ORIGINAL RESEARCH ARTICLE

Seabuckthorn - Nature's Boon against Periodontal Pathogens: An *Ex vivo* Study

Susmitha Santosh[1], G. A. Babitha[1], Shobha Prakash[1], K. Suresh[2], Kishore G. Bhat[3]
[1]Department of Periodontics, College of Dental Sciences, Davangere, Karnataka, India, [2]Department of Microbiology, JJM Medical College and Hospital, Davangere, Karnataka, India, [3]Department of Molecular Biology and Immunology, Maratha Mandal's NGH Institute of Dental Sciences and Research Centre, Belgaum

Email for correspondence: susmithasantosh@gmail.com

ABSTRACT

Background: Seabuckthorn (Hippophae rhamnoides) is an orange-colored fruit which is found in high altitude in Asian and European countries. It is rich in Vitamins A, E, and K whereas its seed-oil has high content of omega-3, 6 and 9 fatty acids. It has been found to be useful in various systemic diseases and oral conditions such as gingivitis and periodontitis. **Aims and Objectives:** The present article aims to evaluate the efficacy of Seabuckthorn against growth of periodontal pathogens (*Porphyromonas gingivalis, Aggregatibacter actinomycetemcomitans*, and *Tannerella forsythia*) and determine the amount of SBT pulp extract for its bactericidal and bacteriostatic action against periodontal pathogens. **Materials and Methodology**: Alcoholic extract of SBT was prepared and its minimum inhibitory concentration (MIC) against periodontal pathogens; *Porphyromonas gingivalis* (Pg), *Aggregatibacter actinomycetemcomitans* (Aa), and *Tannerella forsythia* (Tf), were estimated using serial dilutions of Seabuckthorn pulp extract by tube dilution method; and bactericidal and bacteriostatic activity of Seabuckthorn pulp extract against Pg, Tf, and Aa was determined using Time kill curve assay (at baseline, 10 min, 30 min, and 2 h). **Results:** Minimum inhibitory concentration of SBT was found to be 25 μg/ml for Pg within 2 h interval and significantly lower for the other organisms. **Conclusions:** Seabuckthorn pulp extract shows good inhibitory effect on the major periodontal pathogens and hence is promising in the field of periodontics. In the coming future, it has the potential to be used in home oral care as well.

Key words: Antioxidant, local drug delivery, minimum inhibitory concentration, periodontal pathogens, seabuckthorn

INTRODUCTION

Nature itself acts as a chemist. A variety of antioxidant factors are found in plants and berries, such as alpha-tocopherol, ascorbate, carotenoids, and zinc. Plant based medicines may also contain flavonoids, polyphenols, and flavoproteins. Further, some plants or specific combinations of herbs in formulations may act as antioxidants by exerting superoxide scavenging activity or by activating superoxide dismutase activity in various tissue sites which neutralizes free radical activity (Sawant, 2009, Alok, 2014).

Quick Response Code

Article Info:

doi: 10.5866/2021.12.10011

Received: 04-01-2021
Revised: 08-02-2021
Accepted: 17-03-2021
Available Online: 08-04-2021, (www.nacd.in)

Periodontal disease is defined as a chronic inflammatory disease of supporting tissues of teeth caused by microorganisms eventually resulting in loss of clinical attachment and alveolar bone. Research has shown association of this disease with many other systemic diseases such as diabetes, cardiovascular diseases, respiratory diseases, and rheumatoid arthritis.

Non-surgical therapy remains the cornerstone of periodontal treatment. Sustained plaque control plays an important role in achieving successful long-term results for the care of periodontitis patients. Proper maintenance and patient hygiene play a key role in suppression of re-colonization of micro-organism. Many adjunctive treatment modalities along with mechanical debridement have been

clinically used and investigated for their efficacy. Systemic antioxidants in conjunction with scaling and root planning can offer additional effects and can be used as an adjunctive treatment.

