Original Article

EXPLORING THE IMMUNOMODULATORY EFFECTS OF VARIOUS PHYTOCHEMICALS IN DENTISTRY - AN ENCAPSULATION

ANNIE AROCKIA MARY, PRIYANKA K CHOLAN, JEYVARSHINI KESAVARAM, ANUPAMA TADEPALLI, SANTO GRACE UMESH, D.J.VICTOR

DOI: 10.47750/pnr.2022.13.S09.293

Abstract

Our immune system has a highly sophisticated defence system that protects the host from invading microorganisms by involving the interaction of a number of cell types, products, tissues and organs. Oral health influences the quality of well-being. Phytochemicals are naturally occurring molecules, which significantly play an imperative role in modulating favourable immune responses. A number of phytochemicals act as potential immunomodulators, hence we ought to discuss their mechanism of action and its applications in dentistry in this review article.

Keywords: Immununomodulation, phytochemicals, periodontitis, antimicrobial agents.

INTRODUCTION

Immune response is a result of effective interaction between innate (natural, non-specific) and acquired (adaptive, specific) immune system. Being the first line of defense, if the pathogen manages to dodge the innate immune system, the adaptive immunity will start kicking in, building a myriad of antibodies which is different to different pathogens. Modulation of immune system refers to alteration of immune response that includes stimulation, amplification, expression or inactivation of the immune response. Immune responses takes place in three well defined phases: cognition, activation , and effector (Abbas et al., 1995)(1). In the initial cognitive phase, foreign antigens are bound to specific receptors on the cell wall of mature lymphocytes. B-lymphocytes express specific antibodies that bind soluble foreign proteins, polysaccharides, or lipids, while T-lymphocytes express receptors recognize only short peptide sequences on foreign antigens (including those located on the surfaces of other cells)(2). In the activation phase, lymphocytes which have recognized a foreign antigen proliferate, leading to the formation of lymphocyte clones. Simultaneously, in the cell mediated immunity (CMI) arm of the immune system, antigen-stimulated T-cells differentiate from null cells to helper or suppressor cells or into killer cells (59). CMI serves to support the humoral arm of the immune system, recruit other defensive cells, and kill any cell recognized as foreign. Responding T- and B-lymphocytes eventually travel to site of antigen administration or antigen penetration into body tissues. In the third, effector phase, newly secreted antibodies eliminate the foreign antigen activating the complement cascade, stimulating degranulation of mast cells, and initiate the release of mediators from other cells. Activated T-cells secrete cytokines that enhance the functions of B-cells and phagocytes and stimulate nonspecific inflammatory responses.

Indian medicinal plants and various “Rasayana” possess immunomodulatory effects. Phytochemicals are originally occurring compound with bioactive potentials often referred to as “secondary metabolites”. In addition to compounds that are necessary for the growth and reproduction, plant cells synthesize a number of secondary metabolites, which do not appear to be strictly necessary for the survival of the plant, that include alkaloids, flavonoids, coumarins, glycosides, gums, polysaccharides, phenols, tannins, terpenes, and terpenoids.(3)(4). Phytochemicals also have therapeutic effects like anti-oxidant, anti-diabetic, memory enhancing, cholesterol lowering effects, anticancer, reduce oxidation, reduce inflammation and numerous immunomodulatory activities. Terpenoids, phenolic compounds, and alkaloids dominate among low-molecular immunomodulatory compounds, polysaccharides dominate among the high-molecular weight compounds (5). This article gives us an extensive description of various phytochemicals and their applications in dentistry.

CONCEPT OF IMMUNOMODULATION

Immunomodulation encompasses any intervention directed towards modifying the immune response with a therapeutic intent. Depending on the clinical scenario, it may be advantageous to modulate immune responses as well as therapeutic strategies employed in each therapy. The essence of immunomodulation is the stimulation of immune response which is desired for certain people such as immunocompromised patients or patients with auto immune or inflammatory diseases (6).

