



Antibacterial activity of Methanol and Chloroform extracts of *Spilanthes oleracea* plant on isolated pathogenic oral bacteria

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ABSTRACT: Dental caries is an infectious and transmissible disease caused by the colonization of tooth surfaces by normal oral flora, including *Streptococcus* and *Lactobacillus* species. Routine use of broad spectrum antibiotics in the treatment of dental caries including postoperative prophylactic use, has led to widespread bacteria resistance to antibiotics, a situation that currently pose a global threat to public health. *Spilanthes oleracea*, popularly known as the toothache plant, is widely used for the treatment of toothache, sore throat and gum infections. Although a large number of antimicrobial effects have been reported against gram positive and gram negative species, only a small number of *S.oleracea* have been studied for biochemical activity. The crude extracts of *S. oleracea* showed very potent antimicrobial activity against a population of isolated pathogenic oral organisms. 20mg/ml each of crude methanol and dichloromethane extracts of *S. oleracea* leaves produced average zones of inhibition ranging between 21mm and 29mm (in diameter) against isolated *Streptococcus mutans* and *Lactobacillus* species, while 20mg/ml of crude methanol extracts of *S. oleracea* flowers produced average zones of inhibition of 28mm and 25mm (in diameter) against *S. mutans* and *Lactobacillus* species respectively. Findings indicate that polar and non-polar extracts of *S. oleracea* could potentially function as an alternative to synthetic antibiotics in the treatment of oral infections and dental caries.

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Dental caries is the most common infectious disease of humans, caused by *Lactobacillus acidophilus* and a group of *Streptococcus* species collectively referred to as *Streptococcus viridans*, of which *S. mutans* and *S. sobrinus* are the most important agents of human caries. (Balakrishnan *et al.*, 2000). These bacterial species, together with the Actinomycetes and a host of other microorganisms, are found abundantly in the oral cavity where they colonize tooth surfaces, including pits and fissures of teeth. However, unlike most other human infectious diseases, dental caries is the result of an imbalance of indigenous oral flora, rather than non-indigenous pathogens (Caufield *et al.*, 2005). Furthermore, cariogenic oral bacteria could invade the bloodstream, causing bacterial endocarditis, coronary artery disease, meningitis, atherosclerosis and other systemic infections. Aspiration of oropharyngeal secretions is also believed to be the most common cause of nosocomial pneumonia in the elderly (Kenneth, 2002).

Routine use of antibiotics in the treatment of dental caries, including postoperative prophylactic use, has led to widespread bacteria resistance to antibiotics.

Deepak and colleagues carried out antibiotic susceptibility tests on a group of pathogenic bacteria species isolated from carious lesions in dental patients and observed 48% resistant to Penicillin V; 60% resistance to Erythromycin, 66% resistance to Tetracycline; 78% resistance to Cloxacillin and 90% resistance to Amoxicillin. Antibiotic combinations such as Penicillin V/Amoxicillin and Amoxicillin/Erythromycin were observed to reduce antibiotic resistance to 26% and 30% respectively. (Deepak *et al.*, 2011). Unfortunately, 500mg Amoxicillin (with 90% resistance) is still being widely prescribed as postoperative prophylactic antibiotic in the management of many oral infections in Nigeria. The gravity of the problem of antibiotic resistance was highlighted by the World Health Organization, when it warned that antibiotics resistance poses a 'global threat' to public health. It further warned that the world currently faces a situation where people could begin to die from minor infections, unless urgent steps are taken to address the problem. The first effective antibiotic, Penicillin, was discovered in 1928 by Sir Alexander Fleming, after observing that colonies of *Staphylococcus*

aureus could be destroyed by the mold, *Penicillium notatum* (Bellis, 2015). The ancient Egyptians used plants such as Opium, Castor oil, Garlic and Coriander for medicinal purposes. (Falodun, 2010).

