Evaluation of Antimicrobial Effects of Trona and Alum on Dental Caries Isolates

Onyekachukwu Izuchukwu Udemezue, Chinenye Laureata Alaebo, Evangeline Chinyere Udenweze and Uzoma Odinakachi Okoli

Department of Applied Microbiology and Brewing, Nnamdi Azikiwe University

DOI: https://doi.org/10.51584/IJRIAS.2023.8907

Received: 17 July 2023; Revised: 26 August 2023; Accepted: 04 September 2023; Published: 06 October 2023

ABSTRACT

Trona and alum are chemical substances found abundantly in nature. Dental caries, usually of bacterial origin, is a chronic disease in which microorganisms metabolize sugars from the diet and produce acid which can demineralize enamel, dentine, cementum and inorganic portion of tooth, followed by destruction of organic portions, leading to cavity formation. This research work was aimed at evaluating the antimicrobial effects of trona and alum on microorganisms isolated from dental caries. It was carried out at Microbiology laboratory unit of Nnamdi Azikiwe University, Awka, Nigeria. Eighty-five dental swab specimens were aseptically and properly collected from patients with carious tooth using sterile swab sticks. Microorganisms were isolated and identified using standard macroscopic, microscopic, biochemical and molecular testing methods. The isolates were Streptococcus mutans, Staphylococcus aureus, Bacillus cereus, Lactobacillus spp and Candida albicans. Antimicrobial sensitivity test of trona and alum on the isolates was carried out using the agar-well diffusion method. The results revealed that these isolates were susceptible to the natural compounds as zones of inhibition were observed at different concentrations. Alum showed the highest zone of inhibition of 25mm against Lactobacillus sp. at 200mg while trona showed inhibition of 17mm against Bacillus cereus at 200mg. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the compounds were also determined. The MIC and MBC of alum against the isolates ranges between 50mg/ml to 12.5mg/ml; while trona recorded 200mg/ml against all the isolates. The findings provide the scientific basis for use of alum and trona as antimicrobial agents; thus the need for their production at industrial level.

Keywords: Dental caries, Inhibition, Bactericidal, Antimicrobial, Isolates.

INTRODUCTION

The presence of nutrients, epithelial debris and secretions make the mouth a favorable habitat for a great variety of microbes. The tooth surfaces are unique in that they are the only body part not subject to metabolic turnover. Once formed, the teeth are, under the correct conditions, essentially indestructible, as witnessed by their importance in fossil records and forensic medicine. Yet in the living individual, the integrity of the teeth is assaulted by a microbial challenge so great that dental infections rank as the most universal affliction of humankind (Takahashi and Nyvad, 2011).

Dental caries, also known as tooth decay or cavity, is an infection, usually bacterial in origin that causes demineralization of the hard tissues (enamel, dentin, and cementum) and destruction of the organic matter of the tooth, usually by production of acid resulting from hydrolysis of the food debris accumulated on the tooth surface. There are four main criteria required for caries formation: a tooth surface (enamel or dentin) caries-causing bacteria, fermentable carbohydrates (such as sucrose) and time. If left untreated, the disease can lead to pain, tooth loss and infection. Today, caries remain one of the most common diseases throughout the world (Marya, 2011; Takahashi and Nyvad, 2011; Struzycka, 2014).

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume VII Issue IX September 2023

Alum can mean any of a group of chemical compounds, but usually refers to potassium alum, also known as aluminium potassium sulfate. Alum is both a specific chemical compound and a class of chemical compounds. The specific compound is the hydrated potassium aluminum sulfate (potassium alum) with the formula KAL $(SO_4)_2.12H_2O$. More widely, alums are double sulphate salts, with the formula AM $(SO_4)_2$ $.12H_2O$, where A is a monovalent cation such as potassium or ammonium, and M is a trivalent metal ion such as aluminum or chromium (III). Alum has demonstrated activity against oral bacteria (Marrack *et al.,* 2009; Kanlayavattanakul and Lourith, 2011).

Trona is an evaporite mineral, occasionally encountered as a saline lake deposit or evaporation product and as an efflorescence on arid soil. It is also known as 'Akanwu' in Igbo, 'kanwa' in Hausa and 'kaun' in Yoruba. Trona is the most abundant sodium alkali mineral; an important table salt and second most commonly used in Nigeria (Kutama *et al.,* 2013).

Due to the high prevalence of oral diseases as well as increased microbial resistance to antibiotics, there is need for alternative methods. Thus, the search for viable alternative products is of paramount importance. Abundant natural compounds like alum and trona could serve as better alternatives to the conventional antibiotics if their efficacy is established.

The aim of this study is to evaluate the antimicrobial effects of trona and alum on dental caries isolates.

