



Original Article

# Antimicrobial Activity of Crude Methanol and Chloroform Extracts of *Alchornea cordifolia* Leaves on Cariogenic Bacteria and Other Pathogenic Oral Bacteria

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ABSTRACT

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Dental disease places enormous financial, socio-economic and psychological burden on any society, due to the associated debilitating pain and distress that often interfere with a patient's daily activities and productivity. The situation is further worsened by the current widespread antibiotic resistance, as most organisms that were previously susceptible to common antibiotics used in dentistry, have become resistant. However, public confidence in the use of medicinal plants as alternative remedies for oral infections is rapidly growing and extracts from plants such as *Alchornea cordifolia* have long been used for the treatment of toothache and gum infections. Phytochemical studies have also revealed that extracts from *A. cordifolia* contain various biochemical compounds that mediate several pharmacological effects including antimicrobial and anti-inflammatory activities. Despite these findings, little was known concerning the antimicrobial activity of *A. cordifolia* against some common pathogenic oral bacteria. This to investigate the antibacterial effects of methanol and chloroform extracts of *A. cordifolia* leaves on a group of isolated pathogenic oral bacteria, including *S. mutans* and *Lactobacillus* sp. *A. cordifolia* leaves were collected from their natural habitat in a small swampy garden around the Otite axis of Sapele, Delta State, South-South Nigeria. Plants were collected during the rainy season and authenticated. Using bacterial cell culture and agar well diffusion techniques, crude methanol and chloroform extracts of *A. cordifolia* leaves have potent anti-bacterial effects against isolated pathogenic oral bacteria. 20 mg/ml each of crude methanol and chloroform extracts of *A. cordifolia* leaves produced average zones of inhibition ranging between 21 mm and 23 mm against *Streptococcus mutans* and 18 mm against *Lactobacillus* species. These findings suggest that polar and non-polar extracts of *A. cordifolia* could potentially function as an alternative to synthetic antibiotics in the treatment of oral infections including dental caries and its systemic sequelae.

**Key Words:** Cariogenic, pathogenic, dental pain, medicinal plant, pharmacological effect

## 1. INTRODUCTION

According to the United States Department of Health and Human Services, over 160 million hours of work in the US is lost by employed adults each year, as a result of dental disease, majority of which are associated with dental pain.

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Also, dental caries which is the most common cause of toothache, has been identified as the most common infectious disease affecting humans, caused by a group of normal oral flora including *Streptococcus* species (*S. mutans* and *S. sobrinus*), *Lactobacillus acidophilus* and *Actinomyces*<sup>1</sup>. This worrisome situation, coupled with the current widespread resistance to antibiotics commonly used in the treatment of oral infections, have caused the larger percentage of the populace to seek alternative remedies for the prevention and treatment of oral diseases, including the use of herbal products.

Over the years, extracts from medicinal plants such as *Spilanthes oleracea* and *Alchornea cordifolia* have been applied in the treatment of many oral conditions, including toothache, gum infections, sore throat and mouth ulcers, with positive results<sup>2</sup>. Despite the widespread availability, acceptance and use of various medicinal plant extracts for the prevention and treatment of oral infections, very limited effort is being made to scientifically validate the basis for their use. The paucity of scientific evidence to support the use of extracts from medicinal plants in the treatment of oral diseases could partly be attributed to the fact that oral health professionals have continually shield away from carrying out researches on such medicinal plants and preferred rather to stick to the use of conventional antibiotics, despite the current widespread failure of synthetic antimicrobial agents. The situation is further worsened by the crude methods of preparation of most of these herbal products, lack of knowledge of their active components and poor understanding of their mechanisms of action.

*Alchornea cordifolia* is a widely known medicinal plant belonging to the genus *Alchornea* of the spurge family Euphorbiaceae and comprises about 750 known species of trees, shrubs and herbs<sup>2</sup>. The plant is mainly distributed throughout the tropical forests of Africa, especially around water logged and marshy areas<sup>3</sup>. Also known as Christmas bush, Christmas tree or Dovewood, aqueous extracts of *A. cordifolia* have been used extensively in the treatment of malaria, stomach ulcers, cough, rheumatism, oral ulcers, bronchitis and toothache<sup>4</sup>. Studies have also suggested that *A. cordifolia* could possess some local and systemic anti-inflammatory effects. Phytochemical analysis of various extracts of the plant has identified compounds such as tannin, carbohydrates, flavonoids, glycosides and resins as being responsible for most of the pharmacological effects of *A. cordifolia*<sup>3</sup>.