The herb *Spilanthes oleracea* belongs to the genus '*Spilanthes*' of the Asteraceae family and has over 300 species (Jayaraj *et al.*, 2013). It is known to grow throughout the tropics and has small yellow conical flowers which can be chewed to relieve toothache. (Chakraborty, 2010). The leaves of *Spilanthes oleracea* are also believed to cause numbness of the soft tissues of the mouth when eaten as salad, while extracts from different parts of the plant have been observed to possess some pharmacological effects including anesthetic; antipyretic; anticonvulsant; antioxidant; analgesic; anti-nociceptive; diuretic and antimicrobial effects (Suchita *et al.*, 2013). Despite the wide array of pharmacological effects that have been reported, only a small number of *Spilanthes* species have been studied for biochemical activity. Well planned scientific studies are still largely required, to validate the numerous pharmacological effects that have been linked to various extracts from different *Spilanthes* species. (Jayaraj *et al.*, 2013). An extensive review of the literature on *Spilanthes* species from 1920 to 2013 by Jayaraj and colleagues revealed a large number of documented antimicrobial activities of extracts from *Spilanthes* species against Gram-positive (*Bacillus cereus*, *B. pumilus*, *B. subtilis*, *Staphylococcus aureus*, *Enterobacter faecalis*, *Pseudomonas aeruginosa* and *Corynebacterium diphtheria*) and Gram-negative (*E. coli*) bacteria species. However, despite the widespread use of extracts from different *Spilanthes* species in the treatment of toothache, sore throat, gum infections and other oral diseases, no report was made, concerning their antimicrobial activities against any of the known cariogenic bacteria. The aim of this study therefore is to determine the efficacy and the antimicrobial activity of this plant extracts against cariogenic bacteria.

MATERIALS AND METHODS

Sample collection and isolation of organisms: Informed consent was obtained from six selected patients presenting 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 NA and 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 in order 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 oil.

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 ml 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: Whole plants of *Spilanthes oleracea* 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. Whole plants were washed once under a running tap, after which all leaves and flowers were detached, dried initially in the sun for 72 hours and further air-dried for 24 hours at temperatures below 40°. Dried *S. oleracea* leaves and flowers were grinded using a mortar and pestle, after which 100g each of powdered *S. oleracea* leaves was weighed and introduced into clean flat bottom flasks containing 1L of methanol and 1L of dichloromethane. Similarly, 100g of powdered *S. oleracea* flowers was introduced into flat bottom flasks containing 1L of methanol. 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, labeled and stored in the refrigerator at 4°C until when needed.

Stock preparation of extracts: 100mg/ml of methanol extract of *S. oleracea* leaves was prepared by introducing 1g of extract into a sterile sample bottle and adding 10mls of sterile water. 10mls of sterile water was also added to 1g of methanol extract of *S. oleracea* flowers in a sterile sample bottle to give a 100mg/ml concentration. 5mls of sterile water was used as negative control. Similarly, 100mg/ml concentration of dichloromethane extract of *S. oleracea* leaves was prepared by adding 1g extract to 2mls of Dimethyl sulphur oxide (DMSO), followed by 8mls of sterile water. 10% concentration of DMSO was also prepared by adding 9mls of sterile water to 1ml of DMSO. This served as a negative control for non-polar extracts, while 10µg/ml of Ciprofloxacin was used as positive control for all 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 9mls of sterile water in sterile sample bottles. 5mls 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 liter of nutrient agar was prepared by dissolving 28g of nutrient agar in 1 liter of sterile water. Following sterilization, prepared agar was poured into ninety 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 *S. oleracea* leaves and methanol extracts of *S. oleracea* flowers: 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 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 20mg/ml, 10mg/ml, 5mg/ml and 2.5mg/ml of methanol extract of *S. oleracea* leaves were introduced into four wells in thirty plates. Also, 5mls of sterile water was introduced into the 5th wells to serve as negative control for methanol extracts, while 10µg/ml of Ciprofloxacin was introduced into the sixth wells, to serve as positive control. The above procedure was repeated for methanol extracts of *S. oleracea* flowers

in a second set of thirty nutrient agar plates. Similarly, concentrations of 20mg/ml, 10mg/ml, 5mg/ml and 2.5mg/ml of dichloromethane extracts of *S. oleracea* leaves were introduced into four wells in a third set of thirty nutrient agar plates, while 10% of DMSO was introduced into the fifth wells to serve as negative control for dichloromethane extracts. 10µg/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 - 10µg Ciprofloxacin (figures 1, 2, 3 and 4).

