenic microorganisms.

II. Material and Methods

Plant material: origin and preparation of ethanolic and hydroethanolic extracts

Neem leaves and seeds were collected at Fazenda Pau d`Óleo, in the municipality of Itinga, Jequitinhonha Valley, Minas Gerais State, Brazil, a city located 260 meters above sea level with geographic coordinates: Latitude: 16 °37 '4' 'South; Longitude: 41°45 '54' 'West. The extracts were prepared according to Akeel et al. [20] and Tasanarong et al. [21]. The leaf collection and preparation of the crude and dry extract were carried out and donated by Bioneem® Ltda, a Brazilian company authorized by the Ministry of Agriculture of Brazil under number CNAES 21.10-6/00. To prepare the aqueous extracts, the leaves and seeds of neem, after drying in an oven with air circulation (at 40ºC for 48 hours), were crushed in a mill to obtain the vegetable products (Figure 1). These were added separately to the distilled water in the proportion of 20g/100 mL.The mixtures were kept in an environment without the presence of light, to avoid biodegradation, for 24 hours for the extraction of water-soluble compounds. After this period, the material was filtered, obtaining the extract at 20%, from which the desired concentrations were obtained by diluting with water. In addition to the aqueous extract, an organic ethanol extract was prepared. This extract was obtained by diluting 5g of vegetable powder in 100 mL of 96ºGL ethanol. The suspension was subjected to ultrasound extraction for 40 minutes. After that time, the suspension was filtered with the aid of a vacuum through a Buchner funnel lined with filter paper at the bottom for a kitasato; the solvent was evaporated in a vacuum evaporator route to obtain the pure extract. After weighing the material, it was again diluted in 96ºC ethanol and/or distilled water to obtain the desired concentrations of 5% and 10%. For all experiments, commercial chlorhexidine® digluconate and nystatin® were used as controls [29].

Extracts and products containing neem used in the study:

1- Ethanolic extract 5% Azadirachta indica (neem) – Bioneem®, Brazil.
2- Hydroethanolic Extract 5% Azadirachta indica (nem) – Bioneem®, Brazil).

3- Neem 5% toothpaste with mint - Bioneem®, Brazil – A.indica extract, mint, microcrystalline cellulose, sorbitol, sodium saccharin, sodium carmelose (high viscosity), glycerol, parabens preserving solution, purified water qsp, colloidal silicon dioxide (200 mesh), calcium carbonate (50 mesh), compound flavor, lauryl sulfate sodium.
4-Neem toothpaste 5% without mint- Bioneem®, Brazil – A. indica extract, microcrystalline cellulose, sorbitol, sodium saccharin, carmelose sodium (high viscosity), glycerol, preservative solution of parabenzen, purified water qsp, colloidal silicon dioxide (200 mesh), calcium carbonate (50 mesh), compound flavor, lauryl sulfate sodium.
5- Himalaya® Ethanolic extract Indian Neem 5% - Organic Neem Supercritical CO2 extract leaf Azadirachta indica, Himalaya Drug Company, India
6- Neem mouthwash 5% (Bioneem®, Brasil) – neem extract 5%, ethanol, tween 20, glycerin, aspartame, flavoring, food coloring, deionized water

Figure no 1- Neem leaves and dry extract used in experiments.

Microorganisms

The microorganism samples were provided by the National Institute for Quality Control in Health of the Oswaldo Cruz Foundation (INCQS / Fiocruz), Rio de Janeiro, Brazil. Standard samples of the American Type Culture Collection (ATCC) were selected oral pathogenic microorganisms: *Prevotella intermedia* (ATCC 25611), *Aggregatibacter actinomycetemcomitans* (ATCC 33384), *Porphyromonas gingivalis* (ATCC 33277), *Fusobacterium nucleatum* (ATCC 23726), *Enterococcus faecalis* (ATCC 29212), *Streptococcus mutans* (ATCC 25175), *Neisseria gonorrhoeae* (ATCC 9826), *Lactobacillus casei* (ATCC393). *Candida* sp samples were isolated and kindly provided by Prof. Dr Carlos Augusto Rosa, from the Yeast Laboratory of the Biological Sciences Institute of the Federal University of Minas Gerais (LL / ICB / UFMG): *Candida albicans* (EIA 3M.5), *Candida krusei* (EIA 3M.6) *Candida tropicalis* (HIC 3M.8).

