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REVIEW ARTICLE

Application of plant extracts in oral health

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Abstract

Plant bioactives have been extracted for multiple applications in improving human health. Oral health is primarily affected by the development of caries, which, as a chronic disease, can progress to tooth loss. Many efforts have been made to reduce the incidence of caries; in many countries, high rates of caries are still a reality. Herein, we explore various plant extracts with proven effects on the biofilm. Oral infections and accumulation of biofilm and calculus can also contribute to soft tissue inflammation, leading to periodontitis and gingivitis. Main plant extracts that modulate inflammation are reviewed. Oral cancer is another clinically important condition whose classic treatments (surgery, radiotherapy and chemotherapy) are very invasive and greatly reduce the quality of life of patients. Immunotherapy is used in certain cases alone or in combination with other treatments. All of this carries the possibility of development of a wide variety of side-effects, some of which can severely weaken patients as oral mucositis. This review addresses these main diseases of the oral cavity, describing each of them, as well as the plant extracts that have been studied for their anticancer, anti-inflammatory and antibacterial properties specifically applied to addressing the conditions.

Keywords: extract; plants; oral cancer; periodontitis; caries

Introduction

Since ancient times, plants have been used as alternatives to prevent and cure diseases. In recent years, there has been a surge in the return to nature and the use of plant compounds with biological activity. Plants, like any other living organism, are made up of organic compounds, the main ones being carbohydrates, lipids, proteins and nucleic acids. These are part of the structure and many others are essential for metabolism and survival. Within this framework, it is known that plants change composition depending on where they grow, the climatic conditions and even whether they are suffering from a disease. These conditions cause the compounds to vary and since they have different biological activities for human conditions, plants are ideal for study as potential therapeutic alternatives (1). Some of these extracts have been focused on restoring healthy oral conditions, as shown in Fig. 1 & 2 (2–10). Below, the main oral conditions and the use of plant extracts as therapeutic options are described.

Anticancer

Cancer is an abnormal condition where cell replication is uncontrolled, different genes have been reported that are involved in the failure of regulation in the cell cycle (p53, Rb, caspases, among others), this protein (p 53) has the responsibility of inducing apoptosis in cells that have been damaged during their replication to prevent the proliferation of altered cells, however when this gene is altered, it may not fulfill its function and therefore the damaged (cancerous) cells spread (11). Oral squamous cell carcinoma (OSCC) represents the most frequent malignant neoplasia of the oral cavity and one of the most aggressive within the cervicofacial region. In 2021, the WHO reported that 1.54 million people worldwide suffered from lip and oral cancer (12).

This epithelial tumour originates in the oral mucosa (Fig. 2), mainly in sites such as the lateral border of the tongue, floor of the mouth and buccal mucosa and is strongly associated with risk factors such as chronic tobacco and alcohol use and human papillomavirus infection (especially subtype 16) (13). Its diagnosis is usually made in advanced stages due to the absence of specific symptoms in the initial stages, which leads to a reserved prognosis, with five-year survival rates that do not exceed 60 %. The clinical approach to these pathologies requires a deep understanding of their etiopathogenic mechanisms and the implementation of comprehensive preventive, diagnostic and therapeutic strategies from the field of oral pathology, with an emphasis on early detection and patient education (13).

There are different treatments to combat cancer, such as surgery, radiotherapy and chemotherapy. As in any cancer, these types of therapies are aggressive, causing general discomfort in the patient, which is why plants have been used as a possible treatment. To demonstrate the effect of plants on oral cancer *in vitro* and *in vivo* models, some of the cells used *in vitro* are: the SCC-9 cell line (CRL-1629 ™), the SCC-15 line (CRL-1623) and SCC-25, both of which are squamous cells isolated from tongue cancer (Table 1). In relation to *in vivo* models, they have been used with murine models and a few clinical trials (14).

