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Research Article

Phytochemical Properties and Antimicrobial Activity of Some Selected Methanolic Leaf Extract of *Jathropha multifida* (Coral Plant), *Curcuma longa* (Turmeric) And *Citrus lemon* against Oral Pathogenic Microbes in Nigeria

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Abstract

The aim of this study was to determine the phytochemical composition and antimicrobial activity of *Jathropha multifida*, *Curcuma longa* and *Citrus lemon* (Lemon juice) on dental pathogens. Antibiotic susceptibility against dental pathogens was determined by disc diffusion method. Methanolic extraction of the phytochemical constituents of the plant were analysed as well as its antagonistic activity against some dental pathogens by agar well diffusion methods. The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) was determined. Data were analyzed using ANOVA at $\alpha_{0.05}$. *Streptococcus mutans* and *Streptococcus pneumonia* were found to be resistant to almost all the antibiotics used with the exception of Gentamycin that was sensitive with 18.00mm zone of inhibition to both organisms, while *Staphylococcus aureus* was

resistant to Nitrofurantoin and Ampicillin. On the other hand, *Candida tropicalis* and *Candida alblican* were resistant to Metronidazole. Of all the three medicinal plants used *Citrus lemon* juice demonstrated highest antimicrobial activity with 25mm zone of inhibition against *Streptococcus mutans* and the least was 15 mm zone of inhibition against *Candida alblicans*. *Curcumin longa* leaf extract inhibited *Streptococcus mutans* with 21±0.00mm zone of inhibition while the least (15±0.00 mm) zone of inhibition was against *Candida tropicalis*. *Jathropha multifida* inhibited *Staphlococcus aureus* with 21±0.01mm zone of inhibition. All the plant extract had MIC of 32µg/ml against *Streptoccocus mutans* while MBC of *Curcuma longa* and that of (*Jathtropha multifida*) Coral plant were 16.00µg/ml against *Streptoccocus mutans*. In all the extracts there were presence of Alkaloid's value ranges between 23± to74.10±0.00 and had the Flavonoid's within 350.10 to 401.70±0.01 Phenols 1508.60, Tannins 580 to 490.00±0.00, Terpenoids, Phytate and Saponin in varying quantities. Plant extracts used in this study exhibited a remarkable antimicrobial activity against dental pathogens.

Introduction

Oral hygiene is vital to general well-being and represents the characteristic of life as well as an individual [1]. Different species of microorganisms occupy the dental cavity, of which only very few has been identified (*Streptococcus mutans*) and many of these species have been implicated in oral infection [2]. Despite advancement in the medical sciences, plants are still thought to be a vital source of different drugs in various regions around the globe [3]. The practice of herbal products had become promising technique in treating common oral microbial infections [4]. Therefore, herbal mouthwash of origin is thought to be reducing assembly of pathogenic groups of microbes in the mouth and can serve as a substitute in patients with the tooth, gum [5]. or odontitis challenges [5].

As a result of this, majority of drugs are obtained from various plants, and numerous others are extracted from prototype components gotten from many species of plants. More than two million standard health professionals use more than 7500 species of plants which have curative potentials and medicinal value [6]. The drugs from plants are contributive and defensive in its approach in which the World Health Organization in 2007 reported that community health expenditure specific to dental care was 5-10 Tooth decay and oral infections treatment are likely to be very expensive that people have to struggle for throughout their lifetime.

Natural plant products are used even to correct numerous oral infections. In Burkina Faso, West Africa, more than 62 species of plants belong to 29 families documented to cure oral infections [7]. It is supposed that quarter of recommended drugs contains constituents taken from the plants in industrialized countries [8]. To cure many human diseases, plants were used, and knowledge about these plants is widespread all over the world which has been serving humanity from the time immemorial. These conventional systems are alive today over a large area of the world. About 80% of people from progressing countries rely on this system to cure human diseases [9]. Asian countries have used traditional medicine to correct much oral disease for more than 2000 years [10].

Anaerobic Gram-negative bacteria such as *Actinobacillus, Porphyromonas gingivalis, Fusobacterium* and *Prevotella* have been associated with periodontal diseases which leads to the case of oral infections [11].

