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Herbal mouthwash formulated with the leaf extract of *Jatropha gossypiifolia* Linn. (Euphorbiaceae) exhibited *in vitro* antimicrobial activity against selected oral pathogens

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Abstract
Increase in the incidence of oral disease, resistance to antibiotics and adverse effect to synthetic medicines has made search for alternative safe, effective and cheaper treatment options imperative, preferably from plant sources. This study evaluated the antimicrobial profile of ethanol extracts of leaf and root bark of *Jatropha gossypiifolia* and mouthwash formulation containing the leaf extract. Extraction of plant parts was done by cold maceration with 70% ethanol. The antimicrobial activities of the extracts and the formulated mouthwash were evaluated against *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumonia*, *Bacillus subtilis* and *Candida albicans* using Agar well diffusion method. The minimum inhibitory concentration of the extracts on susceptible organisms was determined according to the Clinical Laboratory Standards Institute protocol. The physicochemical parameters of the formulated mouthwash were assessed using standard methods. *J. gossypiifolia* leaf and root bark extracts exhibited a concentration dependent antimicrobial activity and MIC ranging from 50 to 260 mg/ml against test organisms. The formulated herbal mouthwash showed effective antimicrobial activity and suitable cosmetics properties. This study indicates that the leaf extracts of *J. gossypiifolia* possess bioactive metabolites with strong antimicrobial activities and its herbal mouthwash formulation has potential in the treatment of various diseases caused by oral pathogens.

Keywords: *Jatropha gossypiifolia*; Antimicrobial; Herbal mouthwash; Oral pathogens

INTRODUCTION

Oral disease is a major health problem with dental periodontal diseases among the most important preventable global infectious diseases [1]. Oral health influences the general quality of life because poor oral health has been linked to chronic conditions and systemic disease [1-3]. The association between oral diseases and oral microbiota is well established as more than 750 species of bacteria that inhabit the oral cavity have been implicated in oral diseases [4]. Given the incidence of oral disease, increased resistance by bacteria to antibiotics and adverse effect of some antibacterial agents currently used, there is need for alternative prevention and treatment options that are safe, effective and economical, hence the search for alternative products. In

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this regard, plant derived natural phytochemicals that inhibit the growth of oral pathogens, dental plaque, influence the adhesion to surfaces and reduce symptoms of oral diseases as compared to synthetic chemicals are preferable[5]. Eighty per cent of African populations use some form of traditional herbal medicine [6] and as many as 80% of the people in the developing countries depend on traditional herbal medicine for their primary healthcare needs [7]. Plant-derived medicines have contributed to human health and welfare and are sources of medicinal products that are important to the health of man [7, 8]. Medicinal products and preparation derived from natural sources, particularly from plants, are widespread in history. Herbs and plant materials have been in use for cleaning teeth and treating different oral diseases in different countries and are sources of many potent and powerful drugs [9, 10].

Jatropha gossypiifolia L. (Figure 1), commonly known as bellyache bush, black physic nut or cotton-leaf physic nut is a bushy, gregarious shrub of about 1.8 m in height and contains characteristic latex largely used for medicinal - purposes [11]. It belongs to the family "Euphorbiaceae" [12]. The leaves are used *in natura* or in compresses, and are considered to have anti-malarial, insecticidal [13], anti-inflammatory [14] and antimicrobial [15-16] properties. The root and stem have cytotoxic [17] anti-malarial, leishmanicidal, antimicrobial, insecticidal, molluscicidal and anti-inflammatory [18] properties. The seeds and fruits are used against influenza, and also as laxative [19], sedative, and analgesic or anti-diarrheal agents [20]. The young stem of the plant is used as toothbrush as well as to clean the tongues in treatment of oral thrush and the tuber of the plant grinded into a paste is also locally used in treatment of hemorrhoids.