Table 1. Mechanism of different plants against oral cancer

Plant	Mechanism	model	Cell line
Cynara scolymus	Induction apoptosis	*In vitro*	SCC-25
Asarum sieboldii	Induction apoptosis	*In vitro*	OSCC
Macaranga peltata	Induction genotoxicity	*In vitro*	KB cell line
Plectranthus amboinicus	Inhibition of cell proliferation	*In vitro*	KB cell line
Glycyrrhiza glabra	Inhibition of cell proliferation	*In vitro*	KB cell line
Curcuma longa	Induction apoptosis	*In vitro*	SCC-9 and SCC-15
Sedum oryzifolium	Inhibition of cell migration	*In vitro*	OSCC
Panax ginseng	Reduce tumor size	*In vivo*	Murine model
Bacopa monnieri	Induce apoptosis	*In vitro* and *in vivo*	Cal33 cells and arecoline-induced oral cancer mice model

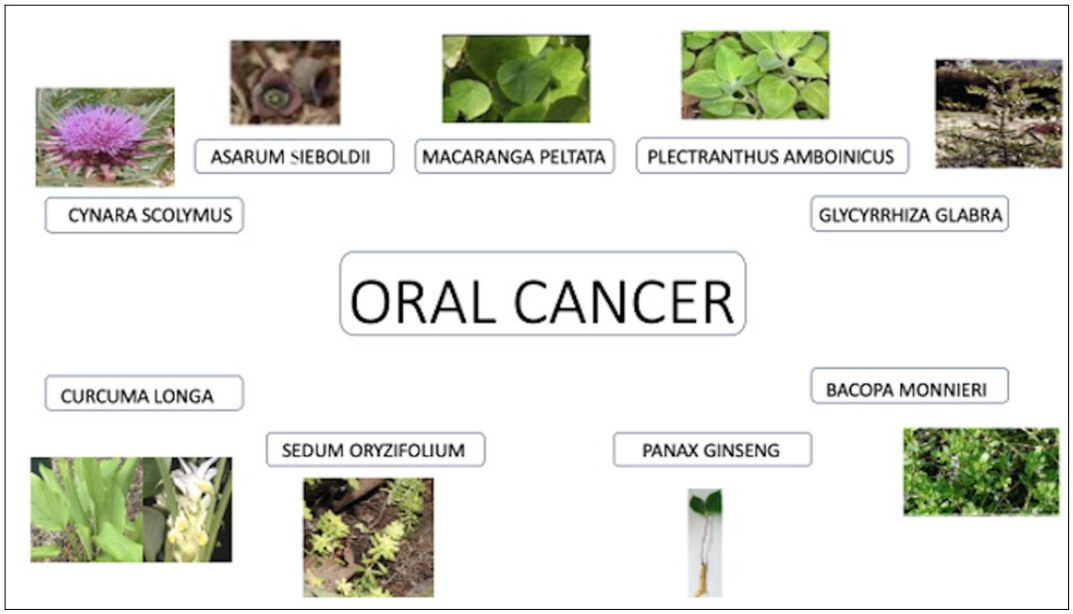

Fig. 1. Plants using for oral cancer (27-35).

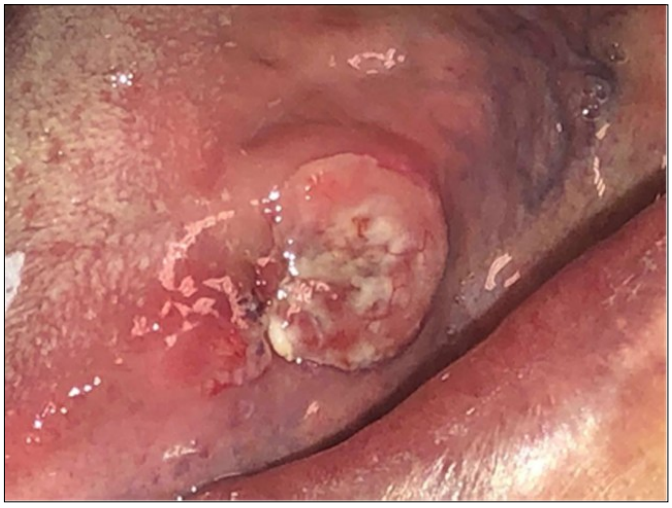

Fig. 2. Oral tongue cancer.

