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Antimicrobial Activity Of *Anacardium occidentale* On Some Microorganisms Associated With Dental Diseases.

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ABSTRACT

Dental disease has become a major problem in all over the world, and current antibiotics has almost become ineffective for its treatment. Hence there is a need to find alternative ways of treatment for dental disease. *Anacardium occidentale L.* having family Anacarddiaceae is frequently used to treat infections. *Anacardium occidentale* is a medium size tree spreading evergreen, much branched, costal sandy areas. There is different information on the pharmacological activitie of *Anacardium occidentale (*cashew tree) byproducts in various dental disease such as periodontal disease, dental plaque, dental biofilm bacteria etc. The objective of this review is the current knowledge on the phytochemestry and pharmacology of *Anacardium occidentale* is updated with some description of their uses in dental diseases.

Keywords: *Anacardium occidentale,* dental disease, periodontal disease, dental plaque, dental biofilm bacteria etc.

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INTRODUCTION

DENTAL DISEASES AND ORAL HEALTH

Dental diseases are the most prevalent chronic diseases worldwide, and a costly burden to health care services. The treatment of dental diseases is expensive, accounting for between 5% and 10% of total health care expenditures in industrialized countries. In most developing low-income countries, the prevalence rate of dental caries is high and more than 90% of caries is untreated. Dental diseases include dental caries, developmental defects of enamel, dental erosion and periodontal disease.

DENTAL CARIES

Dental caries is an infectious microbiological disease of the teeth that results in localized dissolution and destruction of the calcified tissues. It is the second most common cause of tooth loss and is found universally, irrespective of age, sex, caste, creed or geographic location. It is considered to be a disease of civilized society, related to lifestyle factors, but heredity also plays a role. In the late stages, it causes severe pain, is expensive to treat and leads to loss of precious man-hours. However, it is preventable to a certain extent. The prevalence of dental caries in India is 50%–60%.

Aetiology

An interplay of three principal factors is responsible for this multifactorial disease.

- Host (teeth and saliva)
- Microorganisms in the form of dental plaque
- Substrate (diet)

Thus, caries requires a susceptible host, carcinogenic oral flora and a suitable substrate, which must be present for a sufficient length of time.

Host factors

Teeth[1-4]

- *Composition:* Deficiency in fluorine, zinc, lead and iron content of the enamel is associated with increased caries.
- *Morphological characteristics:* Deep, narrow occlusa fissures, and lingual and buccal pits tend to trap food debris and bacteria, which can cause caries. As teeth get worn (attrition), caries declines.
- *Position:* The interdental areas are more susceptible to dental
- *Caries*. Malalignment of the teeth such as crowding, abnormal spacing, etc. can increase the susceptibility to caries.

Saliva [5-8]

Saliva has a cleansing effect on the teeth. Normally, 700–800 ml of saliva is secreted per day. Caries activity increases as the viscosity of the saliva increases. Eating fibrous food and chewing vigorously increases salivation, which helps in digestion as well as improves cleansing of the teeth? The quantity as well as composition, pH, viscosity and buffering.

- *Quantity:* Reduced salivary secretion as found in xerostomia and salivary gland aplasia gives rise to increased caries activity.
- *Composition:* Inorganic fluoride, chloride, sodium, magnesium, potassium, iron, calcium and phosphorus are inversely related to caries. Organic—ammonia retards plaque formation andneutralizes the acid.
- *pH:* A neutral or alkaline pH can neutralize acids formed by the action of microorganisms on carbohydrate food substances.

- *Antibacterial factors:* Saliva contains enzymes such as lactoperoxidase, lypozyme, lactoferrin and immunoglobulin (Ig)A, which can inhibit plaque bacteria.

Dental plaque [9-12]

Dental plaque is a thin, tenacious microbial film that forms on the tooth surfaces. Microorganisms in the dental plaque ferment carbohydrate foodstuffs, especially the disaccharide sucrose, to produce acids that cause demineralization of inorganic substances and furnish various proteolytic enzymes to cause disintegration of the organic substances of the teeth, the processes involved in the initiation and progression of dental caries. The dental plaque holds the acids produced in close contact with the tooth surfaces and prevents them from contact with the cleansing action of saliva.

Substrate[13-16]

The role of refined carbohydrates, especially the disaccharide sucrose, in the aetiology of dental caries is well established. The total amount consumed as well as the physical form, its oral clearance rate and frequency of consumption are important factors in the aetiology. Vitamins A, D, K, B complex (B6), calcium, phosphorus, fluorine, amino acids such as lysine and fats have an inhibitory effect on dental caries.

Causes of dental caries [17-20]

Direct	Indirect	Distant
1. Tooth • Structure·fluoride content and other elements such as zinc, lead, iron. • Morphology·deep pits and fissures · • Alignment·crowding 2. Microorganisms·dental plaque accumulation due to poor oral hygiene 3. Diet •Intake of refined carbohydrates such as sucrose, maltose, lactose, glucose, fructose, cooked sticky starch, etc. -quantity; frequency, physical form; oral clearance rate • Saliva (quantity and quality) ·reduced secretion (xerostomia) increases caries ·Viscosity: more viscous, more caries ·pH: alkaline pH neutralizes acid, less caries ·enzymes: lactoperoxidase, lysozyme lactoferrins ·immunoglobulins IgA	• Poor contact between the teeth resulting in food impaction and caries due to the following causes. -malalignment of the teeth (crowding) -loss of some teeth and failure to replace them • Gingival recession leading to root caries	• Socioeconomic status • Literacy level • Location-Urban, Rural • Age • Sex • Dietary habits • Climatic conditions and soil type • Social and cultural practices • Availability/access to health care facility • Health insurance

Indirect causes

- Loss of some natural teeth and failure to replace them results in drifting of the teeth in the edentulous space. This leads to increased food impaction between the teeth and formation of new carious lesions.

- Malalignment of the teeth, especially crowding, does not allow proper cleaning between the teeth and leads to an increased incidence of caries.
- Gingival recession, abrasion and abfraction defects at the neck of the tooth increase root caries.
- Selenium in the soil increases the formation of caries while molybdenum and vanadium decrease it.
- A high temperature is associated with a lower prevalence of caries. Water has a cleansing effect on the teeth. If the fluoride content of the water is at an optimum concentration, it will also exert an anticaries effect.

Distant causes

- A low socioeconomic and literacy status is associated with caries.
- Urbanization is linked to an increased incidence of caries.
- Caries is more common in childhood and adolescence, and after 60 years of age, when the incidence of root caries is higher.
- Females develop caries more often than males.
- Non-vegetarians develop caries more often than vegetarians.
- Availability/access to a health care facility can affect utilization of health care services.
- Lack of oral health insurance promotes oral neglect and increases disease levels.

PERIODONTAL DISEASE

Periodontal diseases are one of the major causes of tooth loss in India. These include pathological conditions of the supporting structures of the teeth, i.e. gingival, alveolar.

Bone, periodontal ligament and cementum. Gingival and periodontal diseases affect 90% of the population. Gingival disease progresses to periodontal disease, if not checked in time.

Aetiology

Direct causes [21-26]

These include poor oral hygiene leading to accumulation of dental plaque and calculus, and traumatic occlusion.

Indirect causes [27-38]

- Malnutrition (deficiency of vitamins A and C, niacin and protein) is associated with a higher prevalence of periodontal diseases.
- Endocrine disturbances including physiological causes such as puberty, pregnancy, menopause, and pathological causes such as hyperthyroidism, hyperparathyroidism and diabetes may aggravate existing periodontal disease.
- Decreased immunity as in persons with HIV and those on immunosuppressive drugs.
- Blood disorders such as acute monocytic leukaemia and pernicious anaemia can lead to periodontal diseases.
- Malalignment of the teeth interferes with proper plaque control.
- Tobacco smoking and chewing reduce tissue resistance and increase the susceptibility to periodontal diseases.
- An improper brushing technique, besides resulting in inadequate plaque removal, can also cause gingival recession.
- Drugs certain drugs such as phenytoin sodium and nifedipine can cause gingival hyperplasia.

Distant causes [39-45]

These include low socioeconomic and literacy level, difficult access to an oral health care facility, poor oral health awareness, and lack of oral health insurance. Stress is known

to predispose to acute necrotizing ulcerative gingivitis.