Inoculum preparation

The bacterial inoculum was prepared in Brain Heart Infusion - BHI broth (Difco, USA) and the inoculum of *Candida* spp was prepared in Sabouraud Dextrose broth (Difco, USA). Afterwards, the microorganisms were incubated at 37ºC, for 24 hours. The inoculum were prepared according to the 0.5 turbidity of the McFarland standard, which corresponded to $1.0x10^8$ CFU (Colony Forming Units) / mL for bacteria and $1,0x10^6$ UFC/mL for yeasts [30].

Microbiology tests

Microbiological tests were developed according to the standards of the Clinical Laboratory Standard Institute (CLSI) [30]. The pure extracts and industrialized products of commercial brands were tested for antimicrobial properties, and for this, the agar diffusion test was used.

Microorganism-lyophilized samples were kept in a freezer at -20ºC until the moment use. The bacteria were grown in BHI broth and agar (Difco, USA) and left in a microaerophilic environment (5% CO_2) or in anaerobiosis at 37ºC. *Candida albicans*, *Candida tropicalis*, and *Candida krusei* were grown in Sabouraud Dextrose broth and agar (Difco, USA) and left in an aerobic environment at 37ºC. After 18–24 hours, the growth was verified, the inoculum was standardized to 0.5 on the McFarland scale (1.0×10^6 CFU/mL), and 200µL of each microorganism suspension (approximately 1.0×10^6 CFU/mL for yeasts or $1,0 \times 10^8$ CFU/mL for bacteria) was sown in Petri dishes. The neem-content gel was added to cavities measuring 5mm in diameter made on the agar with the aid of a punch. Each well was filled with 20mg of gel. Neem ethanol and hydroalcoholic extracts, 0.12% chlorhexidine digluconate solution (C9394 20% in H_2O - Sigma-Aldrich, USA), Nystatin water-soluble 100 mg/mL (N6803 Sigma-Aldrich, USA), and distilled water were added 20µL to sterile discs (Laborclin, Brazil).

For *N. gonorrhoeae*, antibiogram discs (CECON, São Paulo, Brazil) with Tetracillin 30 mg and Azithromycin 15 mg on blood agar (Difco, USA) were used. The plates containing microorganisms were incubated at 37ºC in aerobiosis, with 5% CO_2, or anaerobiosis, according to the requirements of each microorganism. The inhibition zones (diameters) formed around the products containing Neem and the controls were measured by halometer after 24, 36, and 48 hours, and their means and standard deviations were taken. The zones on the discs less than 8,0mm were considered negative results. The experiments were carried out in triplicate at different times for the diameter measurements around the disks. The results were evaluated through the mean (M) and standard deviation (SD) (M±SD) of three experiments.

Minimal Inhibitory Concentration (MIC) Determination

Minimum inhibitory concentration (MIC) is defined as the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation, and minimum bactericidal concentrations (MBCs) as the lowest concentration of antimicrobial that will prevent the growth of an organism after subculture on to antibiotic-free media and the microdilution plate test was chosen to determine the MIC [30].

III. Result

All microorganisms tested in this study were sensitive to samples of neem collected from Vale do Jequitinhonha (Bioneem®), isolated in extract, or associated with toothpaste or mouthwash. **Table 1** shows that both Gram-positive and Gram-negative bacteria showed high sensitivity to Bioneem and Himalaya extracts and toothpastes when compared to chlorhexidine inhibition zones. With the exception of *F. nucleatum*, which was more sensitive to the hydroethanolic extract, *P. gingivalis, P. intermedia*, and *A. actinomycetemcomitans* were more sensitive, while S. *mutans* was the least sensitive to the ethanolic extracts. No differences were observed between the zones of inhibition against bacteria when comparing neem toothpastes with and without mint in the formulation. However, the zones of inhibition of toothpastes showed intermediate sensitivity when compared to those observed for chlorhexidine digluconate and ethanolic and hydroethanolic extracts. The toothpastes showed an ideal inhibition performance for the control of the pathogenic microbiota available in the oral microbiome. For the daily control of dental biofilm, it is not expected to treat an infection but to control the growth of microorganisms. In **Table 2**, the inhibition zones show that *E. faecalis* exhibited the same pattern of neem sensitivity as seen for the bacteria in Table 1. *Candida* sp. species tested in the study were sensitive to neem. On the other hand, the sensitivity profile presented by zones of inhibition showed similarities between *C. albicans*, *C. krusei*, *C.tropicalis*, and Nystatin. **Table 3** displays the observed results for mouthwash containing 5% neem (Bioneem® Ltd.) and the ethanolic extracts Bioneem® and Himalaya® against *N. gonorrhoeae* and *L. casei*. Both bacteria were sensitive to all the products. *L. casei* showed similar sensitivity to the other bacteria tested in the study, including chlorhexidine, while *N. gonorrhoeae* was sensitive to tetracycline and azithromycin controls, two antibiotics used in the clinical treatment of gonorrhea. **Figure 2** show the inhibition zones of tested products against *N. gonorrhoeae*. The MIC varied between 0.6mg /mL and 0.27 mg/mL for yeasts and 0.54mg /mL and 0.13 mg/mL for bacteria. *N. gonorrhoeae* showed sensitivity to 5% neem extract, and *L. casei* was sensitive to mouthwash containing 5% neem.