Various plants contain phytochemicals with anticancer properties, such as flavonoids, terpenoids, alkaloids, tannins and polyphenols. These compounds exert selective cytotoxic effects on tumour cells through mechanisms that include: Induction of apoptosis mediated by intrinsic and extrinsic pathways, evidenced by caspase activation and regulation of pro- and antiapoptotic proteins (p53, Bcl-2). Inhibition of cell proliferation by arresting the cell cycle in G0/G1 or G2/M phases, blocking the expression of cyclins and cyclin-dependent kinases. Reduction of oxidative stress, neutralising free radicals and modulating endogenous antioxidant enzymes (superoxide dismutase, catalase). Anti-inflammatory modulation of the tumour microenvironment, with a decrease in the production of proinflammatory cytokines (TNF-α, IL-6, IL-1β), which promote tumour progression. The main effects of various extracts are: reduced cell viability, induction of apoptosis, impaired tumour migration and invasion and decreased tumour growth (15).

Artichoke extract has been studied *in vitro* on the oral squamous carcinoma (SCC-25) cell line and a significant induction of late apoptosis was found in cells treated with the extract, as well as a selective cytotoxic mechanism through programmed cell death. This was analysed by flow cytometry with Annexin-V/FITC labelling, as well as morphological observation with inverted light microscopy and cell cycle analysis by propidium iodide staining.

Furthermore, morphological changes compatible with cellular apoptosis were observed, such as cytoplasmic retraction, nuclear pyknosis and the presence of apoptotic bodies, without alterations in normal cell lines, which shows a selective effect on oral neoplastic cells. At the cell cycle level, a significant arrest in the G2/M phase was documented after 48 h of treatment (31.99 % vs. 21.21 % in the control group), a finding compatible with a response to genomic damage or intracellular stress induced by the compounds present in the extract. Activation of the intrinsic mitochondrial apoptosis pathway was also identified through overexpression of proapoptotic genes such as bax and caspase-9 and the inhibition of the expression of bcl-2, a key antiapoptotic gene, reinforcing the hypothesis that artichoke extract acts as a modulator of the molecular pathways of apoptosis and the cell cycle, fundamental elements in the tumor biopathology of oral squamous cell carcinoma (16). Liganan (-) asarinin from *Asarum sieboldii* extracts is also known to induce caspase-dependent apoptosis in oral cancer cell lines (17).

A species native to India, *Macaranga peltata* has been shown to have a cytotoxic effect on oral cancer cell lines, even surpassing the potency of some conventional chemotherapeutic agents. In addition, a significant increase in DNA fragmentation was observed in treated cells, showing genotoxic activity (18). Extracts from *Plectranthus amboinicus* and *Glycyrrhiza glabra* have been studied in oral cancer cell lines such as the KB cell line and both revealed significant concentration-dependent inhibition of cell proliferation. Furthermore, morphological changes consistent with cytotoxicity were observed, such as loss of cell adhesion and progressive cell rounding. These extracts have been studied on other cell lines such as HeLa, T47D and C6 and similar results have been found, suggesting a consistent antiproliferative effect of these extracts on different types of cancer. The literature suggests that the activity of these extracts could be due to the induction of apoptosis and cell cycle arrest (19).

Molecules such as curcumin, epigallocatechin-3-gallate (EGCG), resveratrol, lycopene, isothiocyanates and genistein have demonstrated the ability to modulate oncogenic pathways by inducing apoptosis, inhibiting the cell cycle, suppressing angiogenesis, epigenetic regulation and preventing the progression of dysplasias to carcinoma. Curcumin is a phytocompound that, in cancer cells (SCC-9 and SCC-15), shows a reduction in cell viability by inducing apoptosis. This is reflected in the blockage of the cell cycle, coupled with the decrease in oncogenic signalling through Cox2 and NFkb. In clinical trials, curcumin is one of the compounds used, achieving promising results when used as an adjuvant therapy to conventional therapy (14). Additional studies related to the delivery and release of the molecule are required in order to improve solubility, biodistribution and therapeutic efficacy (20).