Although many studies have been carried out to validate the use of *A. cordifolia* extracts for the treatment of many disease conditions, little is known, concerning the antimicrobial effects of *A. cordifolia* on some common pathogenic oral bacteria. Most of the pain related diseases of the oral cavity are caused by two groups of bacteria – those implicated in the pathogenesis of dental caries and those associated with periodontal diseases. Both groups of bacteria are the most common causes of toothache, through pulp and

periodontal tissue inflammation respectively. While the Gram-positive *Streptococcus mutans*, *S. sobrinus*, *Lactobacillus acidophilus* and *Actinomyces* have been widely implicated in the etiology of dental caries, the Gram-negative *Porphyromonas gingivalis*, *Actinobacillus prevotella* and *Fusobacterium* have been largely associated with periodontal diseases<sup>5</sup>. In the present study, our aim is to investigate the antibacterial effects of methanol and chloroform extracts of *Alchornea cordifolia* leaves on a group of isolated pathogenic oral bacteria, including *S. mutans* and *Lactobacillus* sp.

## 2. MATERIALS AND METHODS

### Sample collection and isolation of organisms

Informed consent was obtained from six selected patients present at the Dental Center of a tertiary healthcare facility. All selected patients had dental caries of various degrees with or without other dental conditions. Plaque samples were collected from the buccal, lingual and palatal surfaces of posterior teeth, using sterile curettes and immediately inoculated in nutrient broth (NB). Samples were properly sealed, labeled and placed in an incubator within one hour of collection. Following incubation at 37 °C for 24 hours, growth was observed in all culture media, after which primary cultures were sub cultured into six nutrient agar (NA) and six blood agar (BA) plates and incubated at 37 °C for 24 hours,

After 24 hours, bacteria growth was observed in all plates. Macroscopic (shape, elevation, surface colony and opacity) and microscopic features (as seen under the 100x magnification of a light microscope following gram staining) of all observed colonies were recorded (table 1). The different bacterial colonies were sub cultured into fresh nutrient agar (NA) and blood agar (BA) plates, incubated at 37 °C and pure bacterial isolates obtained. Slants of all pure isolates were prepared and stored in the fridge at 4 °C until needed.

### **Biochemical characterization of isolated organisms**

Following isolation of pure bacterial strains, biochemical tests including gram staining, catalase tests, coagulase tests, oxidase tests, citrate tests, and hemolytic assays were done to identify isolated pure cultures.

### **Gram staining**

Bacteria smears were prepared for all isolated organisms and heat-fixed by passing slides over flame. Slides were then flooded with crystal violet dye for one minute, after which excess dye was poured off and slides washed gently in tap water. Washed slides were dried against paper towels and smears exposed to Lugol's iodine for one minute by washing with iodine, adding more iodine and leaving it on the smears until the minute was over. After one minute, slides were washed with tap water, drained carefully and washed with 95 % alcohol for 30 seconds to decolorize the smears. Slides were again washed with tap water in order to stop decolorization and drained against paper towels.

Counterstaining was done for 30 seconds, using 0.25 % safranin, after which slides were washed, drained, blotted and examined under the microscope with oil immersion lens.

#### **Catalase test**

Clean glass slides were divided into two sections using grease pencils. One section of each slide was labeled as test and the other labeled as control. Small drops of normal saline were placed on the test and control sections. Using sterile inoculating loops, single colonies were collected from each agar slant and smeared in the drops of normal saline. One drop of hydrogen peroxide was added to each of the test smears and observed for appearance of gas bubbles. No hydrogen peroxide was added to the controls.

#### **Coagulase test**

Single colonies of each of the isolated organisms were placed on the surfaces of clean glass slides. Drops of freshly collected plasma were then placed over each colony and observed for clumping within two minutes. After two minutes, samples were shaken off the slides, with coagulase positive organisms falling off the slides, while coagulase negative organisms stuck to slides.

#### **Oxidase test**

Clean filter papers were placed in sterile petri dishes and oxidase reagent poured over the filter papers until they are adequately soaked. Single colonies were then collected from each agar slant and introduced onto the surfaces of soaked filter papers. Colonies were observed for formation of dark blue coloration after 30 seconds and recorded as oxidase positive. Oxidase negative organisms did not produce dark blue colorations.

