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Antimicrobial effects of blue vitriol and brimstone on dental caries isolates

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Abstract

Natural compounds are organic products synthesized by living organisms or found in nature. The natural compounds of interest were blue vitriol and brimstone. Caries is a chronic, multifactorial disease, which causes destruction and demineralization of hard tissues of teeth by acid production occurring from bacterial fermentation of food. The antimicrobial activity of brimstones and blue vitriol (blue stone) against isolates from dental caries was evaluated. Eighty-five dental swab specimens were aseptically collected from dental caries patients. The isolates were identified based on their microscopic, macroscopic, biochemical and molecular characteristics. They include *Candida tropicalis*, *Streptococcus mutans, Lactobacillus spp*and *Staphylococcus aureus*. Agar-well diffusion and broth dilution methods were used to determine the antimicrobial susceptibility of the isolates. The test showed that the isolates were susceptible to some of the natural stones as zones of inhibition were observed. Blue stone gave the highest inhibition zone diameter of 46.20±1.10 mm against *Lactobacillus spp* at 200 mg/ml. Brimstones showed no zone of inhibition on all the isolates. The Minimal Inhibition Concentration of bluestone was 50 mg/ml; and 200 mg/ml for brimstone against *Staphylococcus aureus*. The minimum bactericidal concentration of bluestone was 50 mg/ml against *Staphylococcus aureus* and 200 mg/ml against the other isolates. Thus, natural stones can be used as antimicrobials in the production of dental care products and in treatment of dental caries.

Keywords: Caries; Brimstone; Blue vitriol; Inhibition; Antimicrobials

1. Introduction

The occurrence and development of oral diseases such as dental caries, periodontal disease and oral cancer are closely related to oral microorganisms. Dental caries is the localized destruction of susceptible dental hard tissues by acidic by-products from bacterial fermentation of dietary carbohydrates. The signs of the carious demineralization are seen on the hard dental tissues, but the disease process is initiated within the bacterial biofilm (dental plaque) that covers a tooth surface. Dental caries is a multifactorial disease that starts with microbiological shifts within the complex biofilm and is affected by salivary flow and composition, exposure to fluoride, consumption of dietary sugars, and by preventive behaviors (teeth cleaning). Dental caries is a chronic disease that progresses slowly in most people. The disease can be seen in both the crown (coronal caries) and root (root caries) portions of primary and permanent teeth, and on smooth as well as pitted and fissured surfaces. It can affect enamel, the outer covering of the crown; cementum, the outermost layer of the root; and dentine, the tissue beneath both enamel and cementum(Karoly *et al.,* 2019; Mosaddad *et al*., 2019; Peres *et al*., 2019).

Despite the rise of combinatorial chemistry as an integral part of drug discovery process, natural products still play a major role as starting material for drug discovery. Natural products may be useful as a source of novel chemical structures for modern techniques in development of antimicrobial therapies. These natural compounds include natural

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stones such as brimstone and blue stones. Copper (II) sulfate commonly called blue stones are the inorganic compounds with the chemical formula $CuSO_4(H2O)x$, where x can range from 0 to 5. The pentahydrate (x = 5) is the most common form. Older names for this compound include blue vitriol, bluestone, vitriol of copper and Roman vitriol. Copper sulfate is highly soluble in water. The largest health benefit of copper sulfate is its use in controlling bacteria and fungus growth on fruits, vegetables, and other crops. It is produced industrially by treating copper metal with hot concentrated sulfuric acid or its oxides with dilute sulfuric acid. Sulfur also known as brimstone or burning stone is a bright yellow, crystalline solid at room temperature. It forms near volcanic vents and fumaroles, where it sublimates from a stream of hot gases. It is the tenth most common element by mass in the universe, and the fifth most common on Earth. Sulphur is sparingly soluble in water though more soluble in warm water. Sulfur (specifically octasulfur, S8) is used in pharmaceutical skin preparations for the treatment of acne and other conditions. It acts as a keratolytic agent and also kills bacteria, fungi, scabies mites and other parasites (Torre *et al*., 2017).

Over the years, infectious diseases have been treated with drugs known as antimicrobial agents. They are substances that kill or inhibit the growth of microorganisms such as bacteria, fungi, viruses or protozoa. Antimicrobial substances can be synthesized or manufactured by chemical procedures independent of microbial activity. They can also be semi-synthesized to make them more potent or less susceptible to inactivation by pathogens.

This research work was aimed at evaluating the antimicrobial effects of blue vitriol and brimstone stone on dental caries isolates.

2. Material and methods

2.1. 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''.

2.2. 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, onto plates of Sabouraud's dextrose agar media supplemented with chloramphenicol (50µg/ml), Blood agar and Nutrient agar media supplemented with nystatin. The media were incubated aerobically at 37 °C for 24 hours (Udemezue and Oyeka, 2021).

2.3. 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).

2.4. 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 °C for 24 hours. The SDA plates were incubated at 28±1 °C 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 °C) on Nutrient agar and SDA slants in Bijou bottles for biochemical tests and identification (Cheesbrough, 2010).

2.5. 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, citrate utilization and hemolysis tests (Cheesbrough, 2010), coagulase test (Varghese and Joy, 2014), sugar fermentation and molecular identification tests (Udemezue and Oyeka, 2021). Germ tube test was also carried out on the fungal isolate ((Moya-Salazar andRojasa, 2018).