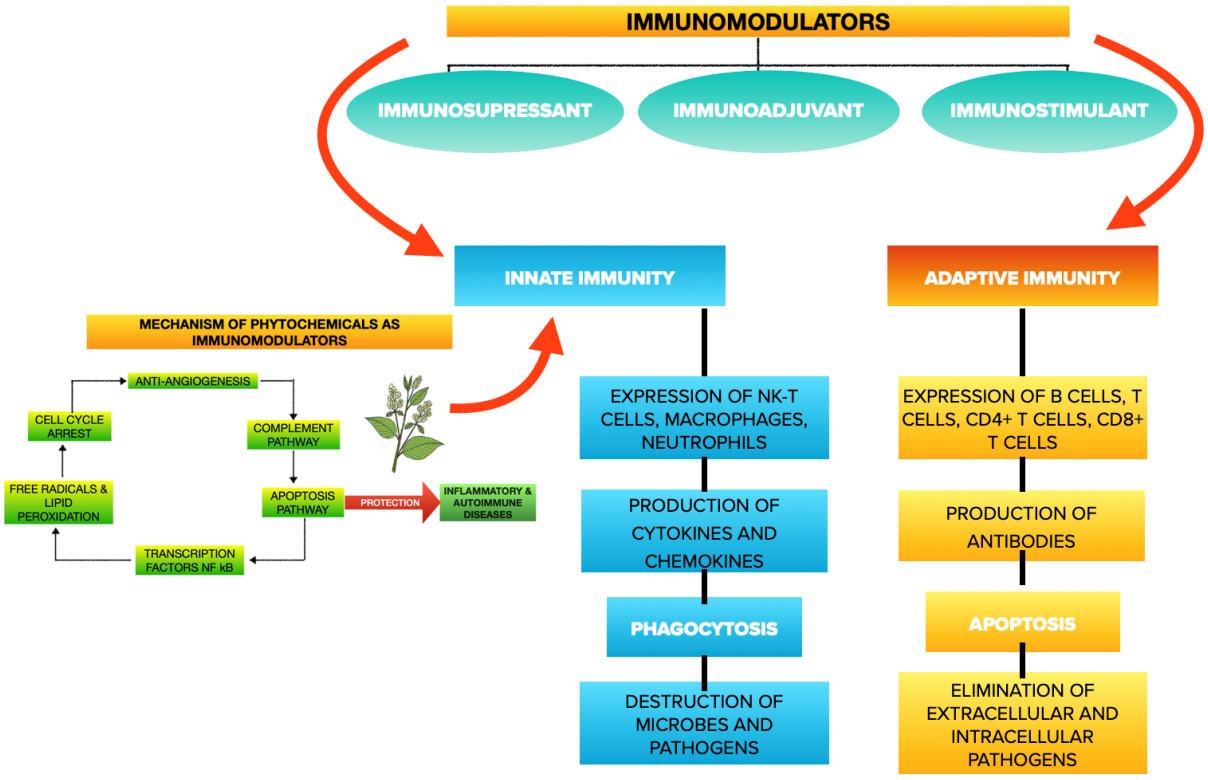

Fig 1. Mechanism of phytochemicals in immunomodulation(6),(7)

CLASSIFICATION OF IMMUNOMODULATORS

IMMUNOSTIMULANTS : Facilitate immune responses against several infections (autoimmunity, allergy, cancer) by activating innate and adaptive immunity(8),(9). They exhibit prophylactic actions in healthy patients by stimulating the primary immune response and act as immunotherapeutic agents in patients having primary and secondary immunodeficiency(10). Immunostimulants do not alter the memory cells of immune system with a brief duration of action. Hence to upregulate therapeutic activity, they are re-administered from time to time (11).

IMMUNOSUPRESSANTS : Suppresses the pathological immune responses in autoimmune diseases, hypersensitivity, graft-versus-host-rejection and other immune mediated diseases. Immunosuppressive agents cure graft rejection and autoimmune disorders. Immunosuppressants weakens the potential of human body which rejects the transplanted organs like kidney, liver and heart. Therefore, known as anti rejection agents (12),(13).

IMMUNOADJUVANTS : Equips the immune system by expanding the magnitude, duration and induction of antigen specific immune response but do not contain any special antigenic activity, administered in combination with specific antigen vaccines. In the absence of such vaccines, these agents do not have any antigen activity (14). They act as a depot for the slow release of antigen, stimulate phagocytosis and improve the immune response produced by the only antigen. These substances display

danger signals to the immune system to respond against specific antigen. Freund's adjuvants are used to distinguish Th1 and Th2, cellular and humoral, IgG and IgE, immunoprotective and immunodestructive immune actions (15),(16),(17).

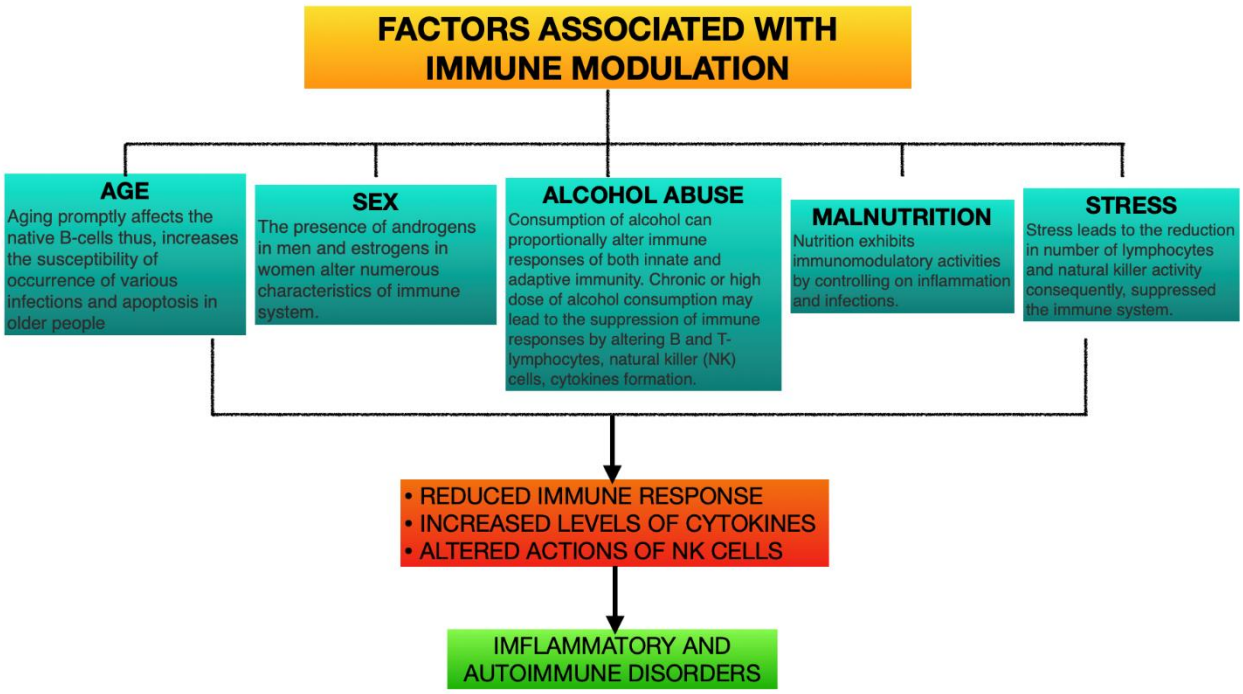

Fig 2. Factors associated with immunomodulation (7)