Mouthwashes are liquid oral products that are gargled for cleaning the mouth and teeth to freshen breath. It may also kill the microorganism in the mouth or whiten the teeth. The use of antimicrobial mouthwashes as chemotherapeutic adjuncts to mechanical oral hygiene regimen has become well established in dental practice [21]. Though the use of plants in folklore medicine to treat infectious diseases is widespread, there are gaps in knowledge about the scientific rationale for the use of these herbs as well as formulating them into effective and suitable dosage forms that would enhance patient acceptability. This research was conducted to investigate the antimicrobial activities of *J. gossypiifolia* leaf and root bark extracts and the activity and the potency of the herbal mouthwash formulations containing the extracts.

EXPERIMENTAL METHODS

Plant collection and identification. Mature leaf and root of *Jatropha gossypiifolia Linn* (Euphorbiaceae) were collected from Ijegun market in Alimosho local government area of Lagos state, Nigeria. They were identified and authenticated by Dr. Nadoza at the Herbarium of the Department of Botany, University of Lagos. The authenticated leaf and root specimen were assigned voucher numbers LUH 9407 and LUH 9406 respectively.

Extraction of leaf and root bark of *Jatropha gossypiifolia.* The fresh leaves of *J. gossypiifolia* were washed with distilled water, cut into suitable small pieces and left for seven days on a clean surface to air dry. The coarse well-dried leaves were shred to coarse powder using a laboratory mill (Christy and Norris Ltd, Chelmsford, England). Eight hundred grams of the coarse powdered leaf were subjected to cold maceration using 70% ethanol for 4 days with frequent agitation [22,23] The maceration process was repeated and the extract was filtered using a fine pored muslin cloth. The filtrate was concentrated in a rotary evaporator (Buchi V-801), dried in an oven at 37^{o}C and stored at 4°C for further analysis. The same maceration process was used to extract the phytonutrients from *J. gossypiifolia* root bark.

Test microorganisms. Clinical isolates of S. *aureus, E. coli, B. subtilis, K. pneumonia* and *C. albicans* obtained from the stock samples at the Department of Pharmaceutical Microbiology Laboratory, Faculty of Pharmacy, University of Lagos, Nigeria were used as test organisms

Evaluation of the antimicrobial activity of the plant extracts. The antibacterial activities of the ethanol extracts of *J. gossypiifolia* leaf and root bark and the control standard (chlorhexidine gluconate) against the test organisms were evaluated using the agar-well diffusion method [24]. Twenty-five millitres of molten sterilized Mueller-Hinton agar was poured into each of the petri dishes containing 1ml of 10^6 cfu of overnight cultures of the microorganisms in saline broth respectively, swirled gently for proper mixing and allowed to solidify. A sterile cork-borer (10 mm diameter) was pushed into the agar to form wells. Each of the wells was filled with 0.15 ml of different concentrations (0.25, 0.5, 1 and 2 µg/ml) of the control standard (chlorhexidine gluconate), then, 125, 250 and 500 mg/ml of the leaf and root bark extracts respectively. A blank well in each of the culture plates was filled with 0.5 ml sterile water which served as the negative control. The agar plates were left at room temperature for one hour to allow for adequate diffusion, incubated at 37^{o}C for 24 h and zone of inhibition measured [25].

Determination of minimum inhibitory concentration (MIC) of *J. gossypiifolia* leaf and root extracts. The minimum inhibitory concentrations of both extracts of leaf and root bark of *J. gossypiifolia* were determined using the Clinical Laboratory Standards Institute [26] method. Different concentrations (30 to 260 mg/ml) of the extracts were used. Twenty-five millilitres of Mueller Hinton agar was poured into each petri dish containing 1 ml of extract and seeded with 1 ml of 2 x 10^6 cfu of overnight clinical cultures of the microorganisms, swirled, allowed to set and incubated at 37^{o}C for 24 h. This procedure was repeated using different concentrations of the leaf and root bark extracts from the highest concentration at which there was growth to the least concentration that showed no growth. The least concentration of the plant extract that prevented growth of an organism was the minimum inhibitory concentration of the extract for that organism.