Table no 1: Antimicrobial **s**usceptibility test of oral pathogenic bactéria: **crude** neem extracts and product containing neem in their formulations. Means and Standard Deviations (M ± SD) = mm of three repetitions for each microorganism.

Products	Microorganisms				
	Inhibition Zones (M) ± Standard Deviation (SD) (M±SD =mm)				
	P. intermedia	*P. gingivalis*	*A.a*	*S. mutans*	*F. nucleatum*
1	35.00±0.00	35.00±0.00	33.33±0.89	22.30±0.62	32.30±0.52
2	23.00±0.73	23.30±0.89	22.30±0.53	21.70±0.77	30.00±0.00
3	12.00±0.00	17.30±0.58	14.00±0.73	15.70±0.15	12.70±0.58
4	13.30±0.53	17.70±0.04	14.53±0.58	14.70±0.58	12.00±0.00
5	21.33±1.73	20.00±0.00	21.30±2.31	16.30±1.23	21.70±0.58
CLO	23.00±0.30	21.30±0.04	22.00±0.46	24.70±0.62	24.00±0.00
ETN	00.00±0.00	00.00±0.00	00.00±0.00	00.00±0.00	00.00±0.00

Legend: 1- Neem 5% ethanol extract(Bioneem®)/ 2- Neem 5% Hydroalcoholic extract (Bioneem)/ 3- Neem 5% Toothpaste with mint (Bioneem)/ 4- Neem 5% Toothpaste without mint (Bioneem)/ 5- Himalaya® Indian Neem extract/ CLO-Chlorhexidin digluconate 0.12% (Sigma, USA)/ ETN- Ethanol 70%.

Table no 2: Susceptibility test of *E. faecalis* and *Candida spp* to neem extracts and toothpastes containing neem. Means and Standard Deviations (M ± SD) = mm of three repetitions.

Products	Microorganisms			
	Inhibition Zones (M) ± Standard Deviation (SD) (M±SD =mm)			
	E. faecalis	*C. albicans*	*C. krusei*	*C. tropicalis*
1	30.30±0.62	18.70±0.06	18.30±0.31	11.30±0.04
2	20.00±0.00	14.70±0.89	15.70±0.16	17.00±0.65
3	14.30±0.58	20.00±0.50	16.30±0.53	17.00±0.73
4	14.00±0.00	16.30±0.58	16.30±0.15	15.70±0.58
5	20.70±1.15	15.30±1.59	14.00±0.50	16.70±1.31
CLO	24.00±0.73	-	-	-
NYS	-	19.70±0.08	20.00±0.00	22.00±0.58
ETN	00.00±0.00	00.00±0.00	00.00±0.00	00.00±0.00

Legend:1- Neem 5% ethanol extract(Bioneem®)/ 2- Neem 5% Hydroalcoholic extract (Bioneem)/ 3- Neem 5% Toothpaste with mint (Bioneem)/ 4- Neem 5% Toothpaste without mint (Bioneem)/ 5- Himalaya® Indian Neem extract/ CLO-Chlorhexidin digluconate 0.12% (Sigma, USA)/ NYS– Nystatin water soluble(Sigma-Aldrich)/ ETN- Ethanol 70%.

Table no 3: Antimicrobial activity of mouthwash and ethanolic extract containing 5% neem against *Neisseria gonorrhoeae* and *Lactobacillus casei*. Means and standard deviations (M±SD) of three experiments.

Products	Microorganisms	
	Inhibition zones (M±SD) = mm	
	N. gonorrhoeae	*L. casei*
Neem 5% mouthwash Bioneem®	16.36±0.22	15.33±0.73
Neem 5% ethanol extract	17.66±0.56	16.39±0.67
Neem Himalaya extract®	17.93±1.33	17.00±0.32
Chlorhexidine 0,12%	-	18.60±0.58
Tetraciclyn	23.00±0.00	-
Azithromycin	18.66±0.53	-
Ethanol 96ºGL70%	00.00±0.00	00.00±0.00

Figure no 2: Antimicrobial susceptibility test of extracts and mouthwash of Neem against *N. gonorrhoeae*.