Sedum oryzifolium extract has been shown to be very important in inhibiting the migration and invasion of oral squamous cell carcinoma cells, with trehalose being one of its main bioactive constituents (21). The effect of the extracts on Slug, an epithelial-mesenchymal transition factor (EMT), which has been implicated in metastasis and tumour progression, has also been studied. *Sedum oryzifolium* extract exerts decreased expression of oral carcinoma cells in cell models and in clinical samples. Slug inhibition suggests that *Sedum oryzifolium* halts EMT, reducing the invasive capacity of OSCC cells. Reversing this inhibition limits cell migration and metastatic potential. Although additional pathways, such as caspases or reactive oxygen species (ROS), were not explored, evidence supports the disruption of the EMT process essential for tumour invasion (21).

In murine cancer models, ginseng has been shown to reduce tumour size by modulating microenvironmental conditions, such as the reduction of VEGF (Vascular endothelial growth factor), which contributes to limiting tumour vascularisation (14). Other compounds are more preventative than therapeutic, such as lycopene, a carotenoid with potent antioxidant action, which protects cells from potential damage by reducing oxidative stress. Within this same antioxidant effect are anthocyanins, which have also been shown to have *in vitro* cytoprotective effects against oral cancer (14). *Bacopa monnieri* extract is known for its antioxidant, anti-inflammatory and neuroprotective properties. Its anticancer properties have also recently been demonstrated through the *in vitro* induction of apoptosis and in *in vivo* models of oral cancer (22). Although natural agents have demonstrated their ability to participate in the complementary therapy of oral cancer, in a particularly less toxic way, multicenter studies are required, with standardisation of doses and formulations for their incorporation into oncological protocols (23).

Anti-inflammatory

Gingivitis represents a superficial inflammation of the gingiva, generally induced by the accumulation of bacterial plaque at the gingival margin. It is clinically characterised by redness, oedema and bleeding upon probing (Fig. 3). It is mediated by the activation of proinflammatory cytokines such as IL-1β, IL-6 and TNF-α and is reversible with adequate plaque control measures (24). However, lack of treatment can facilitate progression to periodontitis, a destructive chronic inflammatory disease that affects the tooths supporting structures-the periodontal ligament, root cementum and alveolar bone-and is associated with dysbiosis of the subgingival microbiome, with a predominance of anaerobic gram-negative bacteria such as *Porphyromonas gingivalis*, *Treponema denticola* and *Tannerella forsythia* (25).

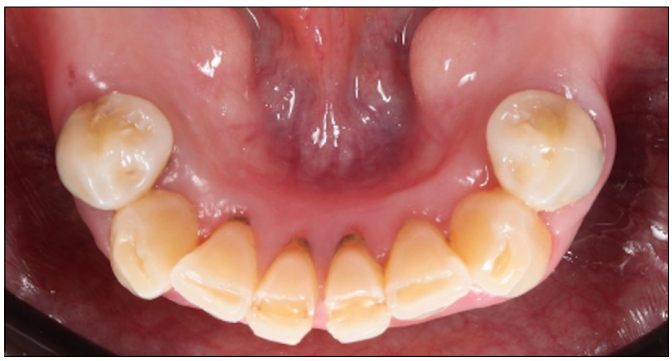

Fig. 3. Gingivitis with the presence of dental plaque.

The exacerbated immune response in periodontitis leads to the release of proteolytic enzymes, matrix metalloproteinases and inflammatory mediators that promote bone resorption, making this disease the leading cause of tooth loss in adults worldwide. Furthermore, there is evidence of a bidirectional association between caries and periodontitis, as both share predisposing factors such as biofilm, sugar consumption and poor oral hygiene, although they differ in their microbiology and immune response (26). Various ethnopharmacological evidence has documented the traditional use of plant species with antiseptic and anti-inflammatory properties. These include *Matricaria chamomilla* L., *Punica granatum* L., *Lippia sidoides* Cham., *Salvadora persica* L. *and Camellia sinensis*. This last plant has been studied as a chemical adjuvant for the control of biofilm and gingivitis. It is recognised for its mechanical and chemical action in the removal of dental biofilm (27).

Nigella sativa essential oil has a high concentration of phenols and flavonoids and its antioxidant capacity has been demonstrated by significantly neutralising reactive oxygen species. It also inhibits the thermal denaturation of proteins and stabilises human erythrocyte membranes against various types of aggressions, which is why it could modulate inflammation without presenting side effects observed with synthetic drugs (20). Phytochemical compounds have been obtained from different plant parts, including roots, leaves, stems and fruit. For example, root extract from *G. glabra*, as well as leaf extracts from both *F. religiosa* and *P. major*, are effective against primary colonisers and periodontal pathogens (28).