Preparation of herbal mouth wash formulations of *J. gossypiifolia* leaf extract. A total of four different formulations (F1, F2, F3 and F4) of oil in water (O/W) emulsion-based control and herbal mouthwashes containing *J. gossypiifolia* leaf extracts and other ingredients at various concentrations were prepared as shown in Table 1 using the modified form of a method previously described [27]. Sodium chloride was added into the mixture of ethyl alcohol, orange oil, polysorbate 80 and propylene glycol to make the oil phase with continuous shaking during mixing and heated to 60°C. The leaf extract, saccharine and Sodium benzoate were mixed in water (water phase) and heated to 30°C. There was no leaf extract in the negative control formulation. The oil phase was added in portions to the water phase with continuous stirring to form homogenous dispersion of oil phase in aqueous phase. The mixture was constantly stirred until homogenous oil in water emulsion was formed, allowed to cool, transferred into a sterile container and sealed.

Evaluation of the antimicrobial activity of the formulated herbal mouthwash containing *J. gossypiifolia* leaf extract. The antimicrobial activity of the formulated herbal mouthwash containing the leaf extract of *J. gossypiifolia* and the positive control (Listerine®) were evaluated by the agar diffusion method [24] using clinical isolates of *S. aureus, K. pneumonia, E. coli, B. subtilis,* and *C. albicans*. 25ml of sterile molten Mueller-Hinton agar prepared according to the manufacturer's instruction was poured into each of the sterile petri-dishes containing 1 ml of 10^6 cfu of overnight cultures of the

microorganisms in saline broth respectively, swirled gently for proper mixing and allowed to solidify. Wells were bored on the seeded agar using a 10 mm sterile cork-borer and filled with 1mleach of herbal mouthwash formulations(F1-F4) and Listerine®, incubated at 37℃ for 24 h and zones of inhibition measured.

Physicochemical evaluation of the mouthwash formulations

Determination of the pH of the cream formulations. To determine the pH of the prepared herbal mouthwash, 1 ml of the mouthwash was diluted with 50 ml of distilled water and the pH measured using a digital pH meter (Mettler Toledo)

Determination of emulsion type of the mouthwash. The emulsion type was determined by the dilution test which evaluates whether the emulsion formed was oil in water (O/W) or water in oil (W/O) [27]. The herbal cream was diluted with water and oil separately to determine the type of emulsion.

Determination of appearance and fragrance of the formulated mouthwash. Formulated herbal mouthwash was examined for its colour by visual examination and the odour by smelling.

Determination of homogeneity. The formulated herbal mouthwash not exposed to light was carefully observed for phase separation for 5 days.

Viscosity. Viscosity of formulated mouthwash was determined by using Brookfield viscometer at 100 rpm using spindle No 7.

Statistical analysis. Results obtained in triplicates were expressed as mean ± standard error of the mean(SEM). Student's t-test was used to determine the statistical significant difference. The difference was regarded as significant when $P < 0.05$.

RESULTS

Antimicrobial activity of the extracts. The ethanol extracts of both the leaf and root bark of *J. gossypiifolia* showed concentration dependent broad-spectrum activity against *S. aureus, K. pneumonia, E. coli, B. subtilis,* and *C. albicans* comparable to the activity of the positive control, chlorhexidine gluconate as shown in Tables 2 and 3. Though both the leaf and root bark extracts were active against all the organisms tested at 500 mg/ml, the spectrum of activity of the leaf was broader than the root bark extract at concentrations of 250 and 125 mg/ml. The MIC of each of the extracts on the various organisms ranged from 50 to 260 mg/ml as presented in Table 4.

Evaluation of antimicrobial potency and physicochemical parameters of the formulated herbal mouthwash

The formulated mouthwash was found to possess good physicochemical properties. The herbal mouthwash formulations were active against a broad spectrum of microorganisms tested. Strong antimicrobial effect similar to the standard control was shown by the formulated mouthwash especially against *S. aureus, B. subtilis* and *C. albicans* as observed from the zones of inhibition obtained (Table 5).