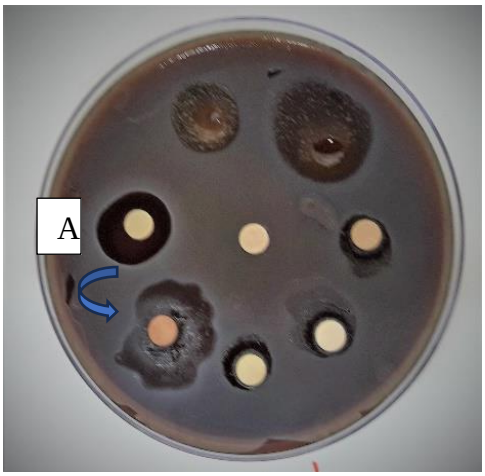

Figure legend: Following the arrow's direction: A: Tetraciclyn, B: Azythromycim, C: Neem 5% ethanol extract, D: Neem 5% mouthwash, E: Neem Himalaya extract, F/G: Neem 5% gel, H(in the centre):Ethanol 96oGL70%.

IV. Discussion

The antimicrobial activities of Brazilian and Indian neem were studied. The Neem plant material used in this work originates from the Jequitinhonha Valley, which extends from the center of Minas Gerais State, where the Jequitinhonha River originates, in the municipality of Serro, with its mouth in the municipality of Belmonte, in the southern region of the State of Bahia. The region comprises 55 municipalities, being one of the 12 mesoregions of Minas Gerais State [31]. The northeast region of the state of Minas Gerais, where the municipality of Itinga is located, is a dry and semi-arid region, hot and dry for most of the year. This region has a bioclimate in which neem seems well adapted, as observed mainly by the good development of neem [32, 33]. Plant species can have their chemical composition altered depending on the type of terrain, sunlight, and seasons [1, 2]. Considering these characteristics, it can be estimated that *A. indica* imported from India adapted very well in the Itinga region of Brazil, favoring and maintaining its arboreal and chemical characteristics similar to those of native Indians.

Ethanolic and hydroethanolic extracts from Bioneem® and Himalaya® showed similar effectiveness in inhibiting the growth of both bacteria and pathogenic yeasts in the oral microbiome. Ethanol extracts provide better extraction of azaradiractin (AZA), the main active component of neem, with better stability than aqueous extracts [34]. Microbiological methods for determining the antimicrobial activity of neem have also been described by several authors [35,36, 37]. Neem seed and leaf extracts inhibited ***in vitro*** the growth of and *Escherichia coli* and, on the other hand, did not inhibit *Bacillus subtilis*, *Salmonella paratyphi*, *Salmonella desynteriae*, or *C. albicans* [38]. Neem leaf extracts were effective against fungal pathogens, inhibiting the protease and growth of *Trichophyton* sp.[39], in addition to antifungal activity against *Penicillium expansum* [40]. Neem seed oil has also shown broad-spectrum antibacterial activity against Gram-positive and Gram-negative bacteria, including strains resistant to streptomycin, and also against *Mycobacterium tuberculosis* [41]. There are reports that neem oil and leaves also inhibited *Vibrio cholerae*, *Klebisiella pneumoniae*, *M. tuberculosis*, and *Micrococcus pyogenes in vitro* [42]. The bacteria tested by different authors are important pathogens; however, some are not related to caries, periodontitis, or purulent infections of the mouth, which is a distinguishing feature of this work. On the other hand, the results obtained by Alves [43] were similar, with the neem extracts prepared with 70% and 80% (V/V) ethanol showing activity against *Staphylococcus aureus*.

All microorganisms tested in this work showed sensitivity to Brazilian neem from the Jequitinhonha Valley, Brazil, and It is the first work that presents a microbiological test using the Bioneem® sample against oral microorganisms. Maintaining biological properties is important when active ingredients are associated with formulations such as toothpastes, rinses, or gels because the chemical composition may alter the activity of the plant, either increasing or decreasing biological properties. In this sense, the antimicrobial activities remained unchanged when comparing the isolated extracts with those associated with pharmaceutical forms. *Candida* sp. samples were isolated and kindly provided by Prof. Dr. Carlos Augusto Rosa from the Yeast Laboratory of the Biological Sciences Institute of the Federal University of Minas Gerais (LL/ICB / UFMG): *C. albicans* (EIA 3M.5) and *C. krusei (*EIA 3M.6), which represent non-Albicans, are less prevalent in oral mucosa and, according to Rossoni et al. [44], are able to inhibit the growth of *C. albicans* in an interaction relationship; *C. tropicalis* (HIC 3M.8), considered the most prevalent among the pathogenic yeasts of the non-Albicans group, shows a drastic increase in the number of infections due to its presence and great resistance to drugs like fluconazole [45]. The differences in the sensitivities of microorganisms observed between the ethanolic and hydroethanolic extracts were already expected, since most of the chemical components of medicinal plants are more soluble in ethanol, which facilitates their availability and diffusion in the agar. It is known that the electrostatic charges present in the culture medium and in extracts and/or pharmaceutical formulations are important for the greater or lesser inhibition of microbial growth [46].