PHYTOCHEMICALS AS IMMUNOMODULATORS

Phytochemicals are chemical compounds produced by plants. They are low molecular weight bioactive compounds widely found in fruits, vegetables and other plant food. Phytochemicals have shown to inhibit the growth of oral pathogens, reduce the development of dental plaque, influence the adhesion of bacteria. Phytochemicals have been classified into many groups based on their structure: alkaloids, sulfur-containing phytochemical, terpenoids and polyphenols(18).

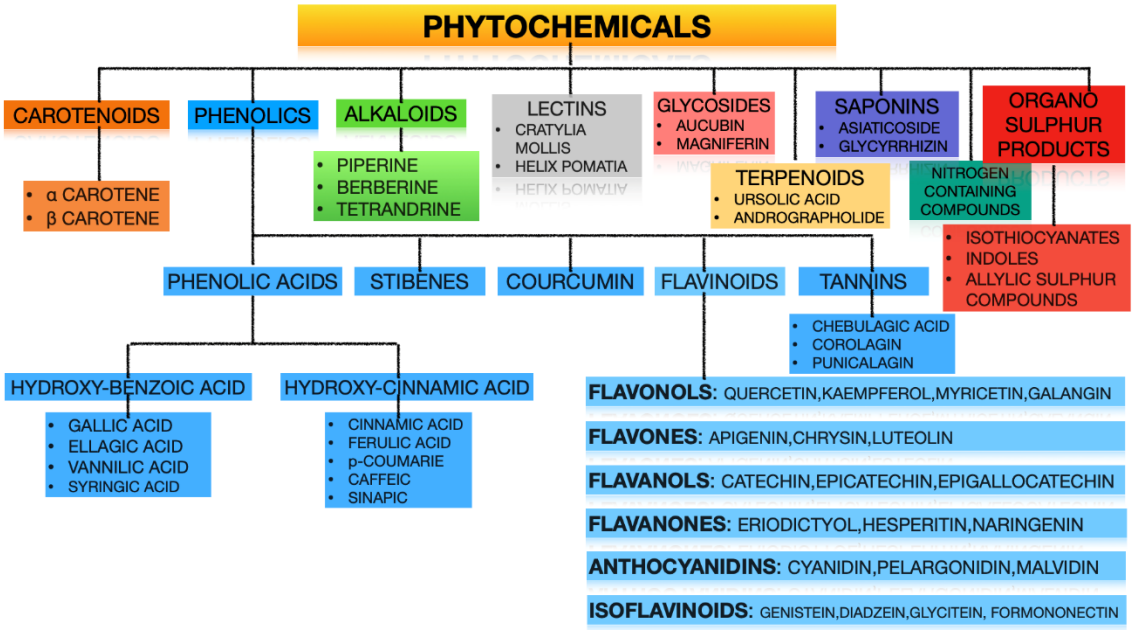

Fig 3. Classification of Phytochemical's

ALKALOIDS

Alkaloids rank among the most efficient and therapeutically significant plant substances comprising the largest single class of secondary plant substances which contain one or more Nitrogen atoms, usually in combination as part of a cyclic structure eg; nicotine, cocaine, morphine and codeine, quinine, reserpine, with large demand worldwide. They exhibit marked physiological activity when administered to animals. Alkaloids possess anti-tumor activity (vinblastine and vincristine), antimicrobial (cepharanthine), and analgesic activity (morphine) are also known to enhance immune response and a large number of alkaloids are being investigated for their immunostimulating properties (19).

Piperine has been found to possess various inhibitory activities on prostaglandin and leukotrienes COX-1 inhibitory effect, thereby exhibiting anti-inflammatory activity (Stohr et al., 2001), reducing inflammation and inhibits osteoclastic activity. Berberine is isolated from C. rhizoma (Ranunculacea) possess bactericidal activity against oral commensals and inhibits collagenase activity against A. actinomycetemcomitans and P. gingivalis(21). Berberine has the potential to inhibit the degradative action of extracellular MMP and in vitro inhibitory effect on P.gingivalis thus slowing periodontal degradation.

Phytochemical and botanical source	**Mechanism of action**
Piperine Piper longum	Increases total WBC count, bone marrow cellularity, total antibody production is enhanced (20)
Berberine Hydrasti canadensis	Significant decrease of plasma TNFα, IFNγ and NO levels (20)
Tetrandrine Stephania tetrandra	Suppress cytokine production Inhibits NFκB mediated release of inflammatory factors (20)
Sinomenine Sinomenium acutum	Essential for graft survival (20)

POLYSACCHARIDES

Polysaccharides modulate innate immunity, specifically macrophage function. The scientific evaluation of polysaccharides provides a unique opportunity for novel therapeutic agents and adjuvants that are beneficial immunomodulatory agent (22). Alginate has been found to be an ideal coating for dental impression materials (Nijhuis et al., 2010). This is an ideal component that binds growth factors and bone-forming cells in aqueous suspensions for the accurate biomimetic modification of implants (Zhitomirsky et al., 2009). Calcium alginate and fibrin gel keep cells in place after seeding and improve the immediate mechanical properties of the graft. Scientific evidences have shown that calcium alginate can be used as a cell carrier for bone marrow mesenchymal stem cells transplantation in periodontal regeneration (Tan et al., 2009).