DISCUSSION

Plants are important sources of potentially useful constituents to the development of new therapeutic agents because most of them are safe with little side effects [28].In this study, ethanol extracts of *J. gossypiifolia* leaf and root bark exhibited concentration dependent antibacterial and antifungal activities against the test organisms of *S. aureus, K. pneumonia, E. coli, B. subtilis,* and *C. albicans* comparable to control standard (chlorohexidine gluconate).

Figure 1: *Jatropha gossypiifolia* L.

Table 1: Composition of mouthwash formulations containing the leaf extract of *J. gossypiifolia*

Ingredients	Formulations			
	F1	F2	F3	F4
Propylene glycol (ml)	10	9.5	8.0	10
Ethyl alcohol (ml)	75	70	70	75
Polysorbate 80 (ml)	2.0	1.8	1.5	2.0
Orange oil (ml)	0.1	0.1	0.2	0.1
Sodium chloride (g)	0.2	0.1	0.15	0.2
Saccharine (g)	0.02	0.03	0.01	0.02
J. gossypifolia (g)	1.5	2.0	2.5	Nil
Sodium benzoate (g)	0.2	0.2	0.1	0.2
Water to 100 (ml)	100	100	100	100

Table 2: Antimicrobial activity of *J. gossypiifolia* leaf and root bark extracts

Organisms	Antimicrobial agent/zone of inhibitions (mm)					
	J. gossypiifolia leaf extract (mg/ml)			*J. gossypiifolia* root bark extract (mg/ml)		
	125	250	500	125	250	500
S. aureus	4.0±0.07	6.2±0.04	10±0.00	9.5±0.07	10±0.05	10.0±0.00
K. pneumonia	3.3± 0.04	5.2±0.05	9.2±0.04	3.3± 0.04	8.8±0.15	8.8±0.04
E. coli	3.0± 00	6.3±0.04	8.6±0.07	-	6.3±0.11	7.5±0.00
B. subtilis	-	6.7± 0.09	8.2±0.05	-	6.7± 00	6.5±0.05
C. albicans	-	6.5±0.00	7.0±0.07	-	-	6.0±0.00

- = No zone of inhibition

Table 3: Antimicrobial activity of positive standard, Chlorohexidine gluconate

Organisms	Chlorohexidine gluconate (mg/ml)/ Zone of inhibition (mm)			
	2.0	1.0	0.5	0.25
S. aureus	17.5±0.64	16.2±0.04	11.2± 0.15	9.8±0.07
K. pneumonia	21.0 ±0.04	11.2±0.05	10.5±0.10	8.7± 0.04
E. coli	20.0±0.07	13.0±0.04	10.0±0.00	8.4± 0.00
B. subtilis	16.0± 0.09	6.5±0.05	10.0±0.00	7.5±0.00
C. albicans	13.0±0.11	12.0 ±0.07	10.0±0.00	6.5±0.50

Table 4: Minimum inhibitory concentration of *J. gossypiifolia* leaf and root extract

Organisms	Minimum inhibitory concentration (mg/ml)	
	J. gossypiifolia leaf	*J. gossypiifolia* root bark
S.aureus	50	60
K. pneumonia	90	100
E.coli	80	180
B. subtilis	150	190
C. albicans	160	260

Table 5: Antimicrobial activities of mouthwash formulations and the standard Listerine®

Microorganisms	Formulations/Zone of inhibition (mm)				
	F1	F2	F3	F4	Listerine®
S. aureus	9.0±0.20	10±0.25	11.9±1.4	-	13.0±0.20
K. pneumonia	7.0±0.05	9.0±0.11	10.6±.13	-	10.7±0.20
E. coli	7.4±0.25	8.0±0.20	9.5±.07	-	11.2± .00
B. subtilis	8.0±0.25	8.2±0.20	8.8±1.0	-	13.7±0.37
C. albicans	7.0±0.25	8.0±0.00	8.4±0.06	-	13.0±0.00