#### **Citrate test**

Thirteen test tubes were washed properly and 5 mls of citrate reagent added into each tube. Tubes containing citrate reagent were properly labeled, sealed and sterilized. Following sterilization, all tubes were allowed to cool and single colonies from each agar slant were inoculated in their respective tubes, with exception of the last tube, which served as a negative control. All tubes were thereafter incubated at 37 °C for 24 hours and observed for turbidity. Following biochemical characterization, pure isolates were identified, (table 2) using methods previously described in Bergey's manual of systematic bacteriology.

#### Preparation of crude plant extracts

*Alchornea cordifolia* leaves were collected from their natural habitat in a small swampy garden around the Otite axis of Sapele, Delta State, South-South Nigeria. Plants were collected during the rainy season and authenticated by a plant taxonomist at the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin. Leaves were washed once under a running tap, after which they were dried initially in the sun for 72 hours and further air-dried for 24 hours at temperatures below 40 °C. Dried *A. cordifolia* leaves were grinded using a mortar and pestle, after which 100 g of powdered *A. cordifolia* leaves was weighed and introduced into clean flat bottom flasks

containing 1L of methanol and 1L of dichloromethane. Samples were left to stand for 24 hours with frequent shaking. After 24 hours, all solvents were decanted into fresh clean glass containers and filtered through fresh cotton, followed by filtration through filter papers. Filtrates were collected in clean glass beakers, after which solvents were evaporated under controlled temperature and pressure in a rotary evaporator. Following solvent evaporation, residual mass of extracts were collected, weighed, labeled and stored in the refrigerator at 4 °C until when needed.

#### Stock preparation of extracts

100 mg/ml of methanol extract of *A. cordifolia* leaves was prepared by introducing 1 g of extract into a sterile sample bottle and adding 9 mls of sterile distilled water. 5mls of sterile water was used as negative control for methanol extracts. Similarly, 100 mg/ml of dichloromethane extract of *A. cordifolia* leaves was prepared by adding 1 g extract to 2 mls of dimethyl sulphur oxide (DMSO), followed by 7 mls of sterile water. 10 % concentration of DMSO was also prepared by adding 9 mls of sterile water to 1ml of DMSO. This served as a negative control for dichloromethane extract, while 10 µg/ml of Ciprofloxacin was used as positive control for both extracts.

#### Serial dilution of organisms and pouring of agar culture plates

Pure cultures from slants were revived in nutrient broth and two-fold serial dilution of all isolated organisms prepared by adding 1ml of revived cultures to 9 mls of sterile water in sterile sample bottles. 5 mls of the above sample was collected and added to 5mls of sterile water in separate sterile sample bottles, after which samples were properly labeled with their appropriate isolates.

1 litre of nutrient agar was prepared by dissolving 28 g of nutrient agar in 1 litre of sterile water. Following sterilization, prepared agar was poured into sixty sterile culture plates and allowed to solidify. All agar plates were further dried for few minutes in an oven.

#### **Evaluation of antimicrobial activity of methanol and dichloromethane extracts of *Alchornea cordifolia* leaves**

Using sterile swabs, diluted organisms were collected and streaked onto solid nutrient agar. Streaking was done in triplicates and all areas of plates were streaked, including the edges. Following streaking, sterile metal cork borers were used to bore six wells in each agar plate, four for the different concentrations of extracts used and two for the negative and positive controls respectively.

Concentrations of 20 mg/ml, 10 mg/ml, 5 mg/ml and 2.5 mg/ml of methanol extract of *A. cordifolia* leaves were introduced into four wells in thirty plates. Also, 5mls of sterile water was introduced into the 5<sup>th</sup> wells to serve as negative control for methanol extracts, while 10 µg/ml of Ciprofloxacin was introduced into the sixth wells, to serve as positive control. Similarly, concentrations of 20 mg/ml, 10 mg/ml, 5 mg/ml and 2.5 mg/ml of dichloromethane extracts of *A. cordifolia* leaves were introduced into four

wells in a second set of thirty nutrient agar plates, while 10 % of DMSO was introduced into the fifth wells to serve as negative control for dichloromethane extracts. 10 µg/ml of Ciprofloxacin was also introduced into the sixth wells, to serve as positive control.