LECTINS

Viscum album has shown to increase the number and cytotoxic activity of NK cells and induces antitumor activity in animal models. Lectin-sugar interactions on the cell surface of immunocompetent cells can induce cytokine gene expression and protein synthesis. Lectin alters mitochondrial transmembrane potential and increases intracellular levels of ROS.(23). According with Teixeira and collegues (24) plant lectins are able to inhibit oral bacteria adherence to enamel acquired pellicles probably through blocking streptococci adhesion. Lectins were found to have an anti-adhesion potential and they can be explored as a biotechnological tool and in therapeutics of dental diseases that are closely related to biofilm formation.

Mangiferin possesses antibacterial activity in vivo against specific periodontal pathogens such as P. intermedia and P. gingivalis. Enhances the production of IgG1 and IgG2b. Use of mango leaf in conjunction with a tooth brush is a good home care device for maintenance of oral hygiene (27). Cytotoxic and antimicrobial activities of methanolic extract of Boerhavia diffusa (28) investigated both stem and leaf extracts showed antimicrobial activity in a dose dependant manner. Leaf extract of Boerhavia diffusa showed maximum antibacterial activity against Staphylococcus aureus sub species aureus and maximum antifungal activity against Fusarium oxysporum. Studies have reported that the use of aucubin may exert its anti-inflammatory effects by inhibiting Ag-induced TNF and IL-6 production and expression by blocking NF-κB activation (29). Moreover, topical application of 0.1% aucubin solution showed antimicrobial activity by inhibiting the RNA and protein biosynthesis of salivary microorganisms (30). Lee et al. reported that application of 0.1% aucubin solution accelerated re-epithelialization and fibrosis on a dermal artificial surgical wound and suggested the possibility of using aucubin as a topical agent.

GLYCOSIDES

These include a wide range of chemical sub groups containing a glycan (sugar) and an aglycan (non-sugar). These are polar compounds which consist of at least one sugar molecule linked to another moiety. Pharmaceutically important glycosides include saponins and anthracin derivatives. Glycosides mainly participate in the stimulation of cardiac system, central nervous system stimulation and immune system, they also possess antimicrobial activity (23).

PHENOLIC COMPOUNDS

These are plant substances possessing an aromatic ring bearing one or more hydroxyl substituents. They are water soluble as they most frequently occur combined with sugar as glycosides. They are usually located in the cell vacuole. The presence of phenols is considered to be potentially toxic to the growth and development of pathogens. This group includes flavonoids, tannins, and other phenols (31).

Turmeric, a rhizome of Curcuma longa, is a herb known for its medicinal properties like anti-inflammatory, antioxidant, antimicrobial, hepatoprotective, immunostimulant, antiseptic, and anti mutagenic. It has a role in the treatment of periodontal diseases and oral cancers. Turmeric can also be used as a pit and fissure sealant, mouth wash (32), and subgingival irrigant (34) in different preparations. It is also used as a component in local drug delivery system in gel form as an adjunct to scaling and root planning (33).The efficacy of pomegranate, Punica granatum (Punicaceae), in the prevention of dental plaque was investigated and experimental data strongly support the antibacterial activity of pomegranate extracts against oral pathogen such as S. mutans (35). Aqueous extracts of Punica granatum show significant decrease in nitric oxide and TNF- α levels (Labsi et al,2016)

Gallic acid	Bcell proliferation, inhibition of mast cell degranulation (36)
Ellagic acid *Punica granatum*	Antiproliferative, antioxidant (36)
Chlorogenic acid *Plantago major*	Enhances lymphocyte proliferation, secretion of IFN (36)
Ferulic acid *Plantago major*	Enhances lymphocyte proliferation, secretion of IFN (37)
PCoumaric acid *Plantago major*	Enhances lymphocyte proliferation, secretion of IFN (37)
Vanilic acid *Plantago major*	Enhances lymphocyte proliferation, secretion of IFN (37)
Curcumin *Curcuma longa*	Enhances bone marrow cellularity, α esterase positive cells and phagocytic activity. Inhibits IL2 expression and NFκB (37)

FLAVONOIDS

Flavinoids, most widely distributed natural products occurring both in free-state and as glycosides with a C6-C3-C6 carbon skeleton (38). Flavonoids are potent water-soluble super antioxidants and free radical scavengers which prevent oxidative cell damage have strong anti-cancer activity and protect against all stage of carcinogens. Flavonoids are known to reduce the risk of heart diseases(39).In terms of anti-cancer activity, they inhibit the initiation, promotion, and progression of tumors. Some isoflavones act as allelochemicals (insecticides) .(40).Flavonoids like quercetrin and naringenin, were added into two pharmaceutical preparations in the form of toothpaste. It was found that the flavonoids inhibit plaque formation and significant decrease in the levels of Gram-positive streptococci by the usage of the toothpaste (Ammar et al) (41). Treatment in surgical wounds it was found that propolis which was found in aqueous solution mouth rinses helps in the repair of intrabuccal surgical wounds and exerted mild anti-inflammatory and analgesic effects (42). Repair of dental sockets and skin wounds numerous studies have pointed out that topical application of 10% hydroalcohol solution of propolis in cutaneous wound healing and socket wound after the extraction of tooth in rats, promoted oral epithelial repair but had no effect on wound healing of the socket.