Increased bacteria resistance to antibiotics, adverse effects of some antibacterial agents currently used in dentistry, financial considerations in developing countries, several agents that are commercially accessible can alter oral microbiota and have undesirable side-effects such as tooth colouring, vomiting and irritations [12]. Therefore, there is a need for substitute that are easily accessible with minimal effort in prevention and treatment that are safe, effective and easily affordable most especially in developing nations [12].

As a result of this, those living in the rural areas, would resort to seeking relief via traditional herbs that has gone through scientific investigations in treating dental infections and its management of human diseases. Therefore, the aim of this study was to determine the phytochemical composition and antimicrobial activity of *Coral plant, Curcumin longa,* and *Citrus lemon* (Lemon juice) *on* dental pathogens.

Materials and Methods

Collection of Samples

The plant material of (*Jathropha multifida*), *Curcumin longa*, and *Citrus lemon* (Lemon juice) were all collected within the premises of Ogun State College of Health Technology Ilese ijebu, Nigeria where they were growing naturally. The authentication of the plant was done at the Department of Botany and microbiology Olabisi Onabanjo University ago iwoye, Ogun State. The indicator organisms (*Streptococcus mutans, Streptococcus pneumoniae, Staphylococcus aureus, Candida tropicalis* and *Candida alblican*) used in this work were from Department of Medical Laboratory Science, Ogun State College of Health Technology Ilese ijebu, the organisms were initially isolated and identified from Dental samples.

Preparation and Sterilization of Media

The media used in this study are Nutrient Agar (NA), Nutrient broth and Muller- Hinton Agar (MHA). The agars were weighed according to manufacturers' specification. The media were autoclave at 121˚C for 15mins before commencement of all laboratory work.

Culture Preservation

The pure cultures of the isolates were sub-cultured into maintenance medium. It was then incubated at 37˚C; the stock culture was stored at 4˚C for subsequent use.

Antibiotic Susceptibility Testing of the Indicator Organisms

Antibiotic susceptibility test for each bacteria pathogen was performed using the disc diffusion method. Actively growing culture (0.1ml) containing 1×10^6cfu/ml of each bacterium pathogen used was introduced into Petri dishes and 20ml of molten agar (Muller- Hinton Agar - MHA) added. The antibiotic sensitivity discs (Abtek Biological Ltd) consisting of different antibiotics namely Augmentin (30µg), Gentamycin (10µg), ofloxacin (5µg), Tetracycline (10µg), Metronidazole (10µg), Ampiclause (10µg), Ampicilline (10µg), Nalidixic (30µg), Cotrimoxazole (25µg), Amoxyline (30µg), Nitrofuratoin (300 µg), Ceflazidine (30µg) and Cefuroxime (30µg) were placed on the solidified agar surface. The plates were incubated aerobically at 37˚C

for 24 h. After this period, the diameter of the zone of inhibition of each disc was measured. The zone of inhibition corresponded to the antibiotic activity of each disc [14]. Resistance was defined by the absence of a zone of inhibition. The relative susceptibility of each isolate to each antibiotic was shown by a clear zone of inhibition.

Preparation of Leaf Extracts

Twenty grams of each of the dried grounded leaves was weighed separately using weighing balance. This was transferred into conical flasks containing 80 ml of methanol. The different mixtures were placed on a mechanical shaker and allowed to macerate for 24 h, filtration was done using sterile filter cloth and the filtrate collected. The total filtrate collected was evaporated to dryness by pouring into sterile stainless plate and was kept in hot air oven at 50° C until whole moisture evaporates completely leaving powder behind [13].

Antimicrobial Susceptibility Studies of Plant Leaves Extracts by Agar Well Diffusion

This was carried out to determine the effects of methanolic leaves extract of selected plants against dental indicator organisms by methods described by [13]. A standardized inoculum of 0.5 McFarland standard containing 1.0 × 106cfu/ml was introduced onto the surface of sterile agar plates (MHA), and a sterile glass spreader was used for even distribution of inoculums. Well was dug on each of the plate with a sterile 6 mm diameter cork borer, 100µL of the crude extracts were introduced into the wells, allowed to stand at room temperature for about 30 mins. The standardized drugs gentamycin and Cotrimoxazole were used as positive control and DMSO solution only as negative control. The plates were incubated aerobically at 37° C and examined for zone of inhibition after 24 h. Each zone of inhibition was measured with a ruler and compared with the control in accordance to the method of [14,15].