The term 'periodontal diseases' encompasses a wide variety of chronic inflammatory conditions of the gingiva (or gums, the soft tissue surrounding the teeth), bone and ligament (the connective tissue collagen fibres that anchor a tooth to alveolar bone) supporting the teeth Periodontal disease begins with gingivitis, the localized inflammation of the gingiva that is initiated by bacteria in the dental plaque, which is a microbial biofilm that forms on the teeth and gingiva In this Primer, the term gingivitis refers to plaque-induced gingivitis. Chronic periodontitis occurs when untreated gingivitis progresses to the loss of the gingiva, bone and ligament, which creates the deep periodontal 'pockets' that are a hallmark of the disease and can eventually lead to tooth loss. Periodontal disease may contribute to the body's overall inflammatory burden, worsening conditions such as diabetes mellitus and atherosclerosis1–3. Chronic periodontitis is classified as generalized chronic periodontitis when it affects >10 of the 32 teeth in the human dentition and localized when fewer teeth are involved [47-50]. Although gingivitis and chronic periodontitis are initiated and sustained by the microbial biofilm of the dental plaque, genetic and environmental host factors influence the rate of the disease. Periodontal diseases are currently considered to share a similar aetiopathogenesis. In this Primer, we focus on the mechanisms, diagnosis, prevention and management of gingivitis and chronic periodontitis, which are the most common types of periodontal diseases; unless otherwisestated, the discussion of peri-implant disease (peri-implant mucositis and peri-implantitis) does not differ. Similarly, we highlight any differences between gingivitis and chronic periodontitis and aggressive and necrotizing forms of periodontal disease.

Epidemiology

Prevalence

Periodontitis is prevalent in adults but may also occur in children and adolescents; the amount of tissue destruction is generally commensurate with dental plaque levels, host defences and related risk factors. A key feature of both chronic and aggressive periodontitis is site specificity: the characteristic periodontal pockets and the accompanying attachment loss and bone loss do not occur uniformly throughout the dentition Consequently, the definition of a case of periodontitis heavily depends on which specific thresholds for both disease extent (the number of affected teeth) and disease severity (the magnitude of pocket depth, clinical attachment loss and alveolar bone loss at the affected teeth) are used. Because no sets of thresholds have been consistently used in epidemiological studies, estimates of the prevalence of periodontitis across populations vary substantially. A frequently used composite case definition of periodontitis, based on a combination of clinical attachment loss and probing depth (that is, the measurement of the pocket depth introduced by the US Centers for Disease Control and Prevention and the American Academy of Periodontology, has resulted in prevalence estimates in excess of 50% in the United States5 and the conclusion that periodontitis is ubiquitous in elderly individuals. Such findings have raised questions whether these case definitions are suitable for estimating prevalence across the entire age range. [51]

By contrast, epidemiological studies that used continuous measures of probing depth and clinical attachment loss (that is, the percentage of teeth in the dentition that present with pockets or clinical attachment loss that are above specific millimetre thresholds) have shown that advanced forms of periodontitis that result in severe loss of supporting structures and substantial tooth loss affect 10–15% of the population globally [52]. This estimated prevalence range includes both severe aggressive periodontitis (which primarily affects adolescents or young adults[53-54] and severe chronic periodontitis (which primarily affects adults and whose prevalence increases with age in all populations [55-56].

Risk factors

Several risk factors have been established, some of which are modifiable (amenable to intervention [57]. Cigarette smoking is a major modifiable risk factor for chronic periodontitis, as shown in association, progression and intervention studies [58-59], with attributable risk estimates ranging between 2.5 and 7.0. Smokers have worse periodontal status and experience more-severe tooth loss than non-smokers, after adjustments for covariates; prospective studies have shown higher progression rates of chronic periodontitis and tooth loss, and treatment studies have shown inferior outcomes of both non-surgical and surgical periodontal therapy in smokers compared with non-smokers. Notably, signs of gingival inflammation can be

less pronounced in smokers than in non-smokers, because of vasoconstriction and enhanced gingival tissue keratinization.

Diabetes mellitus is the most prevalent and researched systemic disease that predisposes to periodontitis. The prevalence and severity of periodontitis are increased in individuals who have diabetes mellitus of long duration, and, in particular, in patients with poorly controlled diabetes mellitus. Conversely, chronic periodontitis can have a negative effect on metabolic control in individuals with diabetes mellitus, as it contributes to an increased inflammatory burden and enhanced insulin resistance [60-61]. Notably, the negative effects of diabetes mellitus on the periodontium manifest at a young age, affecting children and adolescents with type 1 or type 2 diabetes mellitus [62-63].

Epidemiological studies in the United States have shown that low educational attainment, income below the federal poverty line, Mexican-American ethnicity and African-American ethnicity have all been associated with poor periodontal status in multivariable analyses [64]. A systematic review has confirmed the global association between specific socioeconomic and demographic variables and chronic periodontitis. Finally, psychosocial variables have also been associated with various forms of periodontal disease, but most of the literature on stress and periodontal conditions is dated, such as the reports of acute necrotizing ulcerative gingivitis observed in soldiers on the front line during World War I. Stress is considered to be immunosuppressive, and acute necrotizing ulcerative gingivitis can occur in immunosuppressed individuals (for example, patients with HIV infection), but there are insufficient data to precisely determine the role of psychosocial factors as risk factors for periodontitis [65].

Genetic predispositions have been considered to be important for both the onset and the progression of periodontitis [66], with heritability estimates as high as 50% [67]. However, the nine genome-wide association studies that is available so far [68-78] have failed to consistently identify specific single-nucleotide polymorphisms across populations. In contrast to Mendelian diseases, in which the pathological phenotype is typically the result of an abnormality that affects a single gene, genetic predisposition to chronic periodontitis is probably conferred collectively by hundreds or thousands of genes, whereas the clinical phenotype is defined by the interplay between environmental, genetic and epigenetic factors. Epigenetic factors have gained attention only recently, and additional research on their role is expected [79].

Mechanisms/pathophysiology

The dental plaque

Chronic gingivitis and chronic periodontitis are initiated and sustained by the microorganisms of the dental plaque [80]. Indeed, the microbial biofilm has been extensively studied and can comprise around 150 species in a single person, and up to 800 different species have been identified in human dental plaque so far [81]. The debate on which species are particularly virulent and can drive disease onset has lasted decades and is not resolved [82-83]. Putative pathogens include Gram-negative anaerobic bacteria, spirochetes and even viruses, but it is probable that no single pathogen is causative on its own but rather that dysbiosis (an imbalance of the microbial biofilm) itself is the pathogenic 'unit' [85]If periodontal

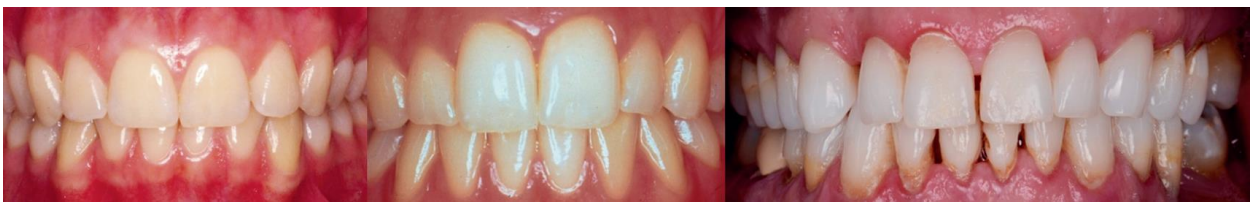

Figure 1 | Healthy and diseased periodontium. a | Healthy periodontal tissues. **b** | Early gingival inflammation (gingivitis; arrow) can be seen in the gingiva between the central incisor teeth. **c** | Clinical appearance of chronic periodontitis, with tissue loss and deep periodontal 'pockets' that are a hallmark of disease (arrow). disease was caused by one or a few specific pathogens, the preferred therapeutic strategy would be a targeted alteration of the plaque microbiota rather than total biofilm removal.[84]

Microbial biofilm

Aggressive forms of periodontal disease have been associated with colonization by specific clones of *Aggregatibacter actinomycetemcomitans* in prospective cohort studies[85]. Other species, including *Porphyromonas gingivalis*, have also been associated with severe or progressive periodontitis[85], but the temporality (the change over time) of the microbial biofilm and its association with periodontitis are less clearly established. A systematic review[86] concluded that aggressive and chronic periodontitis could not be discriminated based on specific periodontal pathogens, a finding that suggests that the causative microbial biofilm is similar in both diseases. High-throughput sequencing technologies that characterize the entire periodontal microbial biofilm are expected to substantially expand our knowledge on the microbial determinants of chronic periodontitis on the population level.