Some of the parameters considered to evaluate the improvement in periodontal disease include: reduction in the gingival index (GI), bleeding and decreased plaque (PI). These parameters have been improved with the use of green tea extract (*Camellia sinensis*), which has been shown to decrease both the GI and PI. Extract from pomegranate (*Punica granatum*) showed significant reductions in GI and PI. Ginger (*Zingiber officinale*) and rosemary (*Rosmarinus officinalis*) showed decreased bleeding and bacterial load in clinical studies. In periodontitis, the role of inflammation is very important since it is a trigger for the development of the pathology, which is why it has been shown that some of the mechanisms induced by plant extracts are inhibition of inflammation mediators such as IL-1β, TNF-a and COX-2.

It is also known that pathogens play an important role in the development of periodontitis; for this reason, the inhibition of pathogens such as *S. mutants* and *P. gingivalis* has been described as crucial for counteracting the disease. The loss of extracellular matrix, in this case the collagen fibres that form the periodontal ligament, is significant, so the inhibition of metalloproteinases is essential for maintaining the periodontal ligament (29). Other options for these plant-based treatments are based on the combination of extracts. A study comparing the efficacy of monoextracts versus polyherbal extracts using *Camellia sinensis* (green tea), *Punica granatum* (pomegranate), *Zingiber officinale* (ginger) and *Rosmarinus officinalis* (rosemary) demonstrated anti-inflammatory efficacy through a significant reduction in the gingival index and bleeding score, in addition to a reduction in plaque and periodontal pathogens (28).

In addition to extracts, essential oils have also been obtained from plants such as Nigella sativa, where, when characterised, the presence of phenols and esters was determined, which are attributed to the inhibition of pathogens such as *P. gingivalis*, *A. actinomycetemcomitans*, *T. forsythia* and *P. intermedia* responsible for periodontitis without presenting toxicity in gingival fibroblasts. Some mechanisms of action have been described, such as rupture of membranes, alteration of adhesins (adhesion proteins in the initial part of the development of caries) and reduction of biofilms (30). There are already options on the market made from extracts of burdock root (*Arctium lappa*), persimmon leaf (*Diospyros kaki*) and quercetin to treat these chronic inflammatory conditions, which have had an inhibitory effect against *Porphyromonas gingivalis in vitro*. In addition, in the rat model with periodontitis, less bone loss was demonstrated, indicating protection against alveolar bone degradation (31).

Antibacterial

Caries is a tooth disease in which the tooth is damaged by the presence of bacteria that destroy the dentin tissue, beginning with demineralisation and then degrading it through acid production (Fig. 4). The main bacteria associated with caries are *Streptococcus mutans*. Ethanol extracts from *Verbascum speciosum* have been shown to inhibit *Streptococcus* growth. The mechanism of action of biofilms, which is important for promoting adhesion to teeth, has also been demonstrated (32). Biofilms represent an adaptive ecological strategy of microorganisms, allowing them to form organised multispecies communities that adhere to biotic and abiotic surfaces. These complex microbial structures constitute a persistent reservoir for chronic infections in the oral cavity, characterised by high resistance to both the host immune response and conventional antimicrobial treatments. The multifactorial aetiology of prevalent pathologies such as dental caries and periodontitis crucially involves the participation of polymicrobial biofilms, whose matrix protects microbial cells and limits the penetration of therapeutic agents, complicating effective clinical management (27).