RESULTS AND DISCUSSION

The aim of the present study is to examine the antibacterial effect of crude methanol extracts of the leaves and flowers of *Spilanthes oleracea* and crude dichloromethane extracts of *Splanthes oleracea* 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).

Table 1: Macroscopic features and gram staining 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

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>

Combining both macroscopic features, gram staining and biochemical characterization, several organisms including *Staphylococcus aureus*, *Streptococcus mutans* (x2), *Neisseria meningitis*, *Lactobacillus species*, *Pseudomonas aeruginosa*, *Allcaligenes species*, *Bacillus subtilis*, *Streptococcus pyogenes* and *Staphylococcus epidermidis* were identified as oral pathogens isolated from collected plaque samples (table 2). Following identification of pathogenic oral bacteria, crude methanol extracts of *S. oleracea* leaves and flowers and dichloromethane extracts of *S. oleracea* leaves were obtained and tested for antibacterial activity against identified bacteria.

Results obtained revealed strong antibacterial activity of methanol extracts of *S. oleracea* leaves and flowers against all identified organisms at all concentrations (20mg/ml, 10mg/ml, 5mg/ml and 2.5mg/ml) used. Antibacterial activity of these extracts was comparable to that of the positive control, Ciprofloxacin (10µg/ml) at their highest concentration of 20mg/ml, as indicated by measured diameters of zones of inhibition (Figures 1, 2, 3 and 4). Antibacterial activity of hydrophobic (dichloromethane) extracts of *S. oleracea* 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 *S. oleracea* 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 *S. oleracea* leaves and their average values taken. The greatest average zone of inhibition was observed for *S. mutans* species (28mm, 29mm), the most important class of pathogenic oral organisms involved in the pathogenesis of dental caries. *Lactobacillus*, another important pathogenic oral bacteria implicated in the pathogenesis of dental caries, had an average zone of inhibition of 24mm in diameter.

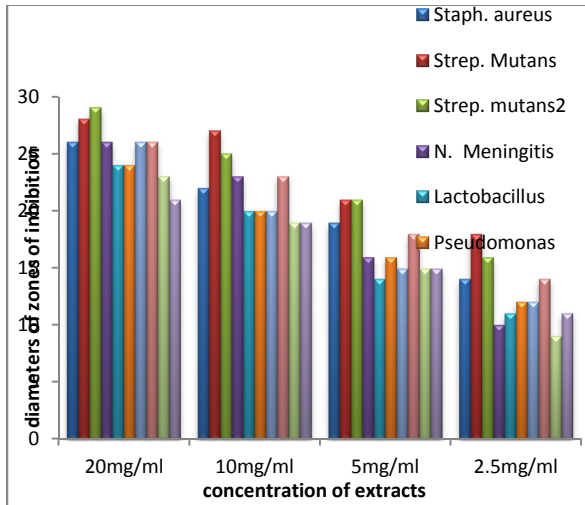


Fig. 1: Average zones of inhibition for methanol extracts of *S. oleracea* leaves

In figure 2 the diameters of the zones of inhibition for all triplet plates were measured for all concentrations of dichloromethane extracts of *S. oleracea* leaves and their average values taken. The greatest average zone of inhibition was observed for *S. mutans* and *N. meningitis* species (27mm each). *Lactobacillus* species had an average zone of inhibition of 21mm for 20mg/ml of non-polar extracts of *S. oleracea* leaves.