Isorhamnetin3Oglucoside *Urtica dioica*	Has in vitro immunomodulatory potential (25)
Eupalitin3OβDgalactopyranoside *Boerhaavia diffusa*	Inhibited PHA stimulated proliferation of peripheral blood mononuclear cells IL2 and TNFα (25)
Aucubin *Plantago major*	Enhances lymphocyte proliferation, secretion of IFNγ (26)
Centaurein *Bidens pilosa*	Augmentation of INF promotor activity (43)
Apigenin 7-0-β-D-neohesperidoside *Jatropha curacas*	Stimulation of humeral and cell mediated immunity (43)
Luteolin *Plantago major*	Enhances lymphocyte proliferation and secretion of INF (44)

Baicalein *Plantago major*	Enhances lymphocyte proliferation and secretion of INF (44)
Quercetin-3-0- rutinoside *Utica dioica*	Immunomodulation (44)
Kaempherol-3-0- rutinoside *Utica dioica*	Immunomodulation (44)

ANTHOCYANINS

Anthocyanins and anthocyanin-rich mixtures of bioflavonoids may provide protection from DNA cleavage, estrogenic activity (altering development of hormone-dependent disease symptoms), enzyme inhibition, boosting production of cytokines (thus regulating immune responses), anti-inflammatory activity, lipid peroxidation, decreasing capillary permeability and fragility, and membrane strengthening (31). Cyanidin-3-glycoside and Peonidin (Blackberry) have anti-oxidant and anti-inflammatory action (45), (46).

The antioxidants from blueberries were shown to protect oral macrophages - WBC that are crucial to the immune system, blocking the molecular pathway involved in inflammation – a factor largely involved in periodontal disease. Strong proteolytic activity of bacteria of red complex causes periodontal destruction by variety of mechanisms including direct tissue degradation and host inflammatory response modulation (47). Bodet in 2006 (48) investigated the effect of cranberry concentrates showing trypsin like activity of T.forsythia and chymotrypsin activity of T.denticola was evaluated using synthetic chromogenic peptides with the potential to reduce the proliferation of P.gingivalis, T.forsythia and T.denticola in periodontal pockets or their proteinase mediated destructive processes occuring in periodontitis. La et.al (2009) (49) investigated the effects of A-type cranberry proanthocyanidins (AC-PACs) on the production of various MMPs by human monocyte derived macrophages stimulated with Aggregatibacter actinomycetocomitans and the catalytic activity of recombinant MMP-1 and MMP-9.The results showed that AC-PACs inhibit the production of MMPs in a concentration dependent manner and also inhibited catalytic activity of MMP-1 and MMP-9.

TANNINS

Amorphous, rare crystalline substances, soluble in water and alcohol having an astringent and bitter taste. Occurs widely in vascular plants; in angiosperms their occurrence is associated with woody tissues. Hydrolysable tannins are based on gallic acid, usually as multiple esters with D-glucose, while the numerous condensed tannins (often proanthocyanides) are derived from flavonoid monomers. Many physiological activities such as stimulation of phagocytic cells, host-mediated tumor activity and a wide range of anti-infective action have been assigned to tannins (38). Chebulagic acid down regulates TNF α and IL-6. Punicalagin scavenges free radicals and causes immunosuppression(50). Oral rinses made from these extracts used in periodontal therapy have anti-microbial and anti-oxidant effect inhibits Streptococcus mutans at concentrations as low as 50µg/ml. This anti-plaque effect probably may be due to the tannic acid in Triphala, which is adsorbed well on the surface of the bacterial cells, which result in protein denaturation and ultimately to bacterial cell death. Triphala mouth rinse when combined with scaling and root planing showed significant reduction in the plaque, gingival, and oral hygiene indices without any evidence of staining of teeth (51).

SAPONINS

Saponins are found in roots, tubers, leaves, blooms or seeds. Based on the carbon skeletons, saponins were classified into triterpenes and steroids. Their glycone parts were mostly oligosaccharides, arranged either in a linear or branched fashion, attached to hydroxyl groups through an acetal linkage (52). Researchers found that saponins have antitumor effect on many cancer cells. Several saponins inhibit tumor cell growth by cell cycle arrest and apoptosis with IC50 values up to 0.2 mM. Saponins in combination with conventional tumor treatment strategies can result in improved therapeutic success rate.(53). Asiaticoside enhances phagocytic index and total WBC count. Glycyrrhizin inhibits classical complement pathway.

A high saponin diet can inhibit dental caries and platelet aggregation. Asiaticoside (54) is a compound isolated from herb Centella asiatica, promotes osteogenic differentiation of human periodontal ligament (hPDL) cells. Thus asiaticoside could be used as a novel therapeutic drug for periodontal tissue regeneration. Liquorice belongs to the genus Glycyrrhiza. The ability of liquorice root polysaccharides to reduce bacterial binding to host cells was observed after pre-treatment of P. gingivalis by Wittschier et al. Bodet et al. investigated the response of liquorice on periodontopathogen - induced inflammatory response and found that liquorice extract exhibited potent anti-inflammatory properties by inhibiting the periodontopathogen (2020) (55).