- = No zone of inhibition

This results suggest it contains bioactive components with strong antimicrobial effects. Although, the mechanism of antimicrobial action of this extract is not yet known, *J. gossypiifolia* L is rich in tannins and saponins [29] which are believed to be responsible for its antimicrobial activity. This is because; phytochemical compounds such as tannins coagulate the cell wall proteins while saponins facilitate the entry of toxic material or leakage of vital constituents from the cell [30]. It has also been proposed that the antibacterial activity may be as a result of inhibition of cell wall formation resulting in leakage of cytoplasmic constituents by the bioactive components of the extract [31]**.** The observation that *J. gossypiifolia* leaf extract exhibited activity comparable to chlorohexidine gluconate standard implies that the extract has potential for use in the treatment of bacterial infections caused by the test organisms. Although both the leaf and root bark extracts showed weak antifungal effect against *C. albicans* as shown by inhibition only at 500mg/ml for the root bark and 250 and 500 mg/ml for the leaf extract, the leaf extract exhibited broader spectrum of activity than the root bark extract at lower concentration of 250 and 125 mg/ml, (Table 2). The low MIC of (50 to 90 mg/ml) for the leaf extract against *S. aureus, E. coli* and *K. pneumoniae* (Tables 4) is an indication that mouthwash formulation with extract of *J. gossypiifolia* leaf could be useful in treatment of oral infections caused by the organisms.

Plants with specific medicinal properties can be used in herbal formulation as active ingredients in order to provide additional value [32]. In this study, the physicochemical properties of the mouthwash formulations with the leaf extract of *J. gossypiifolia* were found to be good. The formulations had appropriate characteristic odour, colour and ideal pH of 6.3-6.79. An ideal product to be used in the mouth should have similar pH with the mouth. The pH of the mouth is maintained near neutrality (6.7-7.3) by saliva and does not fall below 6.3. With more acidity, the mouth can combat harmful microbes while increase in pH increases microbial count [33]. The mouthwash formulations containing *J. gossypiifolia* leaf extract had pH values of 6.30 to 6.79 which fall within the normal pH range of normal healthy mouth, ideal for enhanced antimicrobial activities of the mouthwash. No phase separation was observed in the oil in water emulsion formulated with the ethanol extract of the leaf of *J. gossypiifolia* which indicates that the dosage form is stable and therefore cosmetically acceptable. Oil in water

emulsion remains stable when diluted with water but cracks when diluted with oily liquid since water is the dispersion medium. On the other hand, water in oil emulsion remains stable when diluted with oily liquid and cracks on addition of water because oil is the dispersion medium. Oil in water emulsion can therefore easily be diluted with an aqueous solvent, whereas water in oil emulsion can easily be diluted with an oily liquid.

The formulated mouthwash containing the extracts of *J. gossypiifolia* leaf was active against the clinical isolates of *S. aureus, K. pneumonia, E. coli, B. subtilis,* and *C. albicans* used. It is worthy of note that the activity of the leaf extract formulation against *C. albicans* compared favourably with the standard Listerine® as shown in Table 5. *S. aureus* is notorious for its ability to become resistant to antibiotics. The activity of the prepared mouthwash against *S. aureus* was of significant interest because the organism is commonly found in the mouth and oral cavity. It is a remarkable finding that a natural plant product is active against the organism for obvious advantages of safety, accessibility and affordability.

Conclusion. The ethanol extract of the leaf and rootbark of *J. gossypiifolia* exhibited broad spectrum antibacterial and antifungal activities with the leaf extract showing better activity. The herbal mouthwash formulation showed sufficient potency comparable to the standard, Listerine® in terms of quality and efficacy. The herbal mouthwash formulation can be potent as an antibacterial agent, suitable for use as mouthwash and cosmetically appealing to enhance patient/customer compliance.

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