Most individuals have experienced multiple viral infections in their lifetime, and viral DNA or RNA can still be detected in body tissues long after signs of infection have dissipated, and these dormant viruses can reawaken during inflammation flare-ups[87]. Thus, it is difficult to establish a cause–effect correlation between increased viral presence and periodontal disease, and correlations between periodontal disease and herpes-viruses might simply be epiphenomena[88]. Accordingly, the role of viruses in the aetiopathology of periodontal disease is controversial. However, antiviral therapy reduced the pocket depth and inflammation in patients with periodontal disease when used adjunctively with conventional therapy[89], and is, therefore, recommended for periodontal treatment by some clinicians[90.]

Calcification

Dental plaque is present in both uncalcified (soft) and calcified (calculus) forms: supragingival (on the oral and tooth surfaces) plaque is usually uncalcified, whereas subgingival (in the crevice between the gingival margin and the neck or root of the tooth) plaque is typically dark in colour and calcified. Subgingival calculi are more difficult to remove. Calcification of subgingival plaque is caused by ions from the serum transudate induced by the inflammation in the periodontal tissues, whereas a supragingival calculus results from salivary calcium and phosphate ions that aggregate within the plaque[91]

INTRODUCTION OF PLANTS (*Anacardium occidentale)(Anacarde danida)*

General introduction

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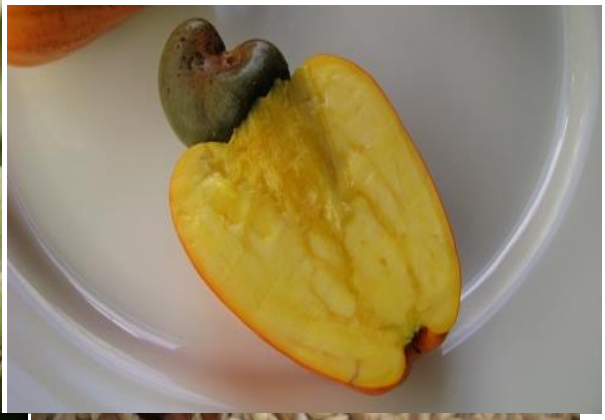
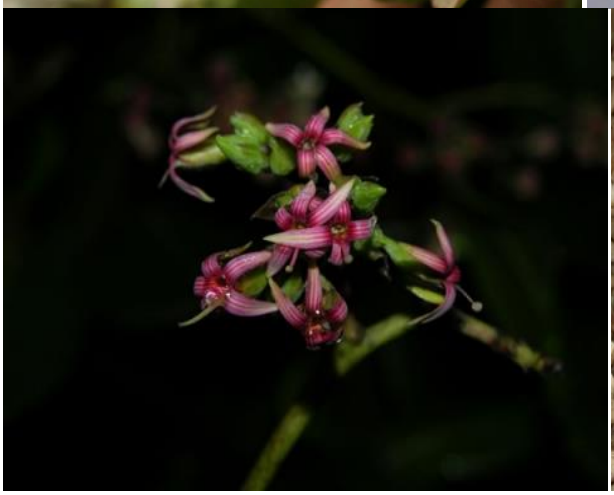


The Anacardiaceae family has 76 genera divided into five tribes (Anacardiaceae, Dobineae, Rhoeae, Semecarpeae and Spondiadeae) covering about 600 species (Correia et al., 2006). *Anacardium occidentale* is an abundant tree in the Northeast of Brazil and the states of Piauí, Ceará and Rio Grande of Norte. It represents 90% of cashew production in Brazil. This species is evident for its antioxidant (Melo-Cavalcante et al., 2003), antigenotoxic, antimutagenic (Melo-Cavalcante et al., 2011), antiulcerogenic (Behravan et al., 2012), anti-inflammatory (Olajide et al., 2004), antibacterial, antifungal and larvicides (Behravan et al., 2012) activities. Also, it is a tree rich in anthocyanins, carotenoids, ascorbic acid (vitamin C), flavonoids and other polyphenols as well as mineral components. The bark and leaves are used in folk medicine (Konan and Bacchi, 2007). In addition, the true fruit of cashew releases a liquid rich in phenolic compounds, known as liquid from chestnut shell- cashew nut shell liquid (CNSL). The components of cashew nut shell liquid depend on the method of production and are classified into two general categories: natural CNSL (LCCI) and technical CNSL (CTCL) liquids. LCCI contains 60-65% of anacardic acid, 15-20% cardol, 10% cardanol and trace amount of metilcardol, while CTCL contains 60-65% of cardanol, 15- 20% cardol, and trace amount of polymeric methyl-cardol l material (Kumar et al., 2002). Both of the liquids also contain trace amount of phytosterols, triacontane and others (Andrade et al., 2011). Though the pharmacological activities reported for CNSL is linked to pharmaceutical consumption, more analysis is needed, especially of its genotoxic effects. Assessment of genotoxic and carcinogenic potentials in drug development is crucial before approving and making it available in the market as pharmaceutical products. Thus, this review aimed to take a picture of *A. occidentale*, highlighting its chemical compositions and related biological activities.[92]

MORPHOLOGY AND GEOGRAPHICAL DISTRIBUTION OF *A. OCCIDENTALE*

A. occidentale has a height of 5-10 m, but in clay land can reach up to 20 m. It has a crooked trunk of 25-40 cm in diameter. The leaves are oval, obovais, leathery, glabrous; rosy when young; it has vináceas flowers, arranged in terminal panicles (Lorenzi, 2008). According to Gomes (2010), cashew tree is spread around the world, between latitudes 27ºN in Southern Florida and 28ºS of South Africa; and also in low latitude regions, near the equator, between the parallel 15ºN and 15ºS, in coastal areas, typically tropical South America, Africa and Asia. *A. occidentale* is common among the Northeastern states such as Ceará, Piauí

and Rio Grande do Norte (Lubi and Thachil, 2000). The family Anacardiaceae covers over 70 genera in which more than 600 species are distributed in tropical, sub-tropical and temperate regions in the world (Engels et al., 2012). The family is rich in important secondary metabolites with varieties of interesting biological activities (Abu-Reidah et al., 2015).

CHEMICAL COMPOUNDS PRESENT IN *A. OCCIDENTALE* AND ITS BIOLOGICAL ROLES

Both yellow and red fruits of *A. occidentale* possess ferulic acid, caffeic acid, sinapic acid, gallic acid, and ellagic myritine (Moo-Huchin et al., 2015). Flavonoid contents in yellow and red cashew may be 12.1 ± 0.3 and 6.4 ± 0.4 mg/g, respectively. The compound, camferol-3- O-glucoside is the major constituent in both varieties, followed by camferol-3-O-arabinofuranoside and quercetin-3-O-glucoside (Shukri and Alan, 2010). The extract of cashew fibers has 11 carotenoids in which auroxantins and β- criptoxantins account for about 50% (Abreu et al., 2014). In the phytochemical analysis of cashew leaves, it is reported that it has (E) -β-ocimene, α-copaene and δ- cadienol; while the fruits contain palmitic, oleic acids, furfural, 4-hydroxydodecanoic acid, lactone, (E) -hexenal, (Z) -hex-3-enol and haxadecanol (Maia et al., 2000). Cashew is rich in anacardic acid, cardanol and cardol along with other alkyl phenolic compounds (Trevisan et al., 2006). It is also evident to have monomeric phenols, flavonoids, glycosides such as myricetin and quercetin hexoside, pentoside, rhamnosides and glycosidic anthocyanidins (Michodjehoun-Mestres et al., 2009). The leaves are rich in alkaloids, essential oils, tannins (Ayepola and Ishola, 2009), saponins, cardenolides and others (Onasanwo et al., 2012). In addition, hydrolysable tannins, phenols, flavones, flavonols, xanthones, chalcones, catechins (Santos et al., 2013), terpenoides and other phenolic compounds (Doss and Thangavel, 2011) have also been reported. Cashew shells also contain a significant amount of gallic acid (345.16 ± 16.24 mg) (De Abreu et al., 2013) and their leaves contain cardanol, cardol (Leitão et al., 2013) and palmitate, oleate, linoleate sitosterol,, sitosterol, stigmasterol, 3-O-β- D-galactopyranoside sitosterol, 3-O-β-Dgalactopyranoside stigmasterol, 3-O-β- Dglucopyranoside.