Recently, it has been observed that the local route of drug delivery can attain 100-fold higher concentrations of an antimicrobial agent at subgingival sites compared with a systemic drug regimen. This reduces the total patient dose by over 400-fold, thereby reducing the potential problems associated with the use of systemic antibiotic drug regimens and development of drug-resistant microbial populations at non oral body sites. These can be safely used in medically compromised patients for whom surgery is contraindicated, in patients with known hypersensitivity to the antimicrobial used, asthmatics, and infective conditions such as AIDS and tuberculosis.[1]

Seabuckthorn (SBT) (Scientific name: Hippophae rhamnoides) is a small orange yellow-to-red colored fruit which are found on high altitude of 2500–4300 m in several Asian and European countries including India, China, Nepal, Britain, Germany, France, Finland, and Russia. According to the literature, the Chinese were the first to use Seabuckthorn oil as a drug and it was then in 1977 that this plant was formally listed in the Chinese pharmacopoeia.

In India, it is found mainly on high altitudes of Ladakh, Lahul-Spiti, parts of Chamba and upper Kinnaur district of Himachal Pradesh, Sikkim, and Arunachal Pradesh.[2] It is known by many local names such as Sastalulu, Shangti, Dhurchuk, Chumma, Tarwaa, Sirmaa, Chhurmak, and Leh berry. On September 23, 2015, a renowned Indian yoga guru, Baba Ramdev of Patanjali Yogpeeth, Haridwar, Uttarakhand, named the plant "Brahmaphal," in a transfer of technology function at the Defence Institute of High Altitude Research (DIHAR), Leh (Jammu and Kashmir), acknowledging the contribution of the author in finding this plant in Ladakh, an area known as a barren cold desert, and popularizing the plant in scientific and business communities in India. Hence, the Hindi name for SBT is also given as Brahmaphal.

This plant is also referred many a times as a "Wonder Plant" (Li and Schroeder 1999) as the seed oil contains high amounts of Vitamins A, E, K, carotenoids, and phytosterols.[3] SBT pulp is an excellent source of omega 7 (palmitic acid and palmitoleic acid), while the seed oil has a high content of omega 3 (α-linolenic acid), omega 6 (linoleic acid), and omega 9 (oleic acid) fatty acids. The fatty acid content of the oil acts as epidermal barrier system which stabilizes cell membrane structure.[4] The oil has an anti-oxidative, antimicrobial, immunomodulatory, cytoprotective, and tissue regenerative properties.[5]

Patients with periodontal disease display increased PMN number and activity, resulting in high degree of free-radical release, culminating in high oxidative damage to gingival tissues, periodontal ligament, and alveolar bone. Damage mediated by free radicals can be mitigated by "antioxidant defense system." SBT oil is an antioxidant with omega-7 fatty acid which has unique nutritional properties thereby benefiting mucous membrane by reducing inflammation, improving wound healing and showing excellent antibacterial property.

In this *in vitro* study, SBT pulp extract was evaluated for,

1. Its efficacy against growth of periodontal pathogens; *Porphyromonas gingivalis* (Pg), *Tannerella forsythia* (Tf), and *Aggregatibacter actinomycetemcomitans* (Aa).
2. To quantify the amount of SBT pulp extract for its bactericidal action against Pg, Tf, and Aa using tube dilution method.
3. To determine its bactericidal and bacteriostatic activity against Pg, Tf, and Aa using time kill curve assay.

MATERIALS AND METHODS

Pure concentrated form of SBT pulp extract was obtained from the berries grown at Spiti valley of Himachal Pradesh, India. Minimum inhibitory concentration of this extract was estimated using serial dilutions of the agent by tube dilution method and its bacteriostatic and bactericidal activity using time kill curve at 10 min, 30 min, and 2 h against the bacteria, *Porphyromonas gingivalis* (Pg), *Tannerella forsythia* (Tf), and *Aggregatibacter actinomycetemcomitans* (Aa).

Tube Dilution Method

Nine dilutions of SBT pulp extract were done with thioglycollate broth. In the initial tube, 20 μL of SBT pulp extract was added into the 380 μL of thioglycollate broth. For dilutions, 200 μL of thioglycollate broth was added into the next nine

tubes separately. Then from the initial tube 200 µL was transferred to the first tube containing 200 µL of thioglycollate broth. This was considered as 10^{-1} dilution. From 10^{-1} diluted tube, 200 µL was transferred to second tube to make 10^{-2} dilution. The serial dilution was repeated up to 10^{-9} dilution for the extract. From the maintained stock cultures of required organisms (Pg, Tf, and Aa), 5 µL was taken and added into 2 ml of thioglycollate broth. In each serially diluted tube 200 µL of above culture suspension was added. The tubes were incubated for 48–72 h in an anaerobic jar at 37 C and observed for turbidity [Figure 1].