TERPENOIDS

These are natural products whose structure may be divided into isoprene units, called as isoprenoids. Triterpenoids have carbon skeleton based on six isoprene units and are derived biosynthetically from the cyclic C30 hydrocarbon, squalene. They are colorless, crystalline, with high melting points and optically active substances. Triterpenes occur in waxy coatings of leaves and on fruit such as apple and pear, and serves a protective function in repelling insects and microbial attack. Terpenoids are reported to possess antiarthritic or antiphlogistic activity, and their biological activities appear to be mediated by immunological processes. Effect of these compounds on immune system appears to be two-fold; first to enhance antibody production and second to suppress T-cell response. Andrographolide enhances the expression of IL-2 and inhibition of NO in endotoxin stimulated macrophages(56). Ursolic acid activates intracellular killing effect of macrophages during Mycobacterium tuberculosis infection.

Andrographolide is a bioactive phytoconstituent found in various parts of A. paniculata, but particularly in the leaves. Nilavembu has the ability to reduce the bacterial growth. Nilavembu is found to be an adjunct to scaling and root planning for the treatment of periodontal diseases. However, further studies on a large-scale population using varied concentrations of the drug may be required to evaluate the long-term effects of nilavembu (57).

FUTURE OF POTENTIAL PHYTOCHEMICALS

By repurposing the traditional Indian medicinal plants and formulations, new treatment options can be identified to combat the deadly COVID-19. Many safe traditional formulations, which are known to be immunomodulators, have been used for centuries in respiratory disorders and in allergic conditions. Many of them are now under clinical trial in COVID-19 patients. Some of the traditional formulations include Berberis aristata (Yan et al, 2018; Wang et al 2017), Glycyrrhiza glabra (Mitra Mazumder et al,2012; Ashraf et al,2017), Piper longum (Jiang et al 2013), Piper nigrum (Mair et al,2016; Tasleem et al,2014), Curcuma longa (Kim et al,2014) that have been recognised to have immunomodulatory effects in COVID-19 patients. Hence a number of traditional herbs have been reported to work as an immunity booster against viral infections and possess anti-allergic/anti-inflammatory activities, its efficacy needs to be tested against COVID-19 (58).

CONCLUSION

Herbal excipients play a major role in pharmaceutical formulation owing to its wide range of availability in developing countries which have rich agro-climatic, cultural and ethnic biodiversity. The effects of phytochemicals on the immune system, have significantly evolved from laboratory testing to clinical studies. Combination therapeutics may be enhanced by exploring more phytochemical adjuvants which will synergistically affect the treatment outcomes with greater efficiency and less side effects. It is therefore, necessary to preserve this indigenous knowledge on traditional medicines by proper documentation, identification of plant species or parts used, herbal preparation, and their dosage to further elaborate on the rich significance of phytochemicals.

REFERENCES

1. Patard JJ, Guille F, Lobel B, Abbou CC, Cheopin D. Current state of knowledge concerning the mechanism of action of BCG. Prog Urol 1998;8:415-21.
2. Brindha P. Role of phytochemicals as immunomodulatory agents: A review. International Journal of Green Pharmacy (IJGP). 2016Mar 5;10(1).
3. Patil US, Jaydeokar AV, Bandawane DD. Immunomodulators: A pharmacological review. Int J Pharm Pharm Sci 2012;4:30-6.
4. Harborne JB. Phytochemical Methods. London: Chapman and Hall, Ltd.; 1973. p. 49-188.
5. Rahman AS, Adel F, Jaganaath MS. Role of levamisole in the induction of cell mediated immune response in experimental caecal coccidiosis of chickens. Indian Vet J 1989;66:706-10.
6. Mukherjee, Pulok & Nema, Neelesh & Bhadra, Santanu & Mukherjee, Debajyoti & Braga, Fernão & Matsabisa, Motlalepula. Immunomodulatory leads