Minimum Inhibitory Concentration (MIC) And Minimum Bacteriocidal Concentration (MBC) Of Plant Leaves Extracts by Tube Technique

Doubling dilution of 256µg/ml of antibiotics solution was made in 1ml volume of broth to 0.125µg/ml. One row of the test was inoculated with 0.02ml of 1 in 100 dilution of the overnight broth culture of the test organism of 0.5 McFarland standard equivalent to 0.1ml actively growing culture containing 1.0 × 106cfu/ml of each bacterium pathogen used. The test was incubated aerobically at 37°C for 24 h. Two control tubes were maintained for each test batch which include tube containing extracts together with the growth medium without inoculum (antibiotic control) and the tube containing the growth medium, physiological saline with the inoculums (organism control). MIC was determined as the lowest concentration of the extracts with no visible growth (no turbidity) when compared with the control tubes. The MBC was determined by sub-culturing the test dilution on fresh solid medium (MHA) and further incubated at 37°C for 18 - 24 h. The lowest concentration of MIC tubes with no visible bacterial growth on solid medium was regarded as MBC as described by [15].

Phytochemical Screening of the Leaves of the Plant Samples

Phytochemical analysis was carried out to determine the quantity of alkaloids, flavonoids, phenols, steroids, tannins, and terpenoids present in the extracts using standard methods [16-18].

Statistical Analysis

One-way analysis of variance (ANOVA) was worked out to find out the significance of the treatments. The treatments were separated by least significance different (LSD) at P 0.05 level.

Results

This study centers on selected medicinal plants extract (*Coral plant, Curcumin longa,* and *Citrus lemon* (Lemon juice) as a curative measure against oral pathogenic fungi and bacteria.

Table 1 Shows antibiotics susceptibility test of the indicator organisms obtained from dental samples. Different antibiotics such as Augmentin, Tetracycline, Ofloxacine, Gentamycine, Cotrimozazole, Ampicillin, Nalidixic, Cotrimoxazole, Nitrofurantoin, Metronidazole and Ampiclause were used. Augmentin showed 20.10mm zone of inhibition against *Staphylococcus aureus*. Gentamycin and Cotrimoxazole inhibited *Staphylococcus aureus* with 20.00 mm zone of inhibition while Ampicillin had 9.00mm zone of inhibition against the same organisms. Augmentin, tetracycline and Cotrimoxazole had 12.00mm zone of inhibition against *Streptococcus mutans*. Ampiclause had 7.00mm zone of inhibition against *Streptococcus mutans* which was the least although, Gentamycin showed 18.00mm zone of inhibition against *Streptococcus mutans* and *Streptococcus pneumoniae*. Metronidazole had 10mm and 12mm zone of inhibition against *Candida tropicalis* and *Candida alblican* respectively while other antibiotics had no effect on *Candida tropicalis* and *Candida alblican*.

Table 1: *Susceptibility of the dental pathogens to Antibiotics*

Organisms	Antibiotics used (µg)/zone of inhibition (mm)								
	Aug 30	Tet 10	Ofl 5	Gent 10	Cot 25	Amp 30	Nit 30	Metro10	Ampi-clause 10
Streptococcus mutans	12.00	12.00	13.00	18.00	12.00	9.00	8.10	NA	7.00
Streptococcus pneumo	12.40	11.30	12.00	18.00	11.20	8.50	9.00	NA	10.20
Staphylococcus aureus	20.10	19.50	19.00	20.00	20.00	0.00	10.00	NA	9.00

Candida tropicalis	NA	NA	NA	NA	NA	NA	NA	12.00	NA
Candida ablican	NA	NA	NA	NA	NA	NA	NA	10.00	NA

key: Zone of inhibition: Sensitive ≥ 20mm, Intermediate between 15-19mm, ≤Resistance Less than 14 mm. Aug -Augmentin., Tet – tetracycline., Ofl – Ofloxacin, Gent – Gentamycin, cot -cotrimoxazole, Amp- Ampicillin, Nit – Nitrofurantoin, metro – Metronidazole and Amp- amp NA clause and NA- Not Applicable.