Stem bark is used for a mixture of sisterol anacardic acids (mono- and diene), alkaloids, tannins and anacardic acids (Chaves et al., 2010). *A. occidentale* is known for its analgesic and gastroprotctive activities. The cashew nut extract at a dose of 200 mg/kg was found to have non-ulcerogenic effect on rats (Behravan et al., 2012). A similar activity was observed with the hydroethanolic extract of cashew leaves, where tannins were suggested as being responsible for moieties (Konan and Bacchi, 2007). Vanderlinde et al. (2009) reported that the acetone extract of cashew stem bark in rodents contains antibodies, and has anti-inflammatory and antinociceptive effects. The dichloromethane extract of cashew leaves is also suggested to have an analgesic effect on rats (Onasanwo et al., 2012). The traditional medicine practitioners in Amazon Region are still using cashew for the treatment of diarrhea, dermatitis, headache, and infectious diseases (Lizcano et al., 2010). One study reported that the methanol extract of cashew stem bark at a dose 200 mg/kg protected mice from lipopolysaccharides induced septic shock (Olajide et al., 2004). Boiled extract from the new leaves of cashew has for wound healing property (Mazzetto et al., 2009), while the adult leaf extract inhibits the action of the enzyme tyrosinase, demonstrating a therapeutic potential for skin pigmentation problems (Abdul et al., 2008). A recent study showed anti-ulcer actions by the hydro-ethanolic extract of cashew (0.1%), leaving the increased gastric acid section. This demonstrates its anti-*Helicobacter pylori* effect (Ajibola et al., 2010). The aqueous extract of leaves of *A. occidentale* showed hypoglycaemic activity in streptozotocin induced diabetic rats at a dose of 175 mg/kg, where repeated administration of this dose (twice/day) significantly reduced the blood glucose level ($p < 0.01$) by 43% in diabetic rats (Sokeng et al., 2001). Petroleum ether and ethanolic extracts of cashew leaves showed antimicrobial activity against *Bacillus subtulis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherchia coli*, *Candida albicans* and *Aspergillus niger* (Dahake et al., 2009; Doss and Thangavel, 2011; Onasanwo et al., 2012); the latter extract had more effect on Gram-positive bacteria (Doss and Thangavel, 2011). The CNSL derivative, 2-hydroxy- 6-pentadecylbenzamide was more active against *S. aureus*, *E. coli* (Pokharkar et al., 2008), *A. flavus*, *Fusarum* sp., *A. fumigafus*, *A. flavus* and *A. niger* (Kannan et al., 2009). The action of anacardic acid (6- pentadecylsalicylic acid) alone and in combination with methicillin was investigated against methicillin-resistant *S. aureus* (Muroi and Kubo, 1996; Tan and Chan, 2014). There are also reports that anacardiac acid and its newly synthesized benzylamine analogs are antibacterial (Kubo et al., 1999; Reddy et al., 2012) and contain anacardiac acid used against *H. pylori* (Castillo-Juarez et al., 2007). CNSL at 2000 mg/kg also suggested its anti-*Aedes aegypti* with the median lethal concentration (LC50) by 90% (Guissoni et al., 2013). The salt, sodium anacardate was also found effective against the same species (Farias et al., 2009). However, Oliveira et al. (2011) suggested cardanol (LC50 = 8.20 ± 0.15 ppm) as a strong

larvicidal agent than the anacardic acid (LC50 12.4 ± 0.10 ppm). The leaf extract (25-250 mg/ml) as well as processed juice (cajuína) inhibits 1,1-dipheny-picrylhydrazyl (DPPH) radicals (Queiroz et al., 2011). CNSL and its compounds were also proved to have antioxidant potential (Andrade et al., 2011; Oliveira et al., 2011). The antioxidant activity order observed for the cashew components was: CNSL > cardanol = hydrogenated cardanol and alkylated> hydrogenated cardanol (Lima et al., 2008). Cashew pulp juice and methanol extract of stem bark (500-2000 µg/ml) have antigenotoxic activity against *Salmonella typhimurium* and Chinese hamster lung fibroblasts (V79 cells), respectively (Barcelos et al., Leite et al. 1857 2007a). According to Melo-Cavalcante et al. (2005), cashew pulp (cajuína) protected *S. typhimurium* (TA102) from damage induced by aflatoxin B1 (AFB1), while the methanolic extract of bark (500-2000 µg/ml) of Chinese hamster (V79) in doxorubicin (0.75 mg/ml) induced damage (Barcelos et al., 2007b). In addition, there are reports for anticlastogenic (*in vivo*) (Melo-Cavalcante et al., 2011) and antimutagenic potentials of the cajuína in *S. typhimurium* TA98 (Chen and Chung, 2000). The latter one may relate to its tannic acid (Chen and Chung, 2000).

Toothpastes without fluoride used by children containing cashew and mango were tested for their antimicrobial activity; they significantly inhibited *Streptococcus mutans*, *S. sobrinus* and *Lactobacillus acidophilus* (Carvalho et al., 2011). Otherwise, the inhibition of the microorganism, *S. mutans* and the formation of its biofilm open the door for its application in dental caries (Furtado et al., 2014). The aqueous extract of cashew has hypoglycemic activity (Alexander-Lindo et al., 2004). Anacardic acid and lunasin, derived from cashew and now seen as having anti-cancer properties, arrest the cell cycle at S mitotic phase (Hsieh et al., 2011). Otherwise, caspase-independent apoptosis inhibition in pituitary adenoma and lung adenocarcinoma cells (Seong et al., 2013) and histone acetyltransferases and nuclear factor kappa B (NF-κB) may be a potential target in chemotherapy (Sung et al., 2008). There are other evidences for its anticancer activity (Schultz et al., 2010; Wu et al., 2011; Huang et al., 2014); where the benzamide derivatives, 2-isopropoxy-6-pentadecyl-Npyridin- 4-ylbenzamide, 2-ethoxy-N-nitrophenyl) -6- pentadecylbenzamide and 2-ethoxy-6-pentadecyl-Npyridin- 4-ylbenzamide strongly inhibit HeLa cell lines (Chandregowda et al., 2009). An alcohol or its metabolites may lead to hyperacetylation of histone thus overexpression of factor GATA4, which is linked to cardiac malfunctions (Wang et al., 2012). In a recent study, the anacardic acid in pregnant female rats at a dose of 5 mg/kg (intraperitoneal) produced an inhibitory effect on histone H3K9 hyperacetylation induced by alcohol. In addition, a reduced acetylation in the promoter region of the GATA4 fetal hearts of mice was also reported. It was also observed that the abortion rates, stillbirths and intestinal timpanismos decreased in mice, thus demonstrating the cardio-protective activity of anacardic acid (Peng et al., 2014). Aurora kinase enzymes play an important role in chromosome segregation and cell division. They are of three types: A, B and C (Bischoff and Plowman, 1999). Deregulation of aurora kinase can result in mitotic abnormalities and genetic instability leading to defects in centromere function in chromosome alignment, and cytokinesis (Fu et al., 2007). In several types of cancer, there is a relationship with overexpression of kinase A and B (Murata-Hori and Wang, 2002). Through a virtual evaluation, it was found that anacardic acid could be fitted into the aurora kinase enzyme A and B; and thus, could activate the aurora kinase A-mediated phosphorylation of histone H3 by modifying the structure of the enzyme and increasing its activity (Kishore et al., 2008). The drug sildenafil (VIAGRA) is a potent inhibitor of 5-fosfadiesterase (Terrett et al., 1996). This is the key enzyme used for the regulation of smooth muscle tone, playing an important role in erectile dysfunction (Beavo and Reifsnyder, 1990). A sildenafil analog was synthesized from anacardic acid (Paramashivappa et al., 2002). However, the resorcinolic lipid (cardol) was also reported for its antimicrobial (Kubo et al., 1999), antitumor, molluscicide, tyrosinase inhibitory (Zhuang et al., 2010), and liposome formation (Przeworska et al., 2001) activities. In addition, it can prevent and repair damage done to DNA (Stepanenko et al., 2004). A recent study indicated that a new resorcinolic lipid, 3-heptyl- 3,4,6-trimethoxy-3H-isobenzofuran-1-one (AMS35AA) alone produced neither genotoxic nor mutagenic effects in mice (Navarro et al., 2014). Otherwise, both cardanol and cardol exhibited antiproliferative properties with LC50 ranging from 41.3 to 52.4 mg/ml and 43.8 to 53.5 µg/ml in cancer cell lines (Teerasripreecha et al., 2012). Nowadays, cardol has gained interest (Kubo et al., 1994) along with other natural compounds such as coumarin (Finn et al., 2005) for their inhibitory activity against tyrosinase (Tocco et al., 2009), a multifunctional enzyme that has copper involved in melanin biosynthesis. Tyrosine catalyzes the ortho-hydroxylation of tyrosine to dopaquinone, which spontaneously polymerizes to melanin. Melanogenesis inhibitors are used to whiten the skin of patients treated with pigmentation disorders, such as over production of melanin (Hartong et al., 2006) and Addison's disease (Pandya and Guevara, 2000). Oliveira et al. (2011) found the anticholinesterase activity of CNSL constituents, although cardol, cardanol, carbachol and anacardic acids were previously reported for their cholinesterase inhibitory activity (Rosenberry et al., 2008) [92].