from medicinal plants. Indian Journal of Traditional Knowledge (2014)13. 235-256.
7. Behl T, Kumar K, Brisc C, Rus M, Nistor-Cseppento DC, Bustea C, Aron RA, Pantis C, Zengin G, Sehgal A, Kaur R. Exploring the multifocal role of phytochemicals as immunomodulators. Biomedicine & Pharmacotherapy. 2021Jan 1;133:110959.
8. F. Clement, S. Pramod, Y. Venkatesh, Identity of the immunomodulatory proteins from garlic (Allium sativum) with the major garlic lectins or agglutinins, Int. Immunopharmacol. 10 (2010) 316–324, https://doi.org/10.1016/j. intimp.2009.12.002.
9. Y. Lu, J. Fan, Y. Zhao, S. Chen, X. Zheng, Y. Yin, C. Fu, Immunomodulatory activity of aqueous extract of Actinidia macrosperma, Asian Pacific J. Cancer Prev. 16 (2007) 261–265, https://doi.org/10.6133/apjcn.2007.16.s1.50.
10. S. Naik, A. Hule, Evaluation of immunomodulatory activity of an extract of andrographolides from Andographis paniculata, Planta Med. 75 (2009) 785–791, https://doi.org/10.1055/s-0029-1185398.
11. H. Wagner, Search for plant derived natural products with immunostimulatory activity: recent advances, Pure Appl. Chem. 62 (1990) 1217–1222, https://doi. org/10.1351/pac199062071217.
12. K. Manu, G. Kuttan, Immunomodulatory activities of Punarnavine, an alkaloid from Boerhaavia diffusa, Immunopharmacol. Immunotoxicol. 31 (2009) 377–387, https://doi.org/10.1080/08923970802702036.
13. K.Yatim,F.Lakkis,A brief journey through the immune system, Clin.J.Am.Soc. Nephrol. 10 (2015) 1274–1281, https://doi.org/10.2215/cjn.10031014.
14. H. Florindo, J. Lopes, L. Silva, M. Corvo, M. Martins, R. Gaspar, Regulatory development of nanotechnology-based vaccines, Micro Nanotechnol. Vacc. Dev. (2017) 393–410, https://doi.org/10.1016/b978-0-323-39981-4.00021-x.
15. M. Lutsiak, G. Kwon, J. Samuel, Biodegradable nanoparticle delivery of a Th2- biased peptide for induction of Th1 immune responses, J. Pharm. Pharmacol. 58 (2006) 739–747, https://doi.org/10.1211/jpp.58.6.0004.
16. N. Petrovsky, Novel human polysaccharide adjuvants with dual Th1 and Th2 potentiating activity, Vaccine 24 (2006) S26–S29, https://doi.org/10.1016/j. vaccine.2005.01.107.
17. C. Trujillo-Vargas, K. Mayer, T. Bickert, A. Palmetshofer, S. Grunewald,
18. Okwu DE. Phytochemicals and vitamin content of indigenous spices of South Eastern. Nig J Sust Agric Environ 2004;6:30-7.
19. Okwu DE. Phytochemical, vitamin and mineral contents of two Nigeria medicinal plants. Int J Mol Med Adv Sci 2005;1:375-81.
20. Lai JH, Ho LJ, Kwan CY, Chang DM, Lee TC. Plant alkaloid tetrandrine and its analog block CD28 costimulated activities of human peripheral blood T cells: potential immunosuppressants in transplantation immunology. Transplantation. 1999Nov 15;68(9):1383-92.
21. J. P. Hu, N. Takahashi, and T. Yamada, "Coptidis rhizoma inhibits growth and proteases of oral bacteria," Oral Diseases, vol6, no5, pp. 297–302, 2000.
22. Schepetkin IA, Quinn MT. Botanical polysaccharides: Macrophage immunomodulation and therapeutic potential. Int Immunopharmacol 2006;6:317-33.
23. Hostanska K, Hajto T, Weber K, Fischer J, Lentzen H, Sütterlin B, et al. A natural immunity-activity plant lectin, Viscum album agglutinin-I, induces apotos is in human lymphocytes, monocytes, monocytic THP-I Cells and murine thymocytes. Nat Immun 1996-1997;15:295-311.
24. Teixeira EH, Napimoga MH, Carneiro VA, De Oliveira TM, Cunha RM, Havt A, Martins JL, Pinto VP, Gonçalves RB, Cavada BS. In vitro inhibition of Streptococci binding to enamel acquired pellicle by plant lectins. Journal of applied microbiology. 2006Jul;101(1):111-6.
25. Pandey R, Maurya R, Singh G, Sathiamoorthy B, Naik S. Immunosuppressive properties of flavonoids isolated from Boerhaavia diffusa Linn. International immunopharmacology. 2005Mar 1;5(3):541-53.
26. Chiang LC, Ng LT, Chiang W, Chang MY, Lin CC. Immunomodulatory activities of flavonoids, monoterpenoids, triterpenoids, iridoid glycosides and phenolic compounds of Plantago species. Planta medica. 2003Jul;69(07):600-4.
27. Bairy, Indira & Reeja, S & Siddharth, Mallapragada & Rao, P & Bhat, Mahalinga & Shivananda, P. (2002). Evaluation of antibacterial activity of Mangifera indica on anaerobic dental microflora based on in-vivo studies. Indian journal of pathology & microbiology. 45. 307-10.
28. Mukesh Kumar Singh, A. Prathapan, Kushagra Nagori, S. Ishwarya, K.G Raghu. Cytotoxic and Antimicrobial Activity of Methanolic Extract of Boerhaavia diffusa L. Research J. Pharm. and Tech.3 (4): Oct.-Dec.2010; Page 1061-1063. Jeong HJ, Koo HN, Na HJ, Kim MS, Hong SH, Eom JW,
29. Kim KS, Shin TY and Kim HM: Inhibition of TNF-· and IL-6 production by aucubin through blockade of NF-κB activation in RBL-2H3 mast cells. Cytokine 18: 252-259, 2002.
30. Lee ES, Ahn JW, Mar WC and Chang IM: Pharmacology of iridoid: Antimicrobial activities of aucubin. Kor J Pharmacog 17: 129-133, 1986.
31. Urquiaga I, Leighton F. Plant polyphenol antioxidants and oxidative stress. Biol Res 2000;33:55-64.
32. Waghmare PF, Chaudhary AU, Karhadkar VM, Jamkhande AS. Comparative evaluation of turmeric and chlorhexidine gluconate mouthwash in prevention of plaque formation and gingivitis: A clinical and microbiological study. J Contemp Dent Pract. 2011;12:221–2.
33. Behal R, Mali MA, Gilda SS, Paradkar AR. Evaluation of local drug delivery system containing 2% whole turmeric gel used as an adjunct to scaling and root planning in chronic periodontitis: A clinical and microbiological study. J Indian Soc Periodontol. 2011;15:35–8
34. Suhag A, Dixit J, Dhan P. Role of curcumin as a subgingival irrigant: A pilot study. PERIO: Periodontal Pract Today. 2007;2:115–21
35. P. Subramaniam, S. Dwivedi, E. Uma, and K. L. Girish Babu, "Effect of pomegranate and aloe vera extract on streptococcus mutans: An in vitro study," Dental Hypotheses, vol3, no3, pp. 99–105, 2012.
36. Serrano A, Palacios C, Roy G, Cespón C, Villar ML, Nocito M, González-Porqué P. Derivatives of gallic acid induce apoptosis in tumoral cell lines and inhibit lymphocyte proliferation. Arch Biochem Biophys. 1998Feb 1;350(1):49-54.
37. Seeram NP, Adams LS, Henning SM, Niu Y, Zhang Y, Nair MG, Heber D. In vitro antiproliferative, apoptotic and antioxidant activities of punicalagin, ellagic acid and a total pomegranate tannin extract are enhanced in combination with other polyphenols as found in pomegranate juice. J Nutr Biochem. 2005Jun;16(6):360-7.
38. Hertog MG, Hollman PC, van de Putte B. Content of potentially anticarcinogenic flavonoids of tea infusions, wines and fruit juices. J Agric Food Chem 1993;41:1242-6.
39. Elangovan V, Sekar N, Govindasamy S. Chemopreventive potential of dietary bioflavonoids against 20-methylcholanthrene-induced tumorigenesis. Cancer Lett 1994;87:107-13.
40. Kandaswami C, Perkins E, Soloniuk DS, Drzewiecki G, Middleton E Jr. Antiproliferative effects of citrus flavonoids on a human squamous cell carcinoma in vitro. Cancer Lett 1991;56:147-52.
41. Ammar N, El Diwany A, Osman N, Gaafar S, Amin N. Flavonoids as a possible preventive of dental plaque. Archives of Pharmacal Research. 1990Jun;13(2):211-3.
42. Magro Filho O, de Carvalho AC. Application of propolis to dental sockets and skin wounds. J Nihon Univ Sch Dent. 1990Mar;32(1):4-13.
43. Chang SL, Chiang YM, Chang CL, Yeh HH, Shyur LF, Kuo YH, Wu TK, Yang WC. Flavonoids, centaurein and centaureidin, from Bidens pilosa, stimulate IFN-gamma expression. J Ethnopharmacol. 2007Jun 13;112(2):232-6.
44. Chiang LC, Ng LT, Chiang W, Chang MY, Lin CC. Immunomodulatory activities of flavonoids, monoterpenoids, triterpenoids, iridoid glycosides and phenolic compounds of Plantago species. Planta Med. 2003Jul;69(7):600-4.
45. Rechner AR, Kroner C. Anthocyanins and colonic metabolites of dietary polyphenols inhibit platelet function. Thromb Res. 2005;116(4):327-34.
46. Wang LS, Stoner GD. Anthocyanins and their role in cancer prevention. Cancer letters. 2008Oct 8;269(2):281-90.
47. Grenier D. Degradation of host protease inhibitors and activation of plasminogen by proteolytic enzymes from Porphyromonas gingivalis and Treponema denticola. Microbiology (Reading). 1996Apr;142 (Pt 4):955-961.