2.6. *In vitro* Evaluation of the Antimicrobial Activity of Trona and Alum

2.6.1. 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 200 mg/ml. A double fold serial dilution of the stock solution was performed to obtain 100 mg/ml, 50 mg/ml, 25 mg/ml and 12.5m mg/ml concentrations (Udemezue and Oyeka, 2021).

2.6.2. 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) (Bauer *et al*., 1966; Cheesbrough, 2010).

2.7. Determination of MIC and MBC using broth dilution method

From the stock concentration of 400 mg/ml of the test agents, various concentrations of the test agents were made in Nutrient broth by double fold serial dilution to obtain 200 mg/ml, 100 mg/ml, 50 mg/ml, 25 mg/ml, 12.25 mg/ml, 6.325 mg/ml, 3.125 mg/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).

From each negative tube in MIC assay, 0.2 ml 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 the minimum bactericidal concentration (MBC) for each isolates (Gahlaut and Chhillar, 2013).

3. Result

Table 1 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	*Lactobacillus spp*

Table 2 Sugar fermentation test

S/N	Glucose	Fructose	Sucrose	Dextrose	Mannitol	Isolates
1	+	+	+	+	+	*Streptococcus mutans*
2	+	+	+	+	+	*Staphylococcus aureus*
3	+	+	-	+	-	*Lactobacillus spp*
4	+	+	+	+	-	*Candida tropicalis*

Table 3 Inhibition zone diameter of Blue stone and Brimstone on *Streptococcus mutans* using agar-well diffusion method

Concentration(mg/ml)	Blue stone (mm)	Brimstone (mm)
12.5	11.5±1.1	-
25	15±0.4	-
50	19±0.4	-
100	21.5±0.4	-
200	28.5±0.4	-

Table 4 Inhibition zone diameter Blue stone and Brimstone on *Staphylococcus aureus* using agar-well diffusion method

Concentration(mg/ml)	Blue stone (mm)	Brimstone (mm)
12.5	8.7±1.1	-
25	11.83±1.1	-
50	17.2±1.1	-
100	32±1.0	-
200	48.67±2.2	-

Table 5 Inhibition zone diameter of Blue stone and Brimstone on *Lactobacillus* species using agar-well diffusion method.

Concentration(mg/ml)	Blue stone (mm)	Brimstone (mm)
12.5	11.83±0.8	-
25	24±1.0	-
50	26.2±1.5	-
100	34.2±1.5	-
200	46.2±1.1	-

Table 6 Inhibition zone diameter of Blue stone and Brimstone on *Candida tropicalis* using agar-well diffusion method

Concentration(mg/ml)	Blue stone (mm)	Brimstone (mm)
12.5	11.7±0.8	-
25	14±0.4	-
50	16±0.4	-
100	20±0.4	-
200	23±1.4	-

Table 7 MIC Determination of Blue stone and Brimstone against the isolates using broth dilution method (mg/ml)

Samples	*Streptococcus mutans*	*Staphylococcus aureus*	*Lactobacillus spp*	*Candida tropicalis*
Blue stone	200	50	200	200
Brimstone	200	200	200	200

Table 8MBC Determination of Bluestone and Brimstone against the isolates (mg/ml)

Samples	*Streptococcus mutans*	*Staphylococcus aureus*	*Lactobacillus spp*	*Candida tropicalis*
Blue stone	200	50	200	200
Brimstone	200	200	200	200

4. Discussion

Dental disease is undoubtedly a public health problem and is among the most prevalent diseases globally, in particular, dental caries which is a biofilm-associated disease (Yadav and Prakash, 2017). This study analyzed the antimicrobial effects of blue stone and brimstone against isolates from human dental caries. The isolates include *Streptococcus mutans, Staphylococcus aureus, Lactobacillus spp* and *Candida tropicalis*. This finding partly agrees with Lubna and Rabia' who noted that *'Streptococcus mutans*, *S. mitis, S*. *constellatus, S. sanguis, S. salivarius, S. anginosus, S. gordonii, S. intermedius, and S. oralis* are some of the primary acid-tolerant bacteria that are associated with dental plaque'(Tahir and Nazir, 2018).

The inhibition zone diameter of the natural stones on all the isolates(tables 3 - 8) showed that blue vitriol had more inhibitory ability when compared to brimstone. This agrees with Street *et al*., 2017 who listed blue vitriol as an active antifungal and toothache relief agent.The minimum inhibitory concentration (table 7) and Minimum bactericidal concentration (table 8) of the natural stones indicated that at 200 mg/ml, all the natural stones inhibited and killed the isolates except blue stone which MIC and MBC was 50 mg/ml against *Staphylococcus aureus*. Based on the findings by Street *et al*. (2017), the efficacy of blue stone could be as a result of its richness in organic (linoelaidic acid, Oleic acid and 6-octadecanoic acid) and inorganic compounds (copper oxide and Sulfur trioxide).

This study proved that natural stones such as blue stone and brimstone can be used as antimicrobials in the production of dental care products and in treatment of dental caries. It aligned with Torre *et al*. (2017) who stated that "despite the rise of combinatorial chemistry as an integral part of drug discovery process, natural products still play a major role as starting material for drug discovery".

5. Conclusion

This research work revealed that natural antimicrobial agents such as blue stones and brimstones appear to be effective in controlling dental caries; thus could be incorporated in dental care products such as toothpastes and mouthwashes.

Recommendation

The Alliance for a Cavity-Free Future has proposed steps to prevent dental caries, which include balancing the levels of oral bacteria; controlling the consumption of sugary and starchy foods; and strengthening the enamel through fluoridated dental products. The need for proper and efficient oral hygiene should not be over emphasized.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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