Antimicrobial Properties of Jatropha Curcas L. against Dental Pathogens

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Abstract

This research is aimed at investigating the in vitro antimicrobial activities of Jatropha curcas twigs against dental carries-causing bacteria. The methanol/methyl chloride crude extract of J. curcas twigs was fractionated into aqueous, n-hexane, ethyl acetate and n-butanol fractions. Using the agar well diffusion and the agar dilution methods, the antimicrobial activities of plant fractions were determined against strains of Streptococcus mutans isolated from dental swabs from patients with dental caries. The ethyl acetate fraction showed best antimicrobial activity with a minimum inhibitory concentration (MIC) of 6.25 mg/ml, followed by the n-butanol and n-hexane fractions, both with MICs ? 25 mg/ml against S. mutans. The aqueous fraction showed no activity against all strains of S. mutans tested. The results of our study show that J. curcas twig possesses antimicrobial against dental caries-causing bacteria strains. This justifies the folkloric use of the plant twigs in oral hygiene as a chewing stick/toothbrush for the prevention of dental caries.

Index terms— jatropha curcas, antimicrobial activity, streptococcus mutans, dental caries.

1 Introduction

atropha Curcas Linn (Euphorbiaceae) is a droughtresistant small tree or large shrub, widely distributed all over the world (Grace et al., 2009;Openshaw, 2000). Various parts of J. Curcas have been used in traditional medicine as a lactagogue, rubefacient, suppurative, purgative, abortifacient, haemostatic, and anthelmint; and for the treatment or prevention of fevers, convulsions, venereal diseases, constipation, skin diseases, rheumatism, malaria, diabetes, wounds, snake bites, haemorrhoids, amenorrhoea and oligomenorrhoea, jaundice and liver troubles **??**Asase et Juices, pastes, decoctions, or other preparations of the plant have been used for oral hygiene in the prevention and treatment of toothaches, mouth ulcer, cracked lips, bleeding gums, carious teeth (Verma and Chauhan, 2007;Silja et al., 2008;Iwu, 1993;Duke and Ayensu, 1985;Yesodharan and Sujana, 2007;Jain and Srivastava, 2005;Rajendran et al., 2008). The twigs of J. Curcas are chewed to prevent pyorrhea, gum and teeth problems, and are used as a toothbrush (Jain and Srivastava, 2005;Dolui et al., 2004).

The twigs of J. Curcas are used in different communities of Imo State, Nigeria as chewing sticks (toothbrushes) for the prevention of pyorrhea and tooth decay (Anyanwu et al., 2018). However, there is paucity of scientific data to validate the folkloric use of J. Curcas for oral hygiene. Our study, therefore, is aimed at evaluating the antimicrobial properties of the plant against human dental caries-causing bacteria.

2 II. Materials and Methods

3 a) Plant Collection

The twigs of Jatropha Curcas were collected in June 2014 from Umuocham, Amudi-Obizi, Imo State, Nigeria. The plant was identified, authenticated, and deposited under the voucher number: PCG423A/022 at the Department

of Pharmacognosy and Traditional Medicine, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria.

4 b) Plant Extraction and Fractionation

The twigs of J. Curcas were cut into smaller pieces and pulverized. The pulverized twig was extracted by cold maceration for 48 hours using methanol/methyl chloride combination in a ratio of 2:1. The crude extract was filtered using Whatman No.1 filter paper and concentrated to dryness under vacuum at 45 o C using a rotary evaporator. Liquid-liquid fractionation of the methanol/methyl chloride crude extract was carried out in a 500 mL separation funnel by dissolving 20 g of the extract in 250 mL of distilled water and then successively adding n-hexane, ethyl acetate, and butanol in increasing order of their polarities. The fractions so obtained were filtered, and then concentrated under pressure 45±5 o C. The water fraction was freeze-dried to dryness.

5 c) Test Organisms

Four strains of Streptococcus mutans (S. mutans 1, 2, 3 and 4) were used in this study. These were clinical isolates obtained from dental swabs of patients with dental caries at the Federal School of Dental Technology and Therapy, Trans-Ekulu, Enugu, Nigeria. The isolates were maintained in Columbia blood agar base (Oxoid, UK) (supplemented with 5% sheep blood).

6 d) Primary Antimicrobial Screening of Fractions of J. Curcas Crude Extract

The antibacterial activity of the fractions of J. Curcas crude extract against the test isolates was determined by the agar well diffusion method as described by Akpotu et al. (2017). Dilutions of 50, 25, 12.5, 6.25, 3.125 and 1.5625 mg/mL were prepared by dissolving the samples in DMSO (100% v/v). Twenty (20) mL of molten Mueller Hinton agar was poured into sterile Petri plates (90 mm) and allowed to set. Standardized concentrations (1.5 x10 8 CFU/mL) of overnight cultures of the test isolates were swabbed aseptically on the agar plates, and holes (6mm) were made in the agar plates using a sterile metal cork-borer. A volume of 20 µl of the various dilutions of the samples and controls were put in each hole under aseptic conditions. DMSO (100% v/v) was used as the negative control, while gentamicin (10 ?g/mL) was used as the positive control. The Petri plates were then incubated at 37 o C for 24 h, and the inhibition zones diameters (IZDs) were measured using a metre rule. The size of the cork borer (6 mm) was deducted from the values recorded to get the actual IZDs. The procedure was conducted in duplicate and the mean IZDs were calculated and recorded.