48. Bodet C, Piché M, Chandad F, Grenier D. Inhibition of periodontopathogen-derived proteolytic enzymes by a high-molecular-weight fraction isolated from cranberry. J Antimicrob Chemother. 2006Apr;57(4):685-90.
49. Bodet C, Chandad F, Grenier D. Anti-inflammatory activity of a high-molecular-weight cranberry fraction on macrophages stimulated by lipopolysaccharides from periodontopathogens. J Dent Res. 2006Mar;85(3):235-9.
50. Lee SI, Kim BS, Kim KS, Lee S, Shin KS, Lim JS. Immune-suppressive activity of punicalagin via inhibition of NFAT activation. Biochem Biophys Res Commun. 2008Jul 11;371(4):799-803.
51. Desai A, Anil M, Debnath S. A clinical trial to evaluate the effects of triphala as a mouthwash in comparison with chlorhexidine in chronic generalised periodontitis patient. Indian Journal of Dental Advancements. 2010Jul 1;2(3):243-8.
52. Sparg SG, Light ME, van Staden J. Biological activities and distribution of plant saponins. J Ethnopharmacol 2004;94:219-43.
53. Bouic PJ. Plant sterols and sterolins. Altern Med Rev2001;6:203-6.
54. Fitri AR, Pavasant P, Chamni S, Sumrejkanchanakij P. Asiaticoside induces osteogenic differentiation of human periodontal ligament cells through the Wnt pathway. J Periodontol. 2018May;89(5):596-605.
55. Sidhu P, Shankargouda S, Rath A, Ramamurthy PH, Fernandes B, Singh AK. Therapeutic benefits of liquorice in dentistry. Journal of Ayurveda and integrative medicine. 2020Jan 1;11(1):82-8.
56. Chiou WF, Chen CF, Lin JJ. Mechanisms of suppression of inducible nitric oxide synthase (iNOS) expression in RAW 264.7 cells by andrographolide. Br J Pharmacol. 2000 Apr;129(8):1553-60.
57. Paulraj, B. & Ramamurthy, Jaiganesh(2019). Antibacterial effect of Andrographis paniculata against oral microbes - An in vitro study. Drug Invention Today12. 91-93.
58. Ahmad S, Zahiruddin S, Parveen B, Basist P, Parveen A, Parveen R, Ahmad M. Indian medicinal plants and formulations and their potential against COVID-19–preclinical and clinical research. Frontiers in pharmacology. 2021Mar 2;11:2470.
59. Balaji S, Cholan PK, Victor DJ. An emphasis of T-cell subsets as regulators of periodontal health and disease. J Clin Transl Res. 2021 Sep 20;7(5):648-656. PMID: 34778595; PMCID: PMC8580519.