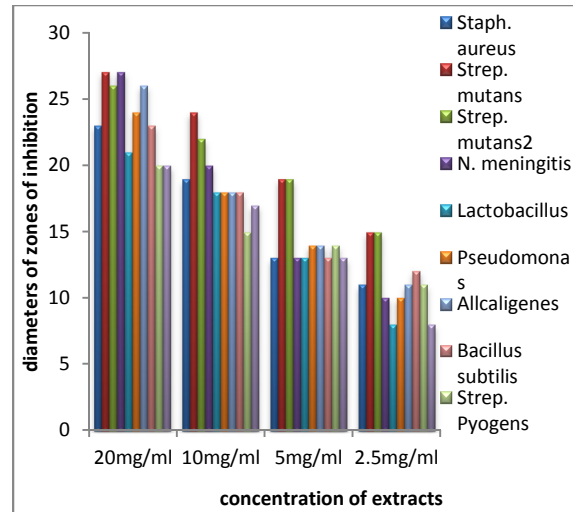


Fig. 2: Average zones of inhibition for dichloromethane extracts of *S. oleracea* leaves

In figure 3 the diameters of the zones of inhibition for all triplet plates were also measured for all concentrations of methanol extracts of *S. oleracea* flowers and their average values taken. The greatest average zone of inhibition was again observed for *Streptococcus mutans* species (28mm each), while *Lactobacillus* species had an average zone of inhibition of 25mm for 20mg/ml of non-polar extracts of *S. oleracea* flowers.

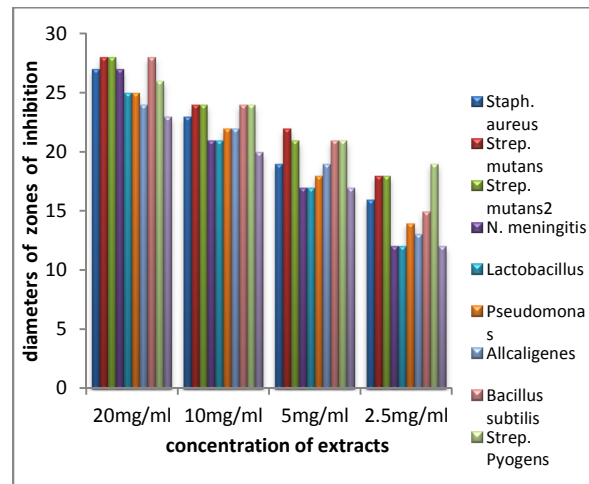


Fig. 3: Average zones of inhibition for methanol extracts of *S. oleracea* flowers

While in figure 4, 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 10µg Ciprofloxacin was observed for *Strep. pyogens* (34mm). *S. mutans* and *Lactobacillus* showed

average zones of inhibitions of 32mm and 31mm respectively.

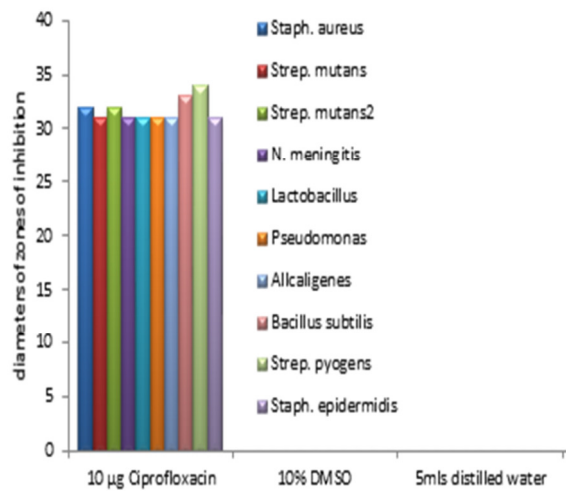


Fig. 4: Controls with 10µg Ciprofloxacin as positive, 10% DMSO and 5mls distilled water as negative