MATERIALS AND METHODS

Study Area

The research was conducted at the General Microbiology Laboratory, Nnamdi Azikiwe University, Awka, Anambra state, Nigeria. It is located in the south-eastern part of Nigeria, with a latitude of 6° 12' 45.68'' N and longitude of 7° 04' 19.16''.

Specimen Collection

With the permission of the ethical committee of Chukwuemeka Odumegwu Ojukwu University Teaching Hospital, Awka, eighty five (85) dental swab specimens were aseptically and properly collected from patients with carious tooth. The specimens were inoculated, using the spread plate method, into plates of Sabouraud's dextrose agar media supplemented with chloramphenicol (50?g/ml), Blood agar and Nutrient agar media supplemented with nystatin. The media were incubated aerobically at 37℃ for 24 hours (Udemezue and Oyeka, 2021).

Natural Compounds

The natural compounds (trona and alum) were hygienically selected after purchase from the Eke-Awka market in Awka South Local Government Area of Anambra State, Nigeria. The samples were transferred into sterile containers and transported immediately to the laboratory for processing and analysis as described by Kamka-Evans *et al*. (2013).

Isolation of the microorganisms

The media preparation was done according to the manufacturer's instructions. The specimens were aseptically inoculated on Nutrient Agar (for bacteria), Blood agar (for fastidious bacteria) and Sabouraud's dextrose agar, SDA (for fungi) using spread plate method. The nutrient and blood agar plates were then incubated at 37℃ for 24 hours. The SDA plates were incubated at 28±1℃ for 3 days. Discrete colonies were selected and sub-cultured onto plates of Nutrient agar and SDA using streak plate method to obtain pure cultures. The pure cultures were stored (at 4℃) on Nutrient agar and SDA slants in Bijou bottles for

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume VII Issue IX September 2023

biochemical tests and identification (Cheesbrough, 2010).

Identification of the Isolates

The isolates were identified using standard methods which include; Colony morphological characteristics on nutrient media (Singleton, 1997), Gram staining (Willey *et al*., 2021), motility test (Smith and Selby, 2017), catalase test (Aryal, 2015), citrate utilization test (Cheesbrough, 2010), coagulase test (Varghese and Joy, 2014), hemolysis (Kato *et al.,* 2017), sugar fermentation and molecular identification tests (Udemezue and Oyeka, 2021). Germ tube test was also carried out on the fungal isolate ((Moya-Salazar and Rojasa, 2018).

In vitro Evaluation of the Antimicrobial Activity of Trona and Alum Preparation of Stock Solution

Stock solutions of the test agents, were prepared by weighing out 2g of each of test agent using electronic weighing balance and dissolving in 10ml of sterile water in test tubes to give a stock concentration of 200mg/ml. A double fold serial dilution of the stock solution was performed to obtain 100mg/ml, 50mg/ml, 25mg/ml and 12.5mg/ml concentrations (Udemezue and Oyeka, 2021).

Sensitivity test

The sensitivity test was conducted using agar-well diffusion method. Plates of Mueller Hinton Agar were aseptically prepared. Using 6mm cork borer, wells were bored through the already gelled agar media. McFarland standards (0.5) of the isolates were added to the surface of the plates and the inoculum evenly spread onto the plate surface using a sterile bent glass rod. The test solutions of the natural compounds (0.5ml) were then added into the wells using sterile hypodermic syringes and then incubated at 37°C for 24 hours. Antimicrobial activity was determined by measuring the inhibition zone diameter (in mm) (Cheesbrough, 2010).

Determination of MIC and MBC using broth dilution method

From the stock concentration of 400mg/ml of the test agents, various concentrations of the test agents were made in Nutrient broth by double fold serial dilution to obtain 200mg/ml, 100mg/ml, 50 mg/ml, 25 mg/ml, 12.25mg/ml, 6.325 mg/ml, 3.125mg/ml and 1.5625 mg/ml. Each dilution in a test-tube was inoculated with 0.5 ml of the broth culture of test isolates (0.5 McFarland standards). All the tubes were incubated at 37°C for 24 hours. The lowest concentration showing no visible growth was recorded as the minimum inhibitory concentration (MIC) for each organism (Gahlaut and Chhillar, 2013; Tripathi, 2013).

From each negative tube in MIC assay, 0.2ml was transferred onto the surface of freshly prepared nutrient agar plates using spread plate method and incubated at 37°C for 24 hours. The lowest concentration showing no visible growth was recorded as minimum bactericidal concentration (MBC) for each isolate (Gahlaut and Chhillar, 2013; Tripathi, 2013).