Toothpastes showed smaller zones of inhibition for both Gram-positive and Gram-negative bacteria and yeasts. This finding may be related to the viscosity of the product, which makes its diffusion difficult. Neem has been studied in Brazil regarding its biological properties against insects and insect larvae, as a pesticide, and regarding cultivation and economic response [47], which makes this work different by studying the properties of antimicrobials against pathogenic microorganisms of the oral microbiome related to caries, periodontal, endodontic diseases, and mucosal changes [48]. *N.gonorrhoeae* was sensitive to extracts and mouthwash containing neem. Gonorrhea is a common and asymptomatic sexually transmitted disease (STD) in the pharynx, which causes the infection to spread through oral sex [49, 50]. This gram-negative bacterium has been shown to be highly resistant to antibiotics [51, 52]. Mouthwash containing neem can be used before and after oral sex in the population at risk to prevent oral infection. The microorganism is unlikely to become resistant due to the complex chemical composition of the plant. However, clinical studies must be conducted to confirm the veracity of the *in vitro* study.

The crude Neem extract 5% demonstrated similar inhibitory activity against the bacterial strains and *Candida* spp and the toothpastes inhibited all microorganisms tested. The mint presence in formulation does not

seem to have influenced the antimicrobial properties. According to the manufacturer's information, mint was added to improve the product's flavor.

Chlorhexidine digluconate 0.12% is considered the gold standard for controlling microorganisms in dentistry, with the vast majority of species of microorganisms in the mouth being sensitive to it. In this study, CLX showed the same pattern of inhibition observed by other authors [53].

In vivo studies must be carried out to confirm these results.

V. Conclusions

Azadirachta indica (neem) cultivated in the Jequitinhonha Valley, Brazil, showed similar or higher antibacterial and antifungal activities than positive control. The interaction of Jequitinhonha Valley Neem with other pharmaceutical components did not alter its antimicrobial activities. All microorganisms tested were sensitive to Neem. Clinical trials studies must be carried out to prove the pharmacological effectiveness of the products tested in these experiments.

REFERENCES

[1]. Sujarwo W, Keim AP, Caneva G, Toniolo C, Nicoletti M. Ethnobotanical Uses Of Neem (*Azadirachta indica* A.Juss.; Meliaceae) Leaves in Bali (Indonesia) and the Indian Subcontinent in Relation With Historical Background And Phytochemical Properties. J Ethnopharmacol. 2016; 189:186-93. Doi: 10.1016/J.Jep.2016.05.014.

[2]. Gupta Sc, Prasad S, Tyagi Ak, Kunnumakkara Ab, Aggarwal Bb. Neem (Azadirachta Indica): An Indian Traditional Panacea With Modern Molecular Basis. Phytomedicine. 2017, 15;34:14-20. Doi: 10.1016/J.Phymed.2017.07.001

[3]. Isah A.B.; Ibrahim Y.K.; Iwalewa E.O. Evaluation Of The Antimalarial Properties And Standardization Of Tablets Of Azadirachta Indica (Meliaceae) In Mice. Phytother Res. 2003; 17(7):807-810.

[4]. Polaquini Sr, Svidzinski Ti, Kemmelmeier C, Gasparetto A. Effect Of Aqueous Extract From Neem (Azadirachta Indica A. Juss) On Hydrophobicity, Biofilm Formation And Adhesion In Composite Resin By Candida Albicans. Arch Oral Biol. 2006; 51(6):482-90. Doi: 10.1016/J.Archoralbio.2005.11.007.

[5]. Wylie Mr, Merrell Ds. The Antimicrobial Potential Of The Neem Tree Azadirachta Indica. Front Pharmacol. 2022; 13:891535. Doi: 10.3389/Fphar.2022.891535.

[6]. Halim E.M. Lowering Of Blood Sugar By Water Extract Of Azadirachta Indica And Abroma Augusta In Diabetes Rats. Indian J. Experimental Biol. 2003; 41(6):636-640.

[7]. Raji Y., Et Al. Effects Of Azadirachta Indica Extract On Gastric Ulceration And Acid Secretion In Rats.J. Ethnopharmacol. 2004; 90(1):167-170.