All plates were incubated at 37 °C for 24 hours, after which they were observed for antimicrobial effect and their zones of inhibition measured. Average zones of inhibition were calculated for all organisms and positive control – 10 µg Ciprofloxacin (figures 1, 2 and 3).

### 3. RESULTS

The aim of the present study is to examine the antibacterial effect of crude methanol and dichloromethane extracts of *A. cordifolia* leaves on some isolated pathogenic oral organisms. Plaque samples were collected from different regions of the oral cavity of patients with varying degrees of dental caries and several oral pathogens from collected samples cultured. Biochemical assays including gram staining, coagulase, catalase, oxidase and citrate tests were also done to characterize and identify isolated organisms. Following macroscopic (shape, colony surface, elevation and opacity) examination and prior to biochemical characterization, gram staining was done and cell arrangements observed under a light microscope at the 100x magnification.

**Table 1: Macroscopic and microscopic features of isolated pathogenic oral bacteria**

Serial number of isolate	Shape of isolate	Colony surface	Opacity	Elevation	Cell arrangement (under light microscope)	Gram reaction
1	Round	Moist	Opaque	Raised	Clustered cocci	Gram positive
2	Round	Moist	Opaque	Raised	Cocci in chains	Gram positive
3	Round	Moist	Opaque	Raised	Cocci in chains	Gram positive
4	Round	Moist	Opaque	Raised	Clustered cocci	Gram negative
5	Short rods	Moist	Opaque	Raised	Singling rods	Gram positive
6	Short rods	Moist	Opaque	Raised	Singling short rods	Gram negative
7	Short rods	Moist	Opaque	Raised	Clustered short rods	Gram negative
8	Short rods	Moist	Opaque	Raised	Clustered short rods	Gram positive
9	Round	Moist	Opaque	Raised	Cocci in chains	Gram positive
10	Round	Moist	Opaque	Raised	Clustered cocci	Gram positive

Combining both macroscopic features, gram staining and biochemical characterization, several organisms including *Staphylococcus aureus*, *Neisseria meningitis*, *Lactobacillus* species, *Pseudomonas aeruginosa*, *Allcaligenes* species, *Bacillus subtilis*, *Streptococcus pyogens* and *Staphylococcus epidermidis* were identified as oral pathogens isolated from collected plaque samples (table 2).

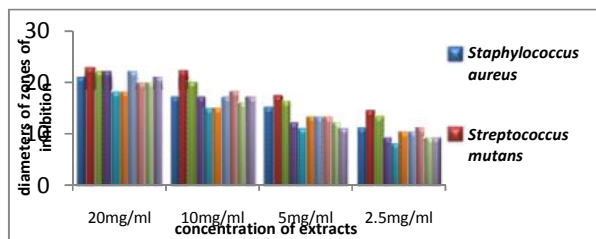
**Table 2: Biochemical characterization/identification of isolated pathogenic oral bacteria**

Serial number of isolate	Catalase test	Coagulase test	Oxidase test	Citrate test	Indole test	Identified organism
1	Positive	Positive	Negative	Positive	Positive	<i>Staph. Aureus</i>
2	Negative	Negative	Positive	Positive	Negative	<i>Strep. Mutans</i>
3	Negative	Negative	Positive	Positive	Negative	<i>Strep. Mutans</i>
4	Positive	Negative	Positive	Positive	Positive	<i>N. meningitis</i>
5	Positive	Negative	Positive	Negative	Positive	<i>Lactobacillus spp.</i>
6	Positive	Negative	Positive	Positive	Negative	<i>Pseudomonas aeruginosa</i>
7	Positive	Negative	Positive	Positive	Negative	<i>Allcaligenes spp</i>
8	Positive	Negative	Negative	Positive	Positive	<i>Bacillus subtilis</i>
9	Negative	Negative	Negative	Positive	Positive	<i>Strep. Pyogenes</i>
10	Positive	Negative	Negative	Positive	Positive	<i>Staph. Epidermidis</i>

Following identification of pathogenic oral bacteria, crude methanol and dichloromethane extracts of *A. cordifolia* leaves were obtained and tested for antibacterial activity against identified bacteria. Results obtained revealed strong antibacterial activity of methanol extracts of *A. cordifolia* leaves against all identified organisms at all concentrations (20 mg/ml, 10 mg/ml, 5 mg/ml and 2.5 mg/ml) used. Antibacterial activity of these extracts was comparable to that of the positive control, Ciprofloxacin (10 µg/ml) at their highest concentration of 20 mg/ml, as indicated by measured diameters of zones of inhibition (figure 1, 2 and 3).