RESULTS

Table 1: Morphological characteristics of the isolates

S/N	Form	Colour	Elevation	Margin	Texture	Transparency	Isolates
1	Rhizoid	Creamy	Raised	Entire	Slimy	Opaque	Streptococcus mutans
2	Irregular	Creamy	Raised	Undulate	Slimy	Opaque	Staphylococcus aureus
3	Rhizoid	Creamy	Raised	Undulate	Slimy	Opaque	Bacillus cereus
4	Circular	Creamy	Raised	Entire	Slimy	Opaque	Lactobacillus spp
5	Circular	Creamy	Raised	Entire	Mucoid	Opaque	Candida albicans

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume VII Issue IX September 2023

Table 2: Microscopic and Biochemical test of the bacterial isolates

S/N	Gram Stain	Rod/Cocci	Motiliy test	Catalase test	Coagulase test	Citrate test	Hemolysis	Isolates
1	+	Cocci	–	–	–	+	Alpha	Streptococcus mutans
2	+	Cocci	–	+	+	+	Beta	Staphylococcus aureus
3	+	Rod	+	+	–	+	Beta	Bacillus cereus
4	+	Rod	–	+	–	–	Beta	Lactobacillus spp

Table 3: Sugar fermentation test

S/N	Glucose	Fructose	Sucrose	Dextrose	Mannitol	Isolates
1	+	+	+	+	+	Streptococcus mutans
2	+	+	+	+	+	Staphylococcus aureus
3	+	+	–	+	–	Bacillus cereus
4	+	+	–	+	–	Lactobacillus spp
5	+	+	–	+	–	Candida albicans

C. albcans was germ tube positive.

Table 4: Inhibition zone diameter of Trona and Alum on *Streptococcus mutans* using agar-well diffusion method

Concentration(mg/ml)	Alum (mm)	Trona (mm)
12.5	6.67±0.47	–
25	11.83±0.62	–
50	12.17±0.62	–
100	15.17±0.62	–
200	19.00±0.82	–

Table 5: Inhibition zone diameter of Trona and Alum on Staphylococcus aureus using agar-well diffusion method

Concentration(mg/ml)	Alum (mm)	Trona (mm)
12.5	6.67±0.47	–
25	11.83±0.62	–
50	14.17±0.62	–
100	18.00±0.82	–
200	21.00±0.82	–

Table 6: Inhibition zone diameter of Trona and Alum on Bacillus cereus using agar-well diffusion method

Concentration(mg/ml)	Alum (mm)	Trona (mm)
12.5	6.00±0.82	–
25	10.50±0.41	–

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume VII Issue IX September 2023

50	12.17±0.62	–
100	19.50±0.41	9.00±0.82
200	23.50±0.41	17.17±0.62

Table 7: Inhibition zone diameter of Trona and Alum on Lactobacillus spp using agar-well diffusion method.

Concentration(mg/ml)	Alum (mm)	Trona (mm)
12.5	5.33±0.47	–
25	10.17±0.62	–
50	12.50±0.41	–
100	16.50±0.41	–
200	24.67±0.47	–

Table 8: Inhibition zone diameter of Trona and Alum on Candida albicans using agar-well diffusion method.

Concentration(mg/ml)	Alum (mm)	Trona (mm)
12.5	6.33±0.47	–
25	8.67±0.47	–
50	11.17±0.62	–
100	17.17±0.62	–
200	19.17±0.62	–

Table 9: MIC Determination of Trona and Alum against the isolates using broth dilution method (mg/ml)

Natural Compounds	Streptococcus mutans	Staphylococcus aureus	Bacillus cereus	Lactobacillus spp	Candida albicans
Alum	25	25	25	12.5	200
Trona	200	200	200	200	200

Table 10: MBC Determination of Trona and Alum against the isolates (mg/ml)

Natural Compounds	Streptococcus mutans	Staphylococcus aureus	Bacillus cereus	Lactobacillus spp	Candida albicans
Alum	50	50	25	25	200
Trona	200	200	200	200	200

DISCUSSION

Dental caries is one of the most prevalent diseases globally. This research was carried out to evaluate the antimicrobial effects of trona and alum on dental caries isolates. The study revealed the presence of Streptococcus mutans, Staphylococcus aureus, Bacillus cereus, Lactobacillus spp and Candida albicans, through their morphological, microscopic, biochemical and molecular characteristics. Staphylococcus aureus and Candida albicans which are normal body flora (though opportunistic pathogens) were isolated from the dental swab specimens. Also, it is important to note that the specie of Streptococcus observed was

oral Streptococci exhibiting beta hemolysis. The bacteria isolates are in agreement with the research work by Wilkins, (2009).

Sensitivity test was carried out on the isolates in triplicates using agar-well diffusion method and the mean deviation was determined. Zones of inhibition were observed thus affirming the use of these natural compounds as a strong basis for the development of novel drugs. There was a notable increase in susceptibility at increasing concentrations. Alum showed high level of activity against the isolates; while trona showed low level of activity. Thus, the use of alum as an alternative to the conventional antibiotics is a step in the right direction. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the natural compounds against the isolates was also determined and the results closely coincide with Udemezue and Oyeka, (2021).