[8]. Altayb Hn, Yassin Nf, Hosawi S, Kazmi I. In-Vitro And In-Silico Antibacterial Activity Of Azadirachta Indica (Neem), Methanolic Extract, And Identification Of Beta.D-Mannofuranoside As A Promising Antibacterial Agent. Bmc Plant Biol. 2022; 22(1):262. Doi: 10.1186/S12870-022-03650-5.

[9]. Joy Sinha D, D S Nandha K, Jaiswal N, Vasudeva A, Prabha Tyagi S, Pratap Singh U. Antibacterial Effect Of Azadirachta Indica (Neem) Or Curcuma Longa (Turmeric) Against *Enterococcus faecalis* Compared With That Of 5% Sodium Hypochlorite Or 2% Chlorhexidine In Vitro. Bull Tokyo Dent Coll. 2017; 58(2):103-109. Doi: 10.2209/Tdcpublication.2015-0029.

[10]. Pereira Em, Gomes Rt, Freire Nr, Aguiar Eg, Brandão Md, Santos Vr. In Vitro Antimicrobial Activity Of Brazilian Medicinal Plant Extracts Against Pathogenic Microorganisms Of Interest To Dentistry. Planta Med. 2011; 77(4):401-4. Doi: 10.1055/S-0030-1250354.

[11]. De Luca Mp, Franca Jr, Macedo Fa, Grenho L, Cortes Me, Faraco Aa, Moreira An, Santos Vr. Propolis Varnish: Antimicrobial Properties Against Cariogenic Bacteria, Cytotoxicity, And Sustained-Release Profile. Biomed Res Int. 2014; 2014:348647. Doi: 10.1155/2014/348647.

[12]. Kumar J, Sharma R, Sharma M, Prabhavathi V, Paul J, Chowdary Cd. Presence Of Candida Albicans In Root Canals Of Teeth With Apical Periodontitis And Evaluation Of Their Possible Role In Failure Of Endodontic Treatment. J Int Oral Health. 2015; 7(2):42-5.

[13]. Slazhneva E, Tikhomirova E, Tsarev V, Orekhova L, Loboda E, Atrushkevich V. Candida Species Detection In Patients With Chronic Periodontitis: A Systematic Review And Meta-Analysis. Clin Exp Dent Res. 2022; 8(6):1354-1375. Doi: 10.1002/Cre2.635.

[14]. Mergoni G, Percudani D, Lodi G, Bertani P, Manfredi M. Prevalence Of Candida Species In Endodontic Infections: Systematic Review And Meta-Analysis. J Endod. 2018; 44(11):1616-1625.E9. Doi: 10.1016/J.Joen.2018.07.016.

[15]. Byun R, Nadkarni Ma, Chhour Kl, Martin Fe, Jacques Na, Hunter N. Quantitative Analyses Of Diverse Lactobacillus Species Present In Advanced Dental Caries. J. Clin. Microbiol. 2004, 42(7):3128-36. Doi: 10.1128/Jcm.42.7.3128-3136.2004.

[16]. Conrads G, About I. Pathophysiology Of Dental Caries. Monog Oral Sci 2018, 27:1-10. Doi: 10.1159/000487826.

[17]. Moncada J, Shayevich C, Philip Ss, Lucic D, Schachter J. Detection Of Chlamydia Trachomatis And Neisseria Gonorrhoeae In Rectal And Oropharyngeal Swabs And Urine Specimens From Men Who Have Sex With Men With Abbott's M2000 Real Time Sex Transm Dis. 2015; 42(11):650-1. Doi: 10.1097/Olq.0000000000000360.

[18]. Van Liere Gafs , Nicole H T M Dukers-Muijrers, Sophie Kuizenga-Wessel, Petra F G Wolffs, Christian J P A Hoebe, Routine Universal Testing Versus Selective Or Incidental Testing For Oropharyngeal Neisseria Gonorrhoeae In Women In The Netherlands: A Retrospective Cohort Study, The Lancet Infectious Diseases, Volume 21, Issue 6, 2021, Pages 858-867, Issn 1473-3099, Https://Doi.Org/10.1016/S1473-3099(20)30594-6.

[19]. Singh, A.; Sharma, J.; Paichha, M.; Chakrabarti, R. Achyranthes Aspera (Prickly Chaff Flower) Leaves- And Seeds-Supplemented Diets Regulate Growth, Innate Immunity, And Oxidative Stress In Aeromonas Hydrophila-Challenged Labeo Rohita. J. Appl. Aquacult. 2020; 32 (3): 250-267.