Antibacterial activity of hydrophobic (dichloromethane) extracts of *A. cordifolia* leaves was slightly less than that of its hydrophilic (methanol) extracts for all concentrations used and also less than that observed for the positive control, Ciprofloxacin. The 5mls of sterile water and 10% DMSO used as negative controls for hydrophilic and hydrophobic extracts of *A. cordifolia* extracts respectively, did not show any significant antibacterial activity when compared with both extracts and positive control.

In figure 1, the diameters of the zones of inhibition for all triplet plates were measured for all concentrations of methanol extracts of *A. cordifolia* leaves and their average values taken. The greatest average zone of inhibition was observed for *Streptococcus mutans* species (23 mm, 22 mm), the most important class of pathogenic oral organisms involved in the pathogenesis of dental caries. *Lactobacillus* sp, another important pathogenic oral bacteria implicated in the pathogenesis of dental caries, had an average zone of inhibition of 18 mm in diameter.



**Fig. 1: Average zones of inhibition for methanol extracts of *A. cordifolia* leaves**



As seen in figure 2, the diameters of the zones of inhibition for all triplet plates were measured for all concentrations of dichloromethane extracts of *A. cordifolia* leaves and their average values taken. The greatest average zone of inhibition was again observed for *Streptococcus mutans* (22 mm). *Lactobacillus* species had an average zone of inhibition of 18 mm for 20 mg/ml of non-polar extracts of *A. cordifolia* leaves.

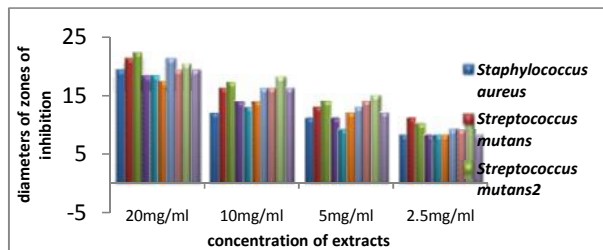


Fig. 2: Average zones of inhibition for dichloromethane extracts of *A. cordifolia* leaves

Also, in figure 3, the diameters of the zones of inhibition for all triplet plates were measured for positive and negative controls and plates with the highest values selected. 10 % DMSO and distilled water had no measurable antibacterial effects. The greatest average zone of inhibition for 10 µg Ciprofloxacin was observed for *Strep. pyogenes* (34 mm). *Streptococcus mutans* and *Lactobacillus* showed average zones of inhibitions of 32 mm and 31 mm respectively.

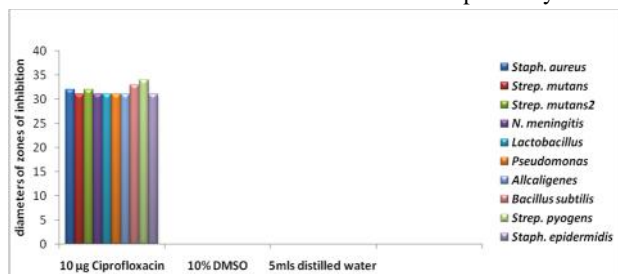


Fig. 3: Positive and negative controls - 10µg Ciprofloxacin, 10% DMSO and 5mls distilled water

#### 4. DISCUSSION

The burden of oral diseases on society is enormous and no effort should be spared towards minimizing the psychosocial and economic impact of associated toothaches on affected individuals. Among the numerous oral flora, organisms such as *Streptococcus mutans* and *Lactobacillus* sp have been implicated in the aetiopathogenesis of dental caries, an important infectious disease characterized by debilitating toothaches that could disturb a patient’s daily activities and sleep at night. These cariogenic organisms take advantage of certain dietary factors such as excessive consumption of refined sugars and compromised immune states, to become pathogenic, causing both localized and systemic diseases. Specifically, cariogenic organisms act on refined sugars such as sucrose to produce acids which degrade dental hard tissues, leading to caries formation. Dental caries is therefore the result of an imbalance between several competing factors including oral micro flora, immune status and dietary