Time Kill Curve

Equal quantity of the broth with organisms (Pg, Tf and Aa) and SBT pulp extract was mixed which was then plated immediately, this was noted as 0 h. Tubes were kept in CO_2 jar till further time slot, that is, 10 min, 30 min, and 2 h. It was cultured or plated, and incubated according to the growth requirement, that is, in CO_2 Jar and anaerobic jar. After 48–72 h of incubation, the plates were removed and the colony count was noted [Figure 2].

RESULTS

In the present study, all three periodontal pathogens, namely, Pg, Tf, and Aa were sensitive to pure Seabuckthorn extract. Aa was sensitive until 0.4 µg/ml dilution and showed resistance to further dilution. Tf was sensitive until 12.5 µg/ml dilution and showed resistance to further dilution. Pg was sensitive until 25 µg/ml dilution and showed resistance to further dilution. Aa was found to be more sensitive than Tf and Pg [Table 1]. Time kill curve assay showed that Pg was inhibited within 10 min, Aa in 30 min, and Tf within 2 h [Table 2].

DISCUSSION

Periodontitis is an inflammatory disease of supporting tissues of teeth caused by specific microorganisms or group of specific microorganisms, resulting in progressive destruction of the periodontal ligament and alveolar bone with periodontal pocket formation, gingival recession or both. Successful periodontal treatment requires suppression or elimination of the subgingival periodontal pathogens.

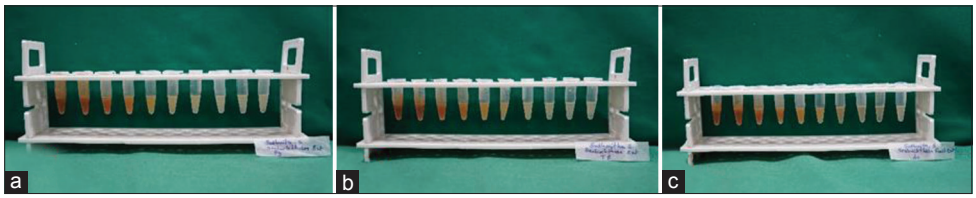

Figure 1: Serial dilutions of seabuckthorn extract (a) *Porphyromonas gingivalis*, (b) *Tannerella forsythia*, (c) *Aggregatibacter actinomycetemcomitans*

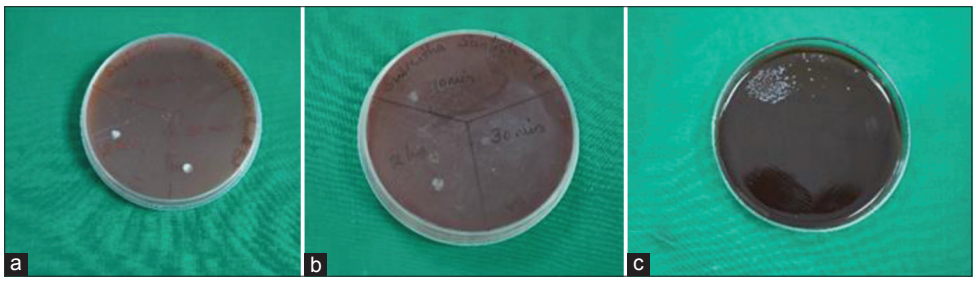

Figure 2: Time Kill Curve of SBT (a) *Porphyromonas gingivalis*, (b) *Tannerella forsythia*, (c) *Aggregatibacter actinomycetemcomitans*

Table 1: MIC of SBT using tube dilution method against Pg, Tf, and Aa

S. No.	Samples	100 µg/ml	50 µg/ml	25 µg/ml	12.5 µg/ml	6.25 µg/ml	3.12 µg/ml	1.6 µg/ml	0.8 µg/ml	0.4 µg/ml	0.2 µg/ml
	Seabuck thorn										
1	Pg	S	S	S	R	R	R	R	R	R	R
2	Tf	S	S	S	S	R	R	R	R	R	R
3	Aa	S	S	S	S	S	S	S	S	S	R