The oral cavity of an adult human is home to about 500 to 1000 bacteria species as components of the normal oral flora, with about 100 to 200 species being present at any given time. (Oyarekua *et al.*, 2015). Different studies have identified numerous organisms, including bacteria species such as *Staphylococcus salivarius*, *Enterococcus faecalis*, *Streptococcus pneumoniae*, *Streptococcus pyogens*, *Neisseria meningitides*, *Escherichia coli*, *Lactobacilli*, *Pseudomonas aeruginosa*, *Corynebacteria*, *Actinomyces* (Oyarekua *et al.*, 2015) and fungal organisms including *Candida* species (75%), *Cladosporium* (65%), *Aspergillus* (35%), *Fusarium* (30%), *Aureobasidium* and *Saccharomycetals* (50%) (Ghannoun *et al.*, 2010). Majority of these organisms are not indigenous to several other sites of the human body and can therefore invade such sites, causing severe infectious diseases in the process. However, unlike most other infectious diseases, dental caries is the result of an imbalance of indigenous oral flora, rather than presence of non-indigenous pathogens. (Caufield *et al.*, 2005). Among these indigenous oral flora, *Streptococcus*, *Lactobacillus* and *Actinomyces* species have been largely implicated in the pathogenesis of dental caries (Balakrishnan *et al.*, 2000). When abundantly present within the oral cavity, these cariogenic organisms colonize the tooth surfaces and act on refined sugars to produce acids, which degrade dental hard tissues, leading to caries formation. Reducing the population of cariogenic bacteria within the oral cavity could therefore function as a viable way of preventing or reducing

the incidence of dental caries, as well as treating the disease.

Spilanthes oleraea is an annual herb belonging to the Asteraceae family of the tribe Heliantheae and sub tribe Spilanthinae (Lavanya *et al.*, 2012). A search through the literature from 1920 to 2013 by Jayaraj *et al* revealed over 300 species of *Spilanthes*, distributed all over the tropics, having originated from tropical Africa and South America. The search also revealed that the different *Spilanthes* species are applied in the treatment of over 60 different disease conditions (Jayaraj *et al.*, 2013). Previous studies on *Spilanthes acmella* was able to demonstrate antimicrobial activity against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pyogens*, *Enterococcus faecalis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Salmonella typhi* and *Shigella dysenteriae*. Agar dilution studies on *Spilanthes acmella* have also revealed antimicrobial activity against 27 strains of gram positive and gram negative bacteria. (Supaluk *et al.*, 2009).

Similar studies in the past have also suggested multiple pharmacological activities including antipyretic, anticonvulsant, antioxidant, vasorelaxant, antimalarial, larvicidal, antinociceptive, immunomodulatory and analgesic activities (Suchita *et al.*, 2013). Extracts from different *Spilanthes* species are used for different purposes in different countries around the world, with treatment of toothache being a universal application of the plant, hence the name 'toothache' plant.

Traditionally, the flower heads or leaves of the plant is chewed or crushed and placed inside carious cavities on affected teeth, to relieve dental pain (Jayaraj *et al.*, 2013). In India, extracts from *Spilanthes* species are also used for treatment of mouth ulcers, boils and wounds (Pushpangadan and Atal, 1986), while traditional medicine practitioners in Ethiopia use extracts from the plant for treatment of external injuries (Teklehaymanot, 2007). The plant is also used for treatment of sialagogue in Nigeria (Dalziel, 1937) and Sri Lanka (Jayaweera, 1981) and for induction of labor during childbirth in Uganda (Kamatenesi-Mugisha and Oryem-Origa, 2007). Chinese traditional healers use extracts from *Spilanthes* species for treatment of amenorrhea, snake bite and rheumatic fever.