factors. Prevention and treatment of dental caries would therefore involve regulating or eliminating any of the contributory factors that favour the development of caries. Such interventional measures include reduction in dietary intake of refined sugars, neutralization of acid content of the oral cavity, reduction of the population of cariogenic bacteria and control of associated inflammation and pain. Although conventional antibiotics have been widely used alongside cavity preparation and restoration for treatment of dental caries, inappropriate use of broad spectrum antibiotics, including post-operative prophylactic use, have resulted in widespread resistance of most pathogenic oral bacteria to common synthetic broad spectrum antibiotics. Consequently, the search for alternative antimicrobial agents against pathogenic oral bacteria has intensified in recent times, with attention being redirected towards soil microorganisms and medicinal plants as potential sources of new antimicrobial agents.

*Alchornea cordifolia* is one of such medicinal plants whose extracts have been observed to be active against toothache and some pathogenic microorganisms, including *Staphylococcus aureus*, *Staphylococcus saprophyticus*, *Salmonella typhi*, *Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa* and *Proteus mirabilis*. The present study, investigated the antimicrobial activity of crude methanol and chloroform extracts of *A. cordifolia* leaves on a group of pathogenic oral organisms, including *Strept. mutans* and *Lactobacillus* sp, two important cariogenic bacteria widely implicated in the pathogenesis of dental caries.

The results revealed antimicrobial activity of both methanol and chloroform extracts of *Alchornea cordifolia* leaves against all isolated organisms including gram positive and gram negative species. This observation suggests a broad spectrum activity of extracts as previously indicated by Okeke *et al.*<sup>6</sup>, who reported a 95.9 % inhibition of 74 isolated microbial strains by 20 mg/ml of 50% aqueous ethanol extracts of *A. cordifolia*. *Strept. mutans*, the most important cariogenic bacteria, was also observed to be the most susceptible organism, with average zones of inhibition of between 21 mm and 23 mm for 20 mg/ml of crude chloroform and methanol extracts respectively hence, validating the use of *A. cordifolia* extracts for treatment of toothaches associated with dental caries. Similarly, 20 mg/ml of both chloroform and methanol extracts of *A. cordifolia* produced average zones of inhibition of 18 mm against *Lactobacillus* sp, another important cariogenic bacteria isolated. Although both *Strept. mutans* and *Lactobacillus* sp were more susceptible to our positive control – 10 µg/ml Ciprofloxacin – with average zones of inhibition of 32 mm and 31mm respectively, the average zones of inhibition of between 18mm and 23 mm obtained for crude extracts of *A. cordifolia* leaves is encouraging, considering the fact ciprofloxacin is a pure compound, as against our extracts, which are crude extract mixtures.

Other gram positive bacteria isolated include *Staph. aureus*, *B. subtilis*, *Strept. pyogenes* and *Staph. epidermidis*, while gram negative species identified were *Neisseria meningitis*, *Pseudomonas aeruginosa* and *Alcaligenes* sp. Overall, crude methanol and chloroform extracts of *A. cordifolia* did not show any preference or better antimicrobial activity against gram positive or gram negative bacteria, as both *Strept. mutans* (a gram positive organism) and *Neisseria meningitis* (a gram negative organism) showed similar diameters of inhibition of 23 mm and 22 mm respectively, for 20 mg/ml of crude methanol extracts; and 22mm and 18mm respectively, for 20 mg/ml of crude chloroform extracts of *A. cordifolia* leaves. This observation suggests that the antimicrobial compounds present in crude methanol and chloroform extracts of *A. cordifolia* leaves may function independently of the thickness and lipid content of the peptidoglycan cell walls of bacteria. In contrast, aqueous extracts of *A. cordifolia* leaves have been observed to contain triterpenes<sup>7</sup>, which are lipophilic and inhibit the membrane structure of microorganisms<sup>8</sup>.