Table 2: Time kill curve of SBT against Pg, Tf and Aa

S. No.	Samples	10 Min	30 Min	2 Hrs
	Seabuck thorn			
1	Pg	NG	NG	NG
2	Tf	>100	>50	NG
3	Aa	>150	NG	NG

Mechanical removal of plaque and calculus has been a gold standard for battling the bacterial challenge in case of periodontitis. Antimicrobial agents attempt to directly reduce the pocket microflora when applied as an adjunct to mechanical debridement. Local delivery of these antimicrobials has been in use currently giving good results and better toleration due to reduced risk of toxicity. Many herbal formulations are making their place in various medical and research fields due to their medicinal properties.

Seabuckthorn (SBT) is one such fruit, found on high altitude in several Asian and European countries, withstanding temperature as low as 43 C. It belongs to the family Elaeagnaceae and genus Hippophae. It mostly grows wild along river beds or wherever there are small water streams and even irrigation channels. Seabuckthorn (SBT) is one of the future crops. The shrub has a remarkable lifespan of more than 100–150 years, and it has a number of eco-environmental and commercial benefits.[6]

As an immunity enhancer, it maintains the immune system and keeps the supervisory role of the system normal and healthy.[5] Studies (Yang *et al.* 2000, Liu *et al.* 1980) on SBT oil have suggested its usefulness in treating damage which is caused by radiation, burns, eye, skin diseases, cardiovascular diseases, oral inflammation, mucous injuries, and gastric ulcers.[7]

This wonder plant is an excellent source of omega 7 (palmitic acid, palmitoleic acid), whereas the seed oil has a high content of omega 3 (α-lenolenic acid), omega 6 (linoleic acid), omega 9 (oleic acid) fatty acids. Olas in 2016 reported that the leaves, fruits, and oils of this plant are sources of many bioactive substances including Vitamins (A, C, and E), unsaturated fatty acids, phenolic compounds, especially flavonoids and phytosterols which confer positive effects to the cardiovascular system. These polyphenolic compounds found in SBT have been reported to have multiple biological effects, including antioxidant activity. Antioxidants can interfere with the oxidation process by reacting with free radicals, chelating catalytic metals and also as reactive species scavenger reducing the oxidative damage induced by them. Indeed, in addition to the antioxidant activity, its oil has provided antibacterial and antiviral properties (Erkkola *et al.*, 2003; Gupta *et al.*, 2011).

Suleyman *et al.* in 2001 tested the antiulcerogenic effect of a hexane extract (HRe-1) from Hippophae rhamnoides (Elaeagnaceae) on indomethacin and stress-induced ulcer models and found HRe-1 to be active in preventing gastric injury.[8] Khajuria *et al.* in 2017 studied the hepatoprotective effects of Seabuckthorn leaf extract supplementation on serum enzymatic levels in streptozotocin induced diabetes mellitus in Wistar rats and observed significant improvement in aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP), and acid phosphatase (ACP) levels.[9]

Sharma *et al.* in 2016 showed a marked reduction in probing pocket depth in chronic periodontitis patients on Seabuckthorn capsules along with mechanical debridement compared to mechanical debridement alone.[5] Smida *et al.* in 2019 demonstrated the bactericidal effect of Seabuckthorn against some periodontal pathogens and its ability to inhibit single and multi-strain biofilms formation.[10] Since no direct assays have been performed on oral biofilms, current research was carried out with the aim of finding the efficacy of SBT pulp extract against periodontal pathogens using tube dilution method and time kill curve wherein we found that at a minimum concentration of 25 μg/ml, all periodontal pathogens were inhibited.

Minimum inhibitory concentration of SBT pulp extract was found at 25 μg/ml for Pg, 12.5 μg/ml for Tf, and as low as 0.4 μg/ml for Aa [Table 1]. Time kill curve assay showed no growth within 2 h for all the organisms tested [Table 2]. Results support the inhibitory effect of SBT pulp extract on periodontal pathogens and application *in vivo* testing. Future therapeutic considerations might include alternative drugs using different antimicrobials from one treatment to the next, as more drugs become available.

Although, traditionally, SBT has been used for various diseases, till date, not much scientific literature is available on SBT