[20]. Akeel Ra, Ayesha Mateen, K. Janardhan, V.C. Gupta. Analysis Of Anti-Bacterial And Anti Oxidative Activity Of Azadirachta Indica Bark Using Various Solvents Extracts. Saudi J Biological Sciences, 24(1):11-14, 2017. Https://Doi.Org/10.1016/J.Sjbs.2015.08.006.

[21]. Tasanarong T, Patntirapong S, Aupaphong V. The Inhibitory Effect Of A Novel Neem Paste Against Cariogenic Bacteria. J Clin Exp Dent. 2021; 13(11):E1083-E1088. Doi: 10.4317/Jced.58781.

[22]. Saigali Ma, Tangutur Ad, Banoth C, Bhukya B. Antimicrobial And Anticancer Potential Of Low Molecular Weight Polypeptides Extracted And Characterized From Leaves Of Azadirachta Indica. Intern J Biol Macromol. 2018; 114:906-921. Https://Doi.Org/10.1016/J.Ijbiomac.2018.03.169.

[23]. Veitch G.E. et al. Synthesis Of Azadirachtin: A Long But Successful Journey. Angew Chem Int Ed Engl. 2007; 46(40):7629- 7632.

[24]. Sengupta P, Desai Pd, Maity I, Mazumdar P, Biswas S, Choudhury Sr, Majumdar Tk. Comparative Evaluation Of Different Probiotic Products On Salivary Streptococcus Mutans And Lactobacillus Level In Caries Risk Population. J Conserv Dent. 2020; 23(6):619-623. Doi: 10.4103/Jcd.Jcd_467_20.

[25]. Gönczi Nn, Strang O, Bagi Z, Rákhely G, Kovács Kl. Interactions Between Probiotic And Oral Pathogenic Strains. Biol Futur. 2021 Dec;72(4):461-471. Doi: 10.1007/S42977-021-00091-3.

[26]. Hook Ew 3rd, Bernstein K. Kissing, Saliva Exchange, And Transmission Of Neisseria Gonorrhoeae. Lancet Infect Dis. 2019; 19(10):E367-E369. Doi: 10.1016/S1473-3099(19)30306-8.

[27]. Xu X, Chow Epf, Ong Jj, Shen M, Wang C, Hocking Js, Fairley Ck, Zhang L. Modelling The Potential Role Of Saliva Use During Masturbation In The Transmission Of Neisseria Gonorrhoeae At Multiple Anatomical Sites. Sex Health. 2022; 18(6):466-474. Doi: 10.1071/Sh21138.

[28]. Santos VR, Pereira EMR. Antifungal Activity Of Brazilian Medicinal Plants Against Candida Species [Internet]. Candida Albicans. Intechopen; 2019. Http://Dx.Doi.Org/10.5772/Intechopen.80076.

[29]. Chinsembu Kc. Plants And Other Natural Products Used In The Management Of Oral Infections And Improvement Of Oral Health. Acta Trop. 2016; 154:6-18. Doi: 10.1016/J.Actatropica.2015.10.019.

[30]. Clinical Laboratory Standards Institute, Performance Standard For Antimicrobial Disk Susceptibility Tests, Weinstein Mp, M02ed13e, Isbn Number 1- 56238-835-5, 13th Edition,92p., 2018.

[31]. Jequitinhonha Valley Mesorregion. https://www.Mg.Gov.Br/Pagina/Geografia#:~:Text=As%2012%20mesorregi%C3%B5es%20estabelecidas%20pelo,Das%20vertentes%20e%20zona%20da. Mg.Gv.Br. Accessed 29/11/2023. (Portuguese)

[32]. Schaaf O. Et Al. Rapid And Sensitive Analysis Of Azadirachtin And Related Triterpenoids From Neem (Azadirachta Indica) By High-Performance Liquid Chromatography-Atmospheric Pressure Chemical Ionization Mass Spectrometry. J. Chromatog. A. 2000; 886(1-2):89-97.

[33]. Dai J. Et Al. Extraction And Colorimetric Determination Of Azadirachtin-Related Limonoids In Neem Seed Kernel. J. Agricult. Food Chem.1999; 47(9):3738-3742.

[34]. Sharma V. Et Al. An Efficient Method For The Purification And Characterization Of Nematicidal Azadirachtins A, B, And H, Using Mplc And Esims. J Agric Food Chem. 2003; 51(14):3966-3972.

[35]. Alzohairy Ma. Therapeutics Role Of Azadirachta Indica (Neem) And Their Active Constituents In Diseases Prevention And Treatment. Evid Based Complement Alternat Med. 2016; 2016:7382506. Doi: 10.1155/2016/7382506.