The widespread use of extracts from *Spilanthes* species and their efficacy is also evidenced by the numerous applications of these extracts in both pharmaceutical and cosmetic industries, just as patents on these products are increasing rapidly, with

over 30 patents already registered by the United States Patent and Trademark office since 1976 (Jayaraj *et al.*, 2013). Healthcare products including skin care, oral health and antifungal products from *Spilanthes* species have also received patents in the United Kingdom and Australia. Examples of such products include Dentaforce herbal mouth spray (containing extracts from *Spilanthes oleracea*), Dentaforce herbal mouthwash, aftershave creams, Gatuline from Gatefosse and skin firming light creams (from *Spilanthes acmella* flower extracts).

Phytochemicals present among *Spilanthes* species include alkaloids, terpenoids, lactones, flavonoids, hydrocarbons, alkyl ketones, coumarins and acetylenes (Jayaraj *et al.*, 2013). These phytochemicals mediate the numerous pharmacological activities of *Spilanthes* species, including *Spilanthes oleracea*. However, alkyl amides are believed to be predominantly present and responsible for the plant's effectiveness against toothache and ability to induce and enhance secretion of saliva (Sharma *et al.*, 2011). Isolated alkylamides, especially spilanthol (E, E, Z) – 2, 6, 8 – decatrienoic acid N-isobutylamide from *Spilanthes acmella*, *Spilanthes mauritiana* and *Spilanthes oleracea*, have also been observed to be largely responsible for the local anesthetic, analgesic, antibacterial, sialagogue and antifungal activities of these *Spilanthes* species (Jayaraj *et al.*, 2013). Spilanthol is also believed to modulate chemosensory receptors and their ligands, with its derivatives having potent sensory effects on trigeminal nerves, including burning, tingling, numbing, warming, cooling, pungency and mouthwatering (salivating) effects (Jakob *et al.*, 2006).

Despite widespread use of extracts from the numerous *Spilanthes* species for treatment of many disease conditions, only a few of these species have been studied for chemical and pharmacological activities. Although believed to have originated from Africa and South America, scientific studies aimed at validating the pharmacological activities of local *Spilanthes* species are either minimal or completely lacking in Africa, especially Nigeria. For instance, an extensive literature survey did not reveal any published study so far on any *Spilanthes* specie in Nigeria, despite the fact that the plant was linked to the country by J. M. Dalziel in faraway London, as far back as 1937. Absence of this plant from existing literature on medicinal plants in Nigeria may be due to a threatening extinction of the plant, resulting from years of neglect. This is coupled with the acute shortage of facilities and expertise needed for extensive and well co-ordinated research aimed at

validating observed pharmacological effects of native *Spilanthes* species in pre and post-independence Nigeria.

However, a few herbal medicine practitioners around the Itsekiri-speaking tribe of Delta State, South-South Nigeria, continue to cultivate the plant, locally referred to as 'Otumale' and apply its extracts for treatment of toothache. Following isolation and biochemical characterization of some pathogenic oral bacteria from different regions of the oral cavity of six patients with varying degrees of dental caries, we evaluated the antibacterial activity of methanol extracts of locally collected *Spilanthes oleracea* leaves and flowers and chloroform extracts of collected leaves. Nine gram positive and gram negative bacteria including *Staphylococcus aureus*, *Streptococcus mutans* (isolated from two samples), *Neisseria meningitides*, *Pseudomonas aeruginosa*, *Streptococcus pyogenes*, *Lactobacilli*, *Allcaligenes*, *Staphylococcus epidermidis* and *Bacillus subtilis* were identified in collected samples.