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DIFFERENT REASERCH WORK ON PLANT ANACARDIUM OCCIDENTALE AGAINST DIFFERENT TYPES OF DENTAL DISEASE

Jothi Varghese et al worked on Antimicrobial effect of *Anacardium occidentale* leaf extract against pathogens causing periodontal disease [93].

In this work The antimicrobial activity at different concentrations of two extracts (methanol and aqueous) of cashew leaf on the periodontopathogens were determined by employing the agar diffusion method. Culture strains of *P. gin- givalis* (ATCC 33277) and *P. intermedia* (ATCC 25611) were maintained on Kanamycin agar plates. The kana-mycin agar plates were prepared in sterile glass petri dishes, seeded with innocula and kept overnight under anaerobic conditions. A total of 33 wells (3 wells for each concentration of the extracts and CHX) of 6.0 mm in diameter and 4 mm deep were cut out on the seeded plates using sterile cork borer and each of the well was filled with the extracts of varying concentrations (5, 10, 25, 50, 75 µl) and 0.2% CHX. Thus, a total of 66 wells were prepared for both the microorganisms. The extracts were allowed to diffuse into the medium and the plates were incubated at 37°C for 72 hours. The sensitivity of the tested pathogenic organisms to aqueous and metha-nolic extracts was shown by zones of inhibition after incubation. The zones of inhibition were measured using a plastic ruler.

For each concentration of the extract, the zone of inhi-bition was measured three times and the mean was re-corded. The statistical analysis was performed using the Kruskal wallis test and the Mann-Whitney "U" test. Significance for all statistical tests was predetermined at $p < 0.05$.

The result of this study revealed that the mean values of growth inhibi- tion of *P. gingivalis* and *P. intermedia*, at different con- centrations of both aqueous and methanolic extract of the leaves of *A. occidentale* were calculated and plotted against 0.2% CHX which was used as a control (**Figures 1** and **2**). There were significant differences in the anti- microbial effects of both aqueous and methanolic extracts, with the aqueous extract being more effective than methanolic ($p > 0.05$). For *P. gingivalis*, at the highest concentration of 75 µl, aqueous extract showed maxi- mum antimicrobial activity than methanolic extract ($p = 0.005$), which was comparable to that of CHX ($p = 0.015$) (**Figure 1**). Similar results were observed with *P. inter- media*, in which the aqueous extract at 75 µl dilution displayed significant inhibitory action than the methanolic extract ($p = 0.007$). However, in comparison to both the extracts, CHX showed better inhibitory action for *P. intermedia* ($p = 0.007$) (**Figure 2**). The absence of zones of inhibition around each well signified resistance or no activity of the extracts, which was observed at the lower dilutions of 5 µl, 10 µl and 25 µl respectively (**Figures 1** and **2**).

The present study demonstrated that the aqueous extract presented better antimicrobial activity than the mentha- nolic extract. The presence of bioactive ingredients in the cashew leaves like carbohydrates, tannins, saponins, resins, alkaloids and flavanoids add to their antimicrobial activities. A phytochemical screening analysis on *Ana- cardium occidentale* leaves have showed the presence of high concentration of tannins in the aqueous extract and its absence in the alcoholic leaf extract. This could probably account for the effective action of aqueous form compared to the methanolic extract in the inhibitory activity against *P. gingivalis* and *P. intermedia*. On the contrary, Ayepola and Ishola evaluated the anti- microbial property of methanol and aqueous extracts of

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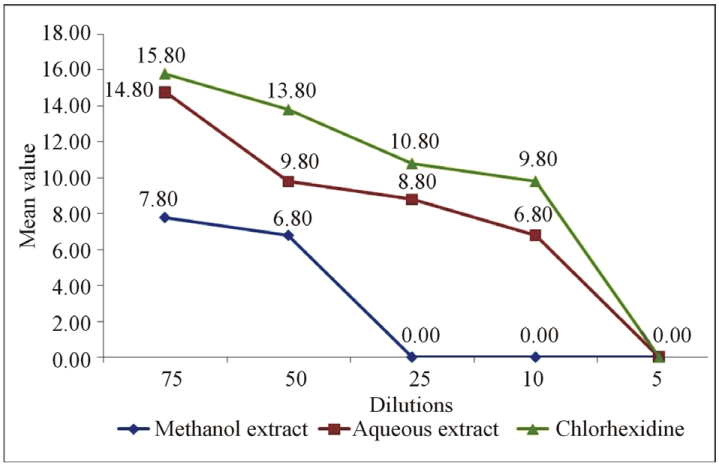

Figure 1. Effect of *A. occidentate* leaf extract on *P. gingivalis*.

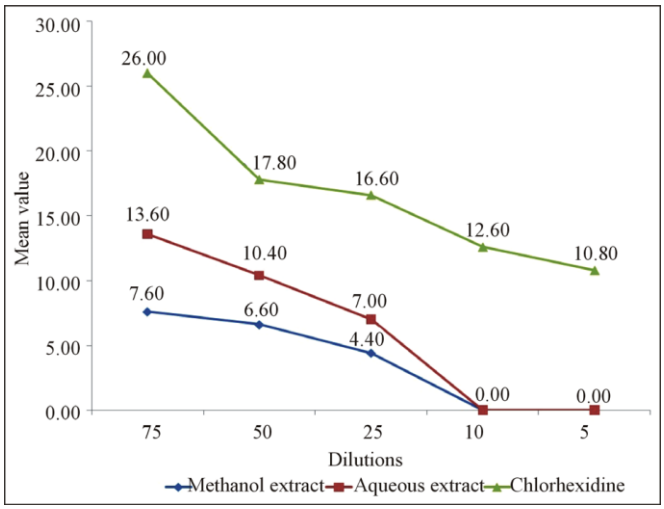

Figure 2. Effect of *A. occidentate* leaf extract on *P. intermedia*.

Karyna de Melo Menezes1 et al worked on Antimicrobial and Anti-Adherent in vitro Activity of Tannins Isolated from Anacardium occidentale Linn. (Cashew) on Dental Biolfilm Bacteria [94].

In this work The antimicrobial activity (MIC) was obtained using the solid medium diffusion method [14] on Petri dishes. The strains were grown in nutrient broth (BHI - Brain Heart Infusion - DIFCO), incubated at 37°C for 18-20 hours in microaerophilic environment through the candle flame method. Mueller Hinton agar plates (Difco) were prepared and after 24 hours (sterility control), they were flooded with saline and inoculated with microorganisms "overnight" at concentration of 10-2 and then holes of approximately 6 mm in diameter were made. Five holes were prepared in each plate that received numbering ranging from 1 to 10, which corresponded to tannin solution diluted in distilled water (mg / ml) (1:1 up to 1:512). After insertion of 50 µL of test substances, the plates were incubated in bacteriological incubator at 37°C for 24 hours. Each assay was performed in triplicate for each selected strain. MIC was defined as the lowest substance concentration capable of inhibiting bacterial growth in halos greater than or equal to 10 mm.

The results showed that S. mutans, S. mitis, S. sanguis, S. oralis, S. salivarius and L. casei bacterial strains were sensitive to the action of tannins isolated from the cashew bark, showing inhibition halos ranging from 15 to 13 mm, 16 to 11 mm, 17 to 11 mm, 15 to 13 mm, 17 to 13 mm and 15 to 12 mm, respectively, as shown in Table 1. The antimicrobial activity of 0.12% gluconate chlorhexidine was more significant, as shown in Table 2.

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Table 1. Minimum Inhibitory Concentration in solid medium of tannins isolated from Anacardium occidentale Linn. (Cashew) on biofilm-forming bacterial strains.

S mutans	*S mitis*	*S sanguis*		*S oralis*	*S salivarius*	*L casei*
Pure Sol	15mm	16mm	**17mm**	15mm	**17mm**	15mm
1:2	14mm	15mm	14mm	14mm	15mm	12mm
1:4	13mm	14mm	13mm	13mm	14mm	0
1:8	0	13mm	11mm	0	13mm	0
1:16	0	**11mm**	0	0	0	0
1:32	0	0	0	0	0	0
1:64	0	0	0	0	0	0
1:128	0	0	0	0	0	0
1:256	0	0	0	0	0	0
1:512	0	0	0	0	0	0

Table 2. Minimum Inhibitory Concentration in solid medium of 0.12% chlorhexidine gluconate (Periogard®) on biofilm-forming bacterial strains.

S mutans	*S mitis*	*S sanguis*		*S oralis*	*S salivarius*	*L casei*
Pure Sol	22mm	21mm	**23mm**	22mm	**24mm**	**23mm**
1:2	20mm	20mm	20mm	21mm	22mm	21mm
1:4	19mm	18mm	19mm	20mm	21mm	19mm
1:8	18mm	17mm	17mm	18mm	20mm	17mm
1:16	15mm	14mm	16mm	17mm	19mm	15mm
1:32	13mm	13mm	0	13mm	15mm	0
1:64	**12mm**	0	0	0	0	0
1:128	0	0	0	0	0	0
1:256	0	0	0	0	0	0
1:512	0	0	0	0	0	0

Tannins isolated from Anacardium occidentale Linn. (Cashew) were effective in inhibiting the adherence of the six test strains, represented by the absence of adhesion to glass in the presence of sucrose. The greatest inhibition potential was observed on Streptococcus sanguis and Lactobacillus casei strains. For S. salivarius and S. mitis strains, inhibition of adhesion was observed up to concentration of 1:16 and for S.oralis and S. mutans of 1:8, acids showed antibacterial activity against all microorganisms, but the greatest inhibitory activity occurred against Streptococcus mutans.

Results from an in vitro study showed that the MIC of extract obtained from Anacardium occidentale L. bark were 12.5 mg / ml for S. mutans and 6.25 mg / ml for S. mitis and S. sanguis. These data are similar to those obtained in the present study with isolated tannins for S. mitis.

The results for the antimicrobial activity of tannins isolated from Anacardium occidentale L. were more effective on S. mitis up to 1:16. The MIC for S. mitis is similar to that obtained in a previous study. In another study, the same bacterial strains used in this study were more sensitive to the action of the extract up to concentrations of 1:16 for S. mutans, 1:8 for S. sanguis and 1:16 for L. casei It is noteworthy that researchers used crude extract of Anacardium occidentale L. stem bark and not the fraction of tannins isolated as in this study and demonstrated that there are other components in the extract able to inhibit bacterial growth.

Thus, it was observed that both the crude extract as tannins isolated from Anacardium occidentale L have inhibitory activity against microorganisms that are part of the composition of the dental biofilm. Among the hypotheses on the mechanisms of antimicrobial action of tannins, the inhibition of enzymes, the modification of cellular metabolism by its action on membranes and the complexation with metal ions, decreasing their availability to the metabolism of microorganisms stand out acids showed antibacterial activity against all microorganisms, but the greatest inhibitory activity occurred against Streptococcus mutans.

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The results for the antimicrobial activity of tannins isolated from Anacardium occidentale L. were more effective on S. mitis up to 1:16. The MIC for S. mitis is similar to that obtained in a previous study In another study, the same bacterial strains used in this study were more sensitive to the action of the extract up to concentrations of 1:16 for S. mutans, 1:8 for S. sanguis and 1:16 for L. casei It is noteworthy that researchers used crude extract of Anacardium occidentale L. stem bark and not the fraction of tannins isolated as in this study and demonstrated that there are other components in the extract able to inhibit bacterial growth. Thus, it was observed that both the crude extract as tannins isolated from Anacardium occidentale L have inhibitory activity against microorganisms that are part of the composition of the dental biofilm. Among the hypotheses on the mechanisms of antimicrobial action of tannins, the inhibition of enzymes, the modification of cellular metabolism by its action on membranes and the complexation with metal ions, decreasing their availability to the metabolism of microorganisms stand out.

Chaithra et al worked on inhibitory effect of leaf and bark of *anacardium occidentale* against clinical isolates of *staphylococcus aureus* and *streptococcus mutans [95]*

Antibacterial activity of leaf and bark extracts:- Agar well diffusion assay was carried out to determine antibacterial activity of leaf extract (LE) and bark extract (BE) against five isolates of *S. aureus* (recovered previously from burn patients) and five isolates *S. mutans* (isolated from dental caries subjects previously). The isolates of *S. aureus* and *S. mutans* were grown in sterile Nutrient broth (HiMedia, Mumbai) and sterile Brain heart infusion broth (HiMedia, Mumbai) respectively for 24 hours at 37oC. The broth cultures of *S. aureus* and *S. mutans* were aseptically swabbed on sterile Nutrient agar (HiMedia, Mumbai) and sterile Brain heart infusion agar (HiMedia, Mumbai) plates respectively using sterile cotton swabs. Wells of 6mm diameter were made in the inoculated plates using sterile cork borer. 100µl of LE and BE (25mg/ml of 25% dimethyl sulfoxide [DMSO]), standard antibiotic (Chloramphenicol, 1mg/ml of sterile distilled water) and DMSO (25%, in sterile water) were filled into labeled wells. The plates were incubated at 37oC for 24 hours and the zone of inhibition was measured using a ruler**.** The inhibitory activity of LE and BE of *A. occidentale* against clinical isolates of *S. aureus*. Among extracts, LE was more effective (zone of inhibition ranging 1.5 to 1.8cm) in inhibiting bacterial isolates when compared to BE (zone of inhibition ranging 1.2 to 1.6cm). Chloramphenicol showed higher inhibition of bacteria than LE and BE. DMSO did not cause inhibition of bacteria. Burn wounds are more vulnerable and suitable sites for multiplication of pathogenic bacteria. These wounds are ideal sources of infection than surgical wounds because of the larger area involved and longer duration of patient stay in the hospital. The major cause of morbidity and mortality in hospitalized burn patients is infection caused by a number of pathogenic microbes. It is estimated that more than 75% of deaths following burn is related to infections. The bacteriology of burn wounds is usually poly-microbial in nature. The most common bacterial pathogens isolated from burn wounds are *Staphylococcus aureus, Pseudomonas aeruginosa, Streptococcus pyogenes* and various coilform bacilli. Among these, *Staphylococcus aureus* is frequently isolated in both community and hospital practices. *S. aureus* is one of the greatest causes of nosocomial infection in burn patients. The pattern of antimicrobial susceptibility of *S. aureus* is changing and therefore antimicrobial agents are becoming ineffective. Majority of strains of *S. aureus* in community as well as hospitals have developed resistance to commonly used antibiotics such as penicillin, methicillin and vancomycin. Infection of burn wounds caused by multidrug resistant pathogens is a serious threat for successful treatment **22-27**. It has been found that plants and their components are effective against *S. aureus* isolates including drug resistant strains **21,25,28-33**. In the present study, the extracts of *A. occidentale* were shown to be potent in causing inhibition of isolates of *S. aureus* recovered previously from burn patients. Dental infections namely dental caries and periodontal diseases are among the most common human diseases. The ubiquitousness and non-life threatening nature have minimized their significance in overall health of humans. Mutans streptococci are found in highest numbers on teeth. Mutans streptococci are the streptococci found in plaque, ferment mannitol and sorbitol, produce extracellular glucans from sucrose and are cariogenic. *Streptococcus mutans* is often considered as the most important and primary aetiological agent among mutans streptococci responsible for causing dental caries in humans. *S. mutans* possesses the property to adhere to pellicle-coated tooth surfaces and to form acids **34-40**. Prevention and treatment of dental caries involves the use of antimicrobial mouth rinses such as triclosan and chlorhexidine and antibiotics such as penicillin, erythromycin, and ampicillin. However, the antimicrobial mouth rinses are reported to cause some undesirable side effects

such as tooth staining, taste alteration and development of hypersensitivity reactions whereas antibiotics suffer from the major drawback that oral microflora are getting resistance against them **33,37,41,42**. This has triggered interest in searching alternatives for treatment of dental caries. Plants have shown to be effective in the treatment of dental caries and a number of studies have shown the potential of plants and their metabolites to inhibit cariogenic flora**21,33,39,42-49.** In our study, leaf and bark extracts of *A. occidentale* displayed inhibition of *S. mutans* isolates recovered from dental caries subjects.

Geethashri Anand, et al, worked on *In vitro* antimicrobial and cytotoxic effects of *Anacardium occidentale* and *Mangifera indica* in oral care [96]

The susceptibility of oral pathogens to plant extracts and to control mouth rinses were determined by well diffusion method.[10] The planktonic cultures of bacteria and fungus were adjusted to 0.5 McFarland constant. The aliquots of the microbial cultures uniformly spread on the surface of solidified MHA and SDA using a sterile swab. With the help of a sterile cork borer, wells of 6 mm were made in the agar plates. The cashew and mango extracts were reconstituted in 99.9% ethanol, and wells were filled with 80 µl (20 mg/ml) of the extract. Subsequently, the plates were placed in an incubator at 37°C. After overnight incubation, the diameter of clear zone produced was measured in mm. The control mouth rinses were also evaluated for antimicrobial activity. Along with the extracts and mouth rinses, ethanol (negative control) also inoculated into the wells to see the possible antimicrobial effects of ethanol.

The minimum inhibitory concentration (MIC) and minimum bactericidal/fungicidal concentration (MBC/MFC) of extracts, mouth rinses, and ethanol were evaluated by microdilution method with slight modifications.[11] Hundred and sixty microliters of the extracts, standards, and ethanol were pipetted into the first row of the plate. Rest of the wells was added with 80 µl of sterile normal saline. The serial dilution of the extracts, positive controls, and ethanol were done in normal saline. 10 µl of resazurin indicator (prepared by dissolving 270 mg in 40 ml of sterile distilled water) was added to all wells. A volume of 30 µl of MHB, TSB, or SDB was pipetted into the wells with respect to the organisms to be inoculated. Then 10 µl of bacterial or fungal suspensions were added to each well. The plates were placed in an incubator at 37°C for 24 h. The highest concentration of extracts taken was 10,000 µg/ml and positive control was 160 µg/ml. The color change from purple to pink or colorless was recorded as the MIC value. In order to find the MBC/MFC value subculturing till MIC value was done on MHA and SDA plates, respectively. After 24 h of incubation, MBC/MFC values were recorded.

Biofilm suppression.

The bacterial and fungal biofilm were grown in 96-well microtiter plate following O'Toole method. The overnight culture of the microorganisms was diluted to 1:100 in the respective broth and known volume of 100 µl was seeded into each well followed by 24 h of incubation at 37°C in an incubator. The unattached or floating cells were removed by giving two gentle wash in sterile double distilled water. The reconstituted extracts were diluted in respective broth. A volume of 100 µl of extract at concentration of 200 µg/ml was added into the wells and incubated for 4 h at 37°C. Simultaneously, 100 µl of positive standards was also evaluated for the efficacy of biofilm suppression. The control wells were treated with 100 µl of broth. After 4 h of incubation, contents of the wells were discarded. Subsequently, staining of biofilm was done by incubating the biofilm with 100 µl of 0.1% crystal violet stain at room temperature for 15 min. The excess stain was removed by gentle washing of the wells with distilled water for three times. The plates were air-dried. The destaining of the microbial cells was done using 125 µl of 30% acetic acid for 15 min. The contents were transferred to another microtiter plate and quantification of biofilm was done by taking the optical density readings at 600 nm using Lisa Chem spectrophotometer.

The cytotoxicity of plant extracts and mouth rinses were determined using microculture tetrazolium (MTT) assay with minor modifications. The confluent monolayer of cells was detached with 0.05% trypsin and 0.001% ethylenediaminetetraacetic acid solution to make single cell suspension of HGF and V79 cells. The viable cells were counted by Tryphan blue (0.4%) assay using a hemocytometer. The cells were diluted in DMEM media to a final density of 1×10^4 cells for HGF and 2×10^4 for V79 cells in 100 µl/well. After seeding with cells, the 96-well plate was incubated to allow the cells attachment at 37°C, 5% CO_2, and with relative humidity.

The extract initially reconstituted in ethanol was diluted in serum free media. In diluted extracts, the concentration of ethanol was not more than 10%. The 100 µl of the extracts (200 µg/ml) was added to the wells and incubated for 4 h. The same was followed for the positive control groups. A 10% of ethanol in serum free media was also evaluated for the possible cytotoxicity of ethanol. On completion of the incubation, the contents of the plates were gently removed and 50 µl of 0.05% MTT was added. Followed by 2 h of incubation, MTT solution was removed and 100 µl of DMSO was added to each well and incubated for 15 min. The viability of cells was quantified by taking the optical density at 545 nm using Lisa Chem spectrophotometer.

Each parameter was repeated twice in triplicates. The statistical analysis of the data was done using GraphPad Prism (version 3.02, San Diego, California). The level of significance was determined by one-way analysis (ANOVA), Bonferroni multiple comparison test. P <0.05 was taken as level of significance.

Percentage inhibition of biofilm was determined using the formula,

Percentage inhibition = 100−(abs sample)/(abs control) ×100
Percentage viability of cells was determined using the formula,
Percentage viability = (abs test − abs blank)/(abs control–abs blank) ×100

The result of this study reveal the antimicrobial activity (mean ± standard deviation [SD]) of cashew and mango leaf extracts is *S. mutans* and *E. coli* showed the highest susceptibility to cashew and mango leaf extracts, respectively The zone of inhibition of cashew and mango against *E. faecalis* was highly significant (P < 0.001) compared to PI-based mouth rinse Although, the extracts produced a greater zone of inhibition against *E. faecalis*; compared to CHX mouth rinse, was found to be not significant (P > 0.05). There was no difference in the antimicrobial action of cashew leaf extract against *S. aureus*, *S. mutans*, *E. coli*, and *C. albicans* compared to CHX-based mouth rinse (P > 0.05). But compared to PI mouth rinse, the cashew leaf extract showed significantly (P < 0.001) greater zone of inhibition against *S. aureus*, *S. mutans*, and *E. coli*. The antimicrobial activity of mango extract against *S. aureus* and *S. mutans* was significant (P < 0.05) as compared to PI mouth rinse. Against *E. coli*, mango leaf extract was highly significant (P < 0.001) compared to both the mouth rinses. The antifungal activity by the cashew leaf extract was comparatively more than the CHX based mouth rinse although no significant difference was noted (P > 0.05). No zone of inhibition was observed around the ethanol against the tested pathogens.

The MIC and MBC/MFC of extracts and mouth rinses are presented in Table 2. The MIC of cashew leaf found in the range of 78.12-1250 µg/ml and MBC/MFC concentration was varied from 156.25 µg/ml to 1250 µg/ml. The MIC of mango leaf varied from 39.06 µg/ml to 1250 µg/ml and MBC/MFC was seen between 78.12 µg/ml and 2500 µg/ml. The CHX mouth rinses MIC value varied from 2.5 to 5 µg/ml, whereas MBC/MFC value varied from 5 to 10 µg/ml. The range of MIC values of PI was found to be between 40 and 80 µg/ml and MBC/MFC 80 to 160 µg/ml. The possible antimicrobial effect of ethanol was ruled out as growth was observed in microtiter plate for all the microbes.