Furthermore, the methanol extracts of *A. cordifolia* showed a slightly better antimicrobial activity against isolated pathogenic oral organisms when compared with chloroform extracts, with average diameters of inhibition ranging from 15 mm to 22 mm, 11 mm to 17 mm and 8 mm to 14 mm for 10 mg/ml, 5 mg/ml and 2.5 mg/ml respectively, of methanol extracts; and from 12 mm to 18 mm, 9 mm to 15 mm and 8 mm to 11 mm for 10 mg/ml, 5 mg/ml and 2.5 mg/ml respectively, of crude chloroform extracts of *A. cordifolia* leaves. These findings largely suggest that the antimicrobial components of *A. cordifolia* that are active against pathogenic oral bacteria are polar, with better solubility in more polar solvents compared to less polar solvents. This observation is in consonance with previous findings by Donatien *et al.*<sup>7</sup> which concluded that aqueous (polar) extracts of *A. cordifolia* leaves produced the most potent antimicrobial activity against some isolated bacteria, with *Pseudomonas aeruginosa* being the most susceptible organism, with a zone of inhibition of 26 mm at 50 mg/ml of extracts. Although the present study did not investigate the antimicrobial activity of aqueous extracts of *A. cordifolia*, it nonetheless, demonstrated an average zone of inhibition of 18mm against *P. aeruginosa*, at 20 mg/ml of crude methanol extracts of *A. cordifolia* leaves. However, based on preceding discussion, it is very unlikely that triterpenes are the polar antimicrobial compounds present in crude methanol extracts of *A. cordifolia* leaves and are therefore equally not likely to be responsible for the extract's activity against *Strept. mutans*, the most important cariogenic organism.

Phytochemicals that have been associated with methanol extracts of *A. cordifolia* leaves in the past include phenolic acids such as gallic acid, quercetin, quercetin arabinose, galactose glycosides and tri isopentenyl guanidine, with gallic acid, protocatechuic acid and tri isopentenyl guanidine

being suggested as compounds with considerable antimicrobial activities<sup>9</sup>. Osadebe *et al.*<sup>10</sup> also demonstrated in 2003 that crude aqueous methanol extract of *A. cordifolia* leaves could possess some anti-inflammatory effects, suggesting that crude methanol extracts of *A. cordifolia* leaves could potentially provide a combined anti-inflammatory and antimicrobial activity against toothache. Other phytochemicals that have been identified in extracts from *A. cordifolia* leaves include saponins, triterpenes, steroids, polyphenols, tanins, anthocyanins and flavonoids<sup>7</sup>. Phytochemical screening of crude methanol extracts of *A. cordifolia* leaves is yet to be done by our team.

Effo *et al.*<sup>11</sup> have also previously investigated the acute toxicity and antipyretic activities of methanol extracts of *Alchornea cordifolia* leaves and observed a significant dose-dependent antipyretic activity that is comparable to that of 100 mg/kg body weight of paracetamol, suggesting a possible inhibition of biosynthesis of prostaglandins in the hypothalamus. Considering the role of prostaglandins in the initiation and maintenance of inflammation through vasodilatation, inhibition of prostaglandin biosynthesis could be a potential mechanism through which methanol extract of *A. cordifolia* leaves protect against toothache, in addition to its observed antimicrobial activities against cariogenic bacteria. This line of thought is also in consonance with submissions of Mavar-Manga *et al.*<sup>12</sup> which highlighted some anti-inflammatory protective effects of *A. cordifolia* against edema. The antipyretic activity of *A. cordifolia* leaves has been linked to the presence of saponins<sup>13</sup>, which are known to be potent inhibitors of prostaglandins<sup>14</sup>.

## 5. CONCLUSION

*A. cordifolia* is a popular African medicinal plant widely used for treatment of several disease conditions including toothaches and other painful inflammatory disorders. The methanol and chloroform extracts of *A. cordifolia* showed potent antimicrobial activity against two important cariogenic bacteria – *Strept. mutans* and *Lactobacillus* sp - as well as other pathogenic bacteria isolated from different aspects of the oral cavity of patients with varying degrees of dental caries. Although the exact mechanism of action of *A. cordifolia* against toothache is yet undefined, previous studies have identified some anti-inflammatory activities in methanol extracts of *A. cordifolia*, mediated through inhibition of prostaglandin biosynthesis. The inflammatory processes associated with dental caries and accompanying toothaches are usually the result of activities of microorganisms referred to as cariogenic bacteria. The antimicrobial activities of methanol and chloroform extracts of *A. cordifolia* leaves against cariogenic oral bacteria therefore suggest a synergistic antimicrobial and anti-inflammatory effect of these extracts hence validate the use of *A. cordifolia* leaves for the treatment of toothaches.

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