Our results indicated a dose dependent antimicrobial activity of *S. oleracea* extracts against all isolated organisms, including gram positive and gram negative species, suggesting a broad spectrum activity of extracts. Overall, methanol extracts of *S. oleracea* flowers showed the most potent antimicrobial activity against isolated organisms, with average diameters of zones of inhibition ranging between 23mm and 28mm for 20mg/ml of extracts and 12mm and 18mm for 2.5mg/ml of extracts respectively. These findings compared favorably with the antimicrobial activity of 10µg/ml of ciprofloxacin, the positive control, which produced average diameters of zones of inhibition ranging between 31mm and 34mm against isolated oral pathogens. The difference in activity between extracts of *Spilanthes oleracea* and the standard antibiotic, ciprofloxacin, is attributable to the presence of mixtures of compounds in crude plant extracts, as against pure compounds present in synthetic antibiotics. No antimicrobial activity was observed for 10% DMSO and 5mls of distilled water, our negative controls.

In contrast, chloroform extracts of *S. oleracea* leaves showed the least observed antimicrobial activity against isolated organisms, with average diameters of zones of inhibition ranging between 20mm and 27mm for 20mg/ml of extracts and between 8mm and 15mm for 2.5mg/ml of crude extracts respectively. These findings suggest that the active antimicrobial components of *S. oleracea* plants are polar hence more soluble in highly polar solvents, when

compared with less polar and non-polar solvents. The antimicrobial activity of aqueous extracts of *S. oleracea* plant is however, yet to be studied by our team.

Furthermore, 20mg/ml of crude methanol extracts of *S. oleracea* leaves and flowers produced the highest average diameter of zones of inhibition of 29mm and 28mm respectively, against *Streptococcus mutans*, the most commonly implicated organism in the pathogenesis of dental caries. Similarly, the greatest antimicrobial activity for 20mg/ml of crude chloroform extracts of *S. oleracea* leaves produced an average diameter of zones of inhibition of 27mm and this was observed against *Streptococcus mutans* as well. These findings suggest that polar and non-polar extracts of *S. oleracea* plant are mostly potent against *Streptococcus mutans*, when compared with other cariogenic oral bacteria like *Lactobacillus*.

Although *Streptococcus mutans* was observed to be the most susceptible organism to both polar and non-polar extracts of *S. oleracea* plant, other pathogenic oral organisms including *Lactobacillus*, *Streptococcus pyogenes* and *Staphylococcus epidermidis* also showed modest and encouraging susceptibility to polar and non-polar extracts of *S. oleracea* plant. These observations largely confirm the effectiveness of the plant's extracts against pathogenic oral organisms and validate their use in the treatment of toothaches and other oral infections such as sore throat and gum infections.

Apart from toothaches and other oral infections, our findings also suggest a potential use of extracts of *S. oleracea* plants against many other infectious diseases caused elsewhere in the body, as a result of systemic spread of normal oral flora. Examples of such infectious diseases include those caused by *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa* and *Neisseria meningitides*, among others. For instance, 20mg/ml of methanol extracts of *S. oleracea* flowers and 20mg/ml of chloroform extracts of *S. oleracea* leaves both produced an average diameter of zones of inhibition of 27mm against isolated *Neisseria meningitides*, suggesting a potential use of these extracts in the treatment of meningitis, an important infectious disease condition of the brain, prevalent in Northern Nigeria.

However, the effectiveness of *S. oleracea* plant extracts against meningitis and other infectious diseases caused by *Staphylococcus aureus*, *pseudomonas aeruginosa*, *Staphylococcus epidermidis* and *Streptococcus pyogenes* is yet to be

elucidated. Further studies are also required, to isolate, identify and characterize the active principles responsible for observed antimicrobial effects of polar and non-polar extracts against cariogenic bacteria as well as other pharmacological activities that have been associated with extracts from *Spilanthes oleracea* plant.

Conclusion: In the area of preventive dentistry, active principles of *S. oleracea* can potentially be incorporated into herbal toothpastes and other oral formulations such as mouthwashes and mouth rinses, in order to provide some antibacterial effects in these oral products. Similarly, oral formulations from *Spilanthes oleracea* extracts can potentially be incorporated into plastic and acrylic dentures and other dental appliances, to provide a slow and continuous antibacterial effect against cariogenic bacteria like *Streptococcus mutans*.

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