These natural compounds hold promise of therapeutic utility, given the findings from the research conducted, especially for alum, which showed strong antimicrobial activity against the dental caries isolates. Also, being a resource that is readily available (Udemezue and Oyeka, 2021), it can be strongly considered for standardized forms that would be acceptable for use in therapy such as in the case of dental caries. It would be comparatively affordable compared to existing medications. Given also the rise in antimicrobial resistance, alum and trona have good position, being naturally occurring compounds, to help reduce the ratio of failed antimicrobial therapy owing to resistance by microbes.

CONCLUSION

There is a dire need for more research to be done on these natural compounds, which holds strong potentials for providing cheaper and readily available alternatives to be used in not just dental caries but antimicrobial therapy in general. Consideration should be made on standardizing preparations of these natural compounds into more effective forms and concentration for therapeutic use, given the strong antimicrobial activity some of the compounds exhibit.

REFERENCES

1. Aryal, K.K., Mehata, S., Neupane, S., Vaidya, A., Dhimal, M. and Dhakal, P. (2015). The Burden and Determinants of Non communicable diseases Risk factors in Nepal: Findings from a nationwide steps survey, Nepal Health research, Kathmandu, Nepal. Pp**.** 56-64.
2. Cheesbrough, M. (2010). Antimicrobial sensitivity test. In: District laboratory Practice in Tropical Countries (part 2). Cambridge University press, United Kingdom. Pp**.** 134-142.
3. Gahlaut, A. and Chhillar, A.K. (2013). Evaluation of antibacterial potential of plant extracts using resazurin based microtiter dilution assay. International Journal of Pharmacy and Pharmaceutical Sciences, **5**(2):372-376.
4. Kamka-Evans, C. I., Ngumah, M. O., Nwachukwu, C. U. and Ugochukwu, N. (2013). Comparative evaluation of phychochemical and antimicrobial activities of Elaeis guineensis tusks, Musa paradisiaca peels and potassium carbonate in bacterial isolate from fermented Pentaclethra macrophylla seeds. Journal of Global Biosciences, **2**(1):17-19.
5. Kanlayavattanakul, M and Lourith, N. (2011). Body malodours and their topical treatment agents. International journal of Cosmetic Science, **33**:298-311.
6. Kato, G.J., Steinberg, M.H. and Gladwin, M.T. (2017). Intravascular hemolysis and pathophysiology of sickle cell disease. Journal of Clinical Investigation, **127**(3):750-760.
7. Kutama, A.S., Auyo, M. I., Umar, M. L. and Hadiza, M.S. (2013). Assessing the antibacterial activity of Marula Stem Bark and Leaf Extract against Some Selected Isolates in Kano, Nigeria. World Journal of Agricultural Science, **1**:209-214.
8. Marya, C.M.A. (2011). Textbook of public health dentistry. 1st ed., JP Medical Ltd, Pp.98-111,273-276,317-322.

9. Marrack, P., McKee, A.S. and Munks, M.W. (2009). Towards an understanding of the adjuvant action of aluminium. Nature Reviews Immunology, **9**:282-287.
10. Moya-Salazar, J. and Rojasa, R. (2018). Comparative study for identification of Candida albicans with germ tube test in human serum and plasma. Clinical Microbiology Infectious Diseases, **3**(3):1-4.
11. Singleton, P. (1997). Bacteria in Biology, Biotechnology and Medicine (4th edn). Wiley, USA. Pp. 300 – 315.
12. Smith, M. and Selby S. (2017). Microbiology for Allied Health Students: Lab Manual. Biological sciences open textbook, **16**:50-61.
13. Struzycka, I. (2014). The oral microbiome in dental caries. Polish Journal of Microbiology, **63**: 127-135.
14. Takahashi, N. and Nyvad, B. (2011). The role of bacteria in the caries process: Ecological perspectives. Journal of Dental Research, **90**:294-303.
15. Tripathi, K. D. (2013). Essentials of Medical Pharmacology (7th ed.). New Delhi, India: Jaypee Brothers Medical Publishers. Pp**.**696-697.
16. Udemezue, O.I. and Oyeka, C.A. (2021). Antifungal Efficacy of Natural Stones Against Clinical Isolates of Candida Species. World Journal of Pharmacy and Pharmaceutical Sciences, **10**(4):289-299.
17. Varghese, N. and Joy, P.P. (2014). Microbiology Laboratory Manual. Kerala Agricultural University, Pineapple Research Station. Pp**.** 18-20, 33-40, 41-56.
18. Wilkins, E. (2009). Clinical practice of the dental hygienist. Philadelphia: Wolters Kluwer Health, **6** (2):79-81.
19. Willey, J. M., Sherwood, L. M. and Woolverton, C. J. (2015). Prescott's Microbiology (10th edition). McGraw-Hill, USA. Pp. 185 – 198.