[36]. Allamed A. Et Al. Effects Of Neem Leaf Extract On Production Of Aflatoxins And Activities Of Fatty Acid Synthetase, Isocitrate Dehydrogenase And Glutathione S-Transferase In Aspergillus Parasiticus. Mycopathologia. 2001; 154(2):79-84.

[37]. Subapriya R.; Nagini S. Medicinal Properties Of Neem Leaves: A Revew. Current Med. Chem. Anticancer Agents. 2005; 5(2):149-160.

[38]. Ahmad I.; Beg A.Z. J. Antimicrobial And Phytochemical Studies On 45 Indian Medicinal Plants Against Multi-Drug Resistant Human Pathogens. J. Ethnopharmacol. 2001; 74(2):113-123.

[39]. Iyer S.R.; Williamson D. Efficacy Of Some Plant Extracts To Inhibit The Protease Activity Of Trichophyton Species. Geobios. 1991; 18: 3-6.

[40]. Mossini Asg, Kemmelmeier Ca. The Neem Tree (Azadirachta Indica A. Juss): Multiple Uses. State University Of Maringá, Londrina, Paraná, 10p, 2004.

[41]. Chopra I.C.; Gupta K.C.; Nazir B.N. Preliminary Study Of Anti-Bacterial Substances From Melia Azadirachta. Indian J. Med. Res. 1952; 40:511-515.

[42]. Satyavati G.V.; Raina M.K.; Sharma M. Medicinal Plants Of India - Volume 1. Editors. Acorus. New Delhi: Indian Council Of Medical Research. 1976; 1:18–22.

[43]. Alves Pd, Brandao Mgl, Nunan Ea, Vianna-Soares Cd. Chromatographic Evaluation And Antimicrobial Activity Of Neem (Azadirachta Indica A. Juss., Meliaceae) Leaves Hydroalcoholic Extracts. Braz J. Pharmacogn. 2009; 19(2b):510-515.

[44]. Rossoni R.D. Et Al. Study Of Microbial Interaction Formed By Candida Krusei And Candida Glabrata: In Vitro And In Vivo Studies. Braz. Dent. J. 2017; 28(6):669-674.

[45]. Kothavade R.J. et al. Candida tropicalis: its prevalence, pathogenicity and increasing resistance to fluconazole. J Med Microbiol. 2010; 59(8):873-880.

[46]. Mahon C.R; Lehman D.C. Textbook of Diagnostic Microbiology, Seventh Edition, Chapter 13. Elsevier, St. Louis, USA. 2023; ISBN: 978-0-323-82997-7.

[47]. Oliveira A.C.M. et al. Use of Melaleuca alternifolia Cheel (Myrtaceae) oil in dentistry: perspectives regarding its use as an alternative antimicrobial for infectious diseases of oral origin. Braz J Med Plants, UNESP. 2011; 13(4):492-499.

[48]. Torrungruang K. et al. Porphyromonas gingivalis, Aggregatibacter actinomycetemcomitans, and Treponema denticola / Prevotella intermedia Co-Infection Are Associated with severe periodontitis in a Thai population. PLoS One. 2015; 10(8):1-13.

[49]. Unemo M, Golparian D, Eyre DW. Antimicrobial Resistance in Neisseria gonorrhoeae and Treatment of Gonorrhea. Methods Mol Biol. 2019;1997: 37-58. doi: 10.1007/978-1-4939-9496-0_3.

[50]. Hook EW 3rd, Bernstein K. Kissing, saliva exchange, and transmission of Neisseria gonorrhoeae. Lancet Infect Dis. 2019; 19(10):e367-e369. doi: 10.1016/S1473-3099(19)30306-8.

[51]. Lin EY, Adamson PC, Klausner JD. Epidemiology, Treatments, and Vaccine Development for Antimicrobial-Resistant Neisseria gonorrhoeae: Current Strategies and Future Directions. Drugs. 2021; 81(10):1153-1169. doi: 10.1007/s40265-021-01530-0.

[52]. Młynarczyk-Bonikowska B, Majewska A, Malejczyk M, Młynarczyk G, Majewski S. Multiresistant Neisseria gonorrhoeae: a new threat in second decade of the XXI century. Med Microbiol Immunol. 2020; 209(2):95-108. doi: 10.1007/s00430-019-00651-4. Erratum in: Med Microbiol Immunol. 2020; 209(5):641.

[53]. Paradella TC, Koga-Ito CY, Jorge AO. In vitro antibacterial activity of adhesive systems on Streptococcus mutans. J Adhes Dent. 2009; 11(2):95-9.