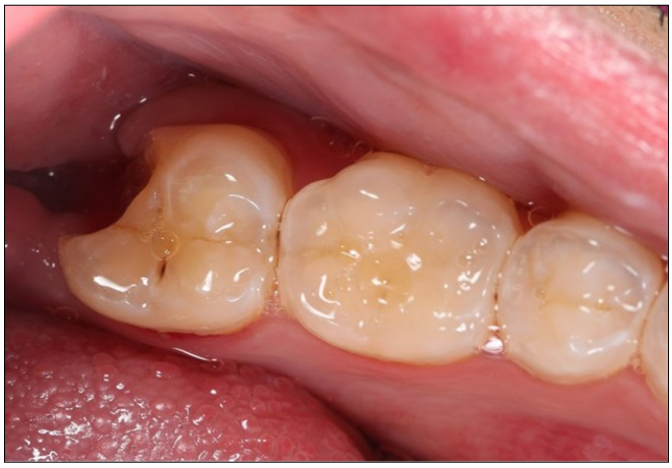

Fig. 4. Tooth decay, tooth destruction due to dental caries.

Faced with this therapeutic challenge, the exploration of plant extracts with antibiofilm activity is emerging as a promising alternative or adjuvant. These bioactive natural products could inhibit microbial growth, modulate virulence factors and degrade the biofilm matrix without promoting antimicrobial resistance, which positions them as innovative therapeutic tools in the prevention and treatment of oral diseases. Furthermore, the use of medicinal plants in dentistry represents an accessible and cost-effective option, particularly in contexts with limited access to traditional preventive measures (27). Extracts from *Camellia sinensis var. assamica*, Zanthoxylum limonella and *Acorus calamus* have demonstrated significant inhibitory activity against Staphylococcus aureus, with minimum inhibitory concentrations comparable to the antibiotic gentamicin. This activity is attributed to the presence of secondary metabolites such as flavonols, limonene and β-asarone, with a potential synergistic effect (33). *Nigella sativa* essential oil possesses potent antibacterial activity against *Staphylococcus aureus* and other commonly present oral microorganisms such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Lactobacillus acidophilus and Candida albicans*. Its antifungal activity is particularly effective. Some bacteria associated with periodontal disease, such as *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, *Tannerella forsythia and Prevotella intermedia*, were significantly inhibited. It is noteworthy that this extract demonstrated cell viability greater than 80 % in human fibroblast cultures, indicating a suitable cell safety profile for topical applications in the oral environment (20).

Gynura procumbens leaf extract demonstrated prominent antibacterial activity against bacteria such as *Chromobacterium sp.*, *Staphylococcus aureus*, *Enterococcus faecium and Escherichia coli*. Furthermore, it did not present any harmful effects evaluated by histopathology in the organs of experimental mice. Therefore, it is a potential source for the treatment of various chronic infectious processes (34). In patient studies, it has been shown that *Mangifera indica* extract, when used as a mouthwash, significantly increases salivary pH and reduces S. mutants present in saliva. It also had a comparative effect with the gold standard, 0.2 % chlorhexidine. Characterising the extracts compounds reveals the presence of tannins, saponins, flavonoids (quercetin, kaempferol) and

mangiferin, which are believed to disrupt pathogen membranes and cause their lysis. It also inhibits the synthesis of bacterial enzymes, decreasing adherence to teeth (35). Overall, methodological heterogeneity was evident among the studies analysed, including differences in dosage forms (mouthwashes, gels, pastes, gummies, powders), standardisation of plant extracts, dose and duration of treatment, as well as in the methodology of randomisation and data collection. Furthermore, the frequent absence of adequate positive controls and placebos limits comparative validity and reflects important methodological deficiencies. Nevertheless, the results suggest a favourable effect of some extracts. Further elucidation of the molecular mechanisms of action of these bioactive compounds is essential to optimise their clinical application in the management of oral pathologies associated with biofilms (27).

Conclusion

This is a review of the bioactive ingredients in different plants that have been studied for their anticancer, antibacterial and anti-inflammatory properties, which have been successfully used to treat major oral pathologies such as caries, periodontitis, gingivitis and even oral cancer. The plants reviewed here are a very important source of natural and non-toxic treatments, leading to improving the quality of life of the patients with compromised oral health and a better cost-effective alternative. In addition, plants with beneficial effects on health have been widely identified throughout the world, which is why their use in oral health is considered accessible for a large part of the population, as well as the development of natural oral hygiene products, looking to minimize side-effects from artificial colorants, flavorings and other ingredients contained in most products used actually.

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Authors' contributions

ARP and SCEG contributed to the writing of the article. DSRU and DFOZ searched for the information. RRH helped in review and writing the article. All the authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

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