7 e) Determination of Minimum Inhibitory Concentrations (MICs)

Minimum inhibitory concentration (MIC) is defined as the lowest concentration of the antimicrobial agent that inhibits the bacterial growth. The MIC of the fractions of J. Curcas crude extract on the test isolates was determined using the agar dilution method previously described by Akpotu et al. (2017). Dilutions of 250, 125, 62.5, 31.25, and 15.625 mg/mL were prepared by dissolving the samples in DMSO (100% v/v). Agar plates were prepared by pouring 4 mL of molten double strength Mueller Hinton agar into sterile Petri dishes containing 1 mL of the various dilutions of the samples making the final plate concentrations to become 100, 50, 25, 12.5, 6.25, 3.125, and 1.5625 mg/mL.

Standardized concentrations (1.5 x10 8 CFU/mL) of overnight cultures of the test isolates were streaked onto the surface of the agar plates containing the different dilutions of the samples. The plates were then incubated at 37 o C for 24 h after which all plates were observed for growth. The minimum dilution (concentration) of the plant fractions completely inhibiting the growth of each organism was taken as the MIC.

8 III.

9 Results and Discussion

Fractionation of the crude methanol/methyl chloride extract of J. Curcas twigs yielded four fractions -aqueous, n-hexane, ethyl acetate and butanol fractions. The antimicrobial activities of the various fractions were determined against strains of S. mutans isolated from dental carries patients (Tables 1-5).

The ethyl acetate fraction showed considerable antimicrobial activity against S. mutans at concentrations ranging from 6.25-100 mg/mL, with IZDs of 3-8 mm (Table 1). The butanol fraction was active at 25-100 mg/mL with IZDs of 2-6 mm (Table 2). Antimicrobial activity of the n-hexane fraction was recorded at 50-100 mg/mL with IZDs of 2-6 mm (Table 3). The aqueous fraction showed no antimicrobial activity at the concentrations tested (Table 4).

The MICs of the extract and fractions of the plant against the test isolates ranged from 6.25-100 mg/mL (Table 5). The lowest MIC (6.25 mg/mL) was recorded for the ethyl acetate fraction. MICs ranging from 25-100 mg/mL were recorded for the butanol and n-hexane fractions. No MIC value was recorded for the aqueous

fraction. Dental caries, also known as also known as a cavity or tooth decay, is the destruction of enamel, dentin or cementum of teeth due to bacterial activities, which if left untreated can cause considerable pain, discomfort, and treatment costs are very high. Colonization of teeth by cariogenic bacteria is one of the most important risk factors in the development of dental diseases with S. mutans being the primary species associated with the early dental caries process (Maripandi et al., 2011).

Maintaining proper oral hygiene, which includes brushing with fluoridated toothpaste and a toothbrush, cleaning or flossing between teeth and gums, is known to inhibit the development of dental caries.

Despite the widespread use of toothbrushes, natural methods of tooth cleaning using chewing sticks prepared from the twigs, stems or roots from a variety of plant species have practiced for thousands of years in different parts of the world including Africa. Also, since conventional dental treatment usually is expensive and not so easily accessible, especially in developing countries, many have turned to the use of chewing sticks to prevent dental caries. Various clinical studies have shown that these chewing sticks, when properly used, can be as efficient as toothbrushes in removing dental plaque due to the combined effect of mechanical cleaning and enhanced salivation (Jyoti et al., 2017;Al-Otaibi, 2004;Wu et al., 2001;Akpata and Akinrimisi, 1977;Homer et al., 1990).

It has been observed that the use of chewing sticks help to inhibit the growth of oral pathogens associated with development of dental caries, gingival and periodontal diseases. During cleaning anti-microbial constituents may get released in the oral cavity and protect teeth and its associated parts against oral microbes (Chandana et al., 2017;Enwonwu, 1974).

The ethyl acetate fraction showed best antimicrobial activity with a minimum inhibitory concentration (MIC) of 6.25 mg/ml, followed by the butanol and n-hexane fractions, both with MICs ? 25 mg/ml against S. mutans. The antimicrobial activity elicited by the ethyl acetate fraction of J. Curcas against the four strains of S. mutans (Tables 2) suggests that the fraction contains important antibacterial compounds that would useful in the treatment of dental pathogens.

Several antimicrobial secondary metabolites of J. Curcas has been reported, and these include Jatropha factor C1 (Hass et al., 2002); Palmarumycins JC1 and JC2 (Ravindranath et al., 2004); and Taraxasterol (Mitra et al., 1970). These compounds may be responsible for the antibacterial activity of the plant against the strains of S. mutans used in this study.

10 IV.

11 Conclusion

The results of our study show that J. Curcas twig exhibits antimicrobial activity against dental caries-causing bacteria strains. These findings give a scientific insight into the traditional use of J. Curcas twigs as chewing stick; a practice believed to prevent dental caries. [1] [2]

Figure 1:

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Concentrations (mg/mL)	Inhibition Zone Diameters (mm) S. Mutans 1	S. Mutans 2	S. Mutans 3	S
100.00	6	6	7	8
50.00	5	5	6	7
25.00	3	3	4	6
12.50	2	2	2	5
6.25	0	0	0	3
3.16	0	0	0	0
1.56	0	0	0	0
Gentamicin (10 ?g/mL)	24	18	22	24
DMSO	0	0	0	0

Figure 2: Table 1 :

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[2] ( )J

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Figure 3: Table 2 :

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Figure 4: Table 3 :

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Figure 5: Table 4 :

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Concentrations (mg/mL)	S. Mutans 1	Inhibition Zone Diameters (mm) S. Mutans 2 S. Mutans 3	S. Mutans 4

Figure 6: Table 5 :

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