Table 2 shows antagonistic activity of medicinal plants extract against the bacterial and fungi indicator organisms. (Lemon juice) Citrus lemon has the highest zone of inhibition (25mm) against *Streptococcus mutans*, closely followed by (Tumeric) *Curcuma longa* which has 21mm zone of inhibition against *Streptococcus mutans* while (*lemoin juice*) *Citrus lemon* had 15mm as the zone of inhibition against *Candida albican*. (*Tumeric*) Curcuma longa has 21mm zone of inhibition against *Streptococcus mutans*, followed by 18mm zone of inhibition against Staphylococcus aureus while the lowest zone of inhibition was 15mm against *Candida tropicalis*.

Table 2: *Antagonistic activity of plant extract against selected oral diseases pathogens*

Organism	Tumeric	Lemon Juice	Jathropha mutifida
Streptococcus mutans	21±0.00	25±0.00	16±0.00
Streptococcus pneumoniae	17±0.00	21±0.10	18±0.00
Staphylococcus aureus	18±0.58	18±0.00	21±0.01
Candida albican	16±0.02	15±0.01	17±0.00
Candida tropicalis	15±0.00	16±0.01	16±0.00

(*Jathropha multifida*) Coral plant had 21±0.01mm zone of inhibition against Staphylococcus aureus followed by 18±0.00mm zone of inhibition against *Streptococcus pneumoniae* while the lowest zone of inhibition of 16±0.00mm was against *Candida tropicalis*.

The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the three different species of plant extract were investigated against selected oral diseases pathogens (Table 3). All the plant extract had MIC of 32µg/ml against *Streptococcus mutans* while MBC of *Curcuma longa* and that of (*Jathropha multifida*) Coral plant were 16.00µg/ml against *Streptococcus mutans* while (*lemon juice*) *Citrus lemon* had MBC of 32.00µg/ml against *Streptococcus mutans*. All the plant extract except (*Jathropha multifida*) *Coral plant* had MIC and MBC of 64.00µg/ml against *Streptococcus pneumoniae* while (*Jathropha multifida*) *Coral plant* had MBC of 32µg/ml. *Curcuma longa* and (*lemon juice*) *Citrus lemon* had equal value of MIC (128.00µg/ml) against *Staphylococcus aureus* while that of (*Jathropha multifida*) *Coral plant* against *Staphylococcus aureus* was 64.00µg/ml. The MIC and MBC of the three plants extract against *Candida alblican* was 32.00µg/ml with the exception of *Curcuma longa* that had MBC of 8.00µg/ml. The MIC and MBC all the plant extract was 32µg/ml against *Candida tropicalis* with the exception of MBC of *Jathropha multifida Coral plant* and (*lemon juice*) *Citrus lemon* that had 8.00µg/ml against *Candida tropicalis*.

Table 3: *Minimum Inhibitory Concentration (MIC) and Minimum Bacteria Concentration (MBC) of plant extract against selected oral diseases pathogens*

Test Organism	MIC (µg /ml) /MBC (µg /ml)	Tumeric	Lemon Juice	*Jathropha mutifida*
Streptococcus mutans	MIC	32. 00	32.00	32.00
	MBC	16.10	32.00	16.00
Streptococcus pneumoniae	MIC	64.00	64.00	64.00
	MBC	64.00	64.00	32.00
Staphylococcus aureus	MIC	128.00	128.00	64.00
	MBC	64.00	128.00	64.00
Candida albican	MIC	32.00	32.00	32.00
	MBC	8.00	32.00	32.00
Candida tropicalis	MIC	32.00	32.00	32.00
	MBC	32.00	8.00	8.00

The phytochemicals constituents of selected plants extract were alkaloids, Flavonoids, Phenols, Tannins, Terpenoids, Saponins and phytate which were all present in substantial quantities in the methanolic extract of the plant as shown in Table 4. Phenol's content of (*Jathropha muitifida*) *Coral plant* was 1650.40±0.10mg/kg, that of (*Lemon juice*) *Citrus lemon* was 1601.20±0.00mg/kg while *Curcuma longa* had 1508.60±0.01 mg/kg. *Coral plant* had the highest Alkaloid content of 74.10±0.01%, followed by *Lemon juice*) *Citrus lemon* (50.20±0.00%) and the lowest of 23.00±0.00% in *Curcuma longa*. *Curcuma longa* had 580.00±0.1mg/kg of Tannins, followed by (*Lemon juice*) *Citrus lemon* which had 520.10±0.00mg/kg and (*Jathropha multifida*) Coral plant had 490±0.00mg/kg of Tannins.