The percentage viability of HGF cells and V79 Chinese hamster lung fibroblast cells is presented Cashew leaf extract significantly (P < 0.001) reduced the viability of HGF and V79 cell lines. Mango leaf reduced the cell viability of HGF and V79 with a significance level of P < 0.05 and P < 0.001, respectively. The lowest survival rate was observed for PI-based mouth rinses in both the cell lines. The mouth rinses found to be more toxic to the cells compared to the plant extracts with lower survival rates. There was significantly (P < 0.01) higher viability of HGF cells and V79 cells treated with plant extracts, as compared to CHX and PI-based mouth rinses. 10% ethanol in serum free media was not cytotoxic to both the cell lines.

Dental plaque in the oral cavity is recognized as the most complex oral biofilm.Microorganisms such as *E. faecalis*, S aureus, *S. mutans*, *E. coli*, and *C. albicans* are known to cause wide range of oral infections such as dental caries, periodontal diseases, and peri-implant diseases due to the formation of biofilm. The structural organization of cells and thick exopolysaccharide matrix reduces the efficacy of topically applied agents and results in emergence of multidrug resistant microbes.

Chlorhexidine gluconate is proven to be the gold standard in oral care; certain undesirable effects such as taste disturbance, tooth discoloration, and mucosal erosions do exist. PI used in mouth rinses also stains teeth, burning sensation of mouth, drying of oral mucosa, and has possible potential for carcinogenic

effects. The emergence of multidrug resistant strains and potential side effects of conventional mouth rinse have shifted the focus of oral health care to plant-based products.

Mango and Cashew leaves were used in indigenous system of oral care in India. Ethanol extract of *A. occidentale* Linn. Leaves exhibited significant antimicrobial and antifungal activity. The strong *in vitro*antibacterial activity of the separated compound against methicillin resistant *S. aureus* suggests the wide pharmaceutical applications. According to ayurveda, varied medicinal properties are attributed to different parts of mango tree. Pharmacologically and medicinally, important chemical like mangiferin, being a polyphenolic antioxidant and a glucosyl xanthone, it has strong antioxidant, antilipid peroxidation, immunomodulation, cardiotonic, hypotensive, wound healing, antidegenerative, and antidiabetic activities. Various effects such as antibacterial, antifungal, anthelmintic, and antiparasitic have been studied.

In vitro agar diffusion technique was done to evaluate the antimicrobial properties of mango and cashew plant extracts. The results of this study confirmed the antimicrobial efficacy of these plant extracts in par with the CHX and superior to PI-based mouth rinses on all test microbes. The results of our study was in accordance with Dahake *et al*., Parasa *et al*. and Stoilova *et al*. whose reports confirmed the antibacterial and antifungal efficacy of plant extracts.

Periodontal disease is a common, slow, progressive inflammatory disease resulting from a complex biofilm of resident commensal and pathogenic bacteria and their products. Along with the environment and host-related factors, the microbes also form an integral part of the disease. Cashew contains high concentration of tannins (poly phenols) which bind and precipitate proteins. Mango contains Mangiferin (polyphenol); with potent antioxidant, antilipid peroxidation activities. Thus, the presence of polyphenolic compound of plant extracts may have caused disruption of microbial biofilm. The Study results were in accordance with Varghese *et al*.proving that these plant extracts be utilized for chemical plaque control formulations.

Minimum inhibitory concentration is a sensitive and quantitative approach to distinguish between bacteriostatic and bactericidal effects. Test results of our study showed that CHX- and PI-based mouth rinses were better than the plant extracts. The logical reasoning for this may be that mouth rinses are miscible in saline and exerted their antimicrobial activity effectively. The ethanol extracts of the plants was sparingly soluble in saline to exert their antimicrobial spectrum. The study results were not in accordance with Dahake *et al*. who reported ethanolic extracts and petroleum ether extracts performed well on test microorganisms at a concentration of 15 µg/ml. Parasa *et al*., stated that pure and 70% acetone extracts of Cashew nut shell exerted lower MIC than methanol extract.

Microculture tetrazolium assay is a sensitive, quantitative, and reliable method to assess the cellular metabolic activity, where methyl trizolyl tetazolium is converted into a dark purple colored formazan by cellular mitochondrial dehydrogenase enzyme. In our study, the percentage survival rate of HGF cells and V79 cells treated with cashew and mango was found to be significantly more than CHX-based and PI-based mouth rinses. This indicates less toxicity and long time usage of active components of these plants as an alternative to commercial mouth rinses.

CONCLUSIONS

Herbal extracts contain a store of chemical constituents, which have been utilized on levels of their biological activities. These active ingredients present in medicinal plants have shown positive results in the management of various types of diseases such as oral and dental diseases and may serve as an adjunctive treatment. This review article is a preliminary study which was done to investigate and collect the study of antimicrobial property of different parts of the plant *Anacardium occidentale* on various types of oral and dental disease. Based on different reaserch article the following conclusions were drawn:

The leaf extract of *A. occidentate* has beneficial antimicrobial effects against pathogenic organisms involved in causing periodontal disease. Also further research has to be conducted for a wider understanding of these medicinal plants and for utilizing these cashew extract for prophylactic use.

The extracts from leaf and bark of *A. occidentale* displayed inhibition of clinical isolates of *S. aureus* and *S. mutans*. The inhibitory effect could be ascribed to the presence of bioactive components present in extracts. The plant can be employed as a potential candidate for the development of inhibitory agents active against clinical isolates.

The ethanol extracts of cashew and mango displayed antimicrobial activity on all test microorganisms. The inhibitory effect could be attributed to the presence of bioactive components present in extracts. Plant extracts or phytochemicals inhibit the growth of oral pathogens, reduce the development of biofilms. In addition, they found to be biocompatible. Further bioactive component isolated from these plant extracts should be evaluated for their antimicrobial and cytotoxicity with the goal of developing effective adjuvant for treatment of gingival and periodontal diseases with lesser side effects.

Tannins isolated from Anacardium occidentale Linn. (cashew) stem bark showed in vitro antimicrobial and anti-adherent activity against biofilm-forming bacterial strains. Thus, tannic solution can be considered as an alternative treatment for oral diseases, especially those caused by the presence of biofilm such as caries and periodontal disease.

FUTURE SCOPE OF ANACARDIUM OCCIDENTALE ON ORAL AND DENTAL DISEASES

Oral or dental diseases still are a serious ill health world-wide. Tooth decay and dental medicine diseases area unit the foremost usually seen international oral health issues. The link between oral diseases and also the activities of microorganism species that type a part of the small accumulation of the oral cavity is well-established. Despite many chemical agents being commercially accessible, these will alter oral small accumulation and have undesirable side-effects like regurgitation, diarrhea and tooth staining. Moreover, the quality Western medication has had solely restricted success within the bar of periodontitis and within the treatment of a range of oral diseases. Hence, the seek for various product continues and natural phytochemicals isolated from plants like *Anacardium occidentale* employed in ancient medication area unit thought of pretty much as good alternatives to artificial chemicals.

All of these oro dental diseases are often treated with material that advocates procedures like oral cleansing, extractions, excisions, flap surgeries etc., written material conjointly recommends daily use of assorted therapeutic procedures for the hindrance and maintenance of oral health. These include: Dant Dhavani (Brushing), JivhaLekhana (Tongue scrapping) Gandoosha or Oil propulsion/Oil Pulling (Gargling) Dant Dhavani (Brushing). Ayurveda recommends chew sticks within the morning yet as when each meal to forestall diseases. Ayurveda insists on the utilization of flavoring brushes, just about 9 inches long and also the thickness of one's little finger These herb sticks ought to be either "kashaya" (astringent), "katu" (acrid) or "tikta" (bitter) in style. The tactic of use is to crush one finish, chew it and eat it slowly.

Various Ayurvedic herbs and natural products have been used for their pharmacological applications viz. antiulcer, wound healing, anti-inflammatory, antimicrobial and antioxidant properties and have been proven to be safe and effective for oral disease and hygiene including various therapeutic Ayurvedic procedures.

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