Table 4: *Quantity of active ingredient present in the selected plants extracts*

Phytochemicals	*(Tumeric) Curcuma longa*	*(Lemon juice) Citrus lemon*	*(Jathropha mutifida) Coral plant*
Alkaloid (%)	23.00±0.00	50.20±0.00	74.10±0.01
Flavonoid (mg/kg)	401.70±0.01	398.90±0.01	350.10±0.00
Phenols (mg/kg)	1508.60±0.01	1601.20±0.00	1650.40±0.10
Tannins (mg/kg)	580.00±0.1	520.10±0.00	490.00±0.00
Terpenoids (mgkg)	300.80±0.00	280.00±0.00	260.10±0.00
Phytate (mg/kg)	40.01	60.00	74.00
Saponin (%)	50.01	65.00	74.00

Discussion

The use of old-style medicine and plants that are medicinal as a substitute therapy for the treatment of microbial infections in most developing countries is a major basis for the maintenance of good health. Three different plant species were investigated in this study namely, (Tumeric) *Curcumin longa*, (*Lemon juice*) *Citrus lemon* and (*Jathropha multifida*) *Coral plant* using methanol for the extraction in order to evaluate their antibacterial activity against dental pathogens such as *Streptococcus mutans, Streptococcus pneumoniae, Staphylococcus aureus, Candida albican*, and *Candida tropicalis*.

Most of the pathogens used in this study were multidrug resistant to antibiotics. The pathogens were observed to be resistant to more than three antibiotics used in this research. Bacterial resistance is the ability of pathogens to prevent antibiotic bactericidal or bacteriostatic effect [19]. Excessive and unintentional usage of antibiotic contributed to resistance development in bacteria [20]. Resistance of these antibiotic could be due to extrachromosomal genes which can be transfered through plasmid, transposon, transduction, transformation and integron [21]. Resistance of antibiotic such as Augmentin, Tetracycline, Ofloxacine, Gentamycine, Cotrimozazole, Ampicillin, Nalidixic, Nitrofurantoin, Metronidazole and Ampiclause may also occur via mutation that change arbitrarily by certain physical or chemical factor [22,23].

Significant effect of antagonistic activity of *Curcuma longa*, (*Lemon juice*) *Citrus lemon* and (*Jathropha multifida*) *Coral plant* against dental pathogen reveal that it is more active than the commercially sold antibiotic which agreed with the research findings of [24] and [25] who demonstrated the methanolic extracts of thyme to be more suppressing and inhibitory against food pathogens. More so the extracts could bind to membrane proteins by hydrophobic and hydrogen bonding of the pathogens and thus changing the permeability of the membranes leading to their death [25]. The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) had close similarity with disc diffusion result. This result aligns with Bartelt *et al.*, [26] who reported that MIC and MBC interrelated with agar diffusion method. However, MIC result could help a physician in choosing from among a group of similar drugs for treatment.

The phytochemicals such as alkaloids, Flavonoids, Phenols, Tannins, Terpenoids, Saponins and phytate were all present in considerable amounts in the methanolic extract of the plant and shows promising antimicrobial effect [27]. These findings aligned with the research of Ramona Barbieri *et al.* [28] who showed that these phytochemicals are capable to affect multiple target site against the pathogens. The research shows that all plants extracts were potentially active in suppressing microbial growth of dental with variable potency [29]. The phytochemical components detected in the present study have been documented by other researchers for different antibacterial properties [30,31]. This study was also in agreement with the findings of other researchers who showed that phytochemical constituents were reported to be responsible for many antimicrobial activities of different plant species [32,33]. Flavonoids and tannins have been reported to be synthesized by plants in response to microbial infections and are good antibacterial agents [34].

Conclusion

Medicinal plants use in this research possess antimicrobial compounds that inhibited dentals pathogens. The presence of phytochemicals such as alkaloids, Flavonoids, Phenols, Tannins, Terpenoids, Saponins and

phytate suggested the usefulness of plants in drug production with better healing capacity for various dental infections and can therefore serve as antimicrobial agents in new drug formulation and recommended as an alternative medicine for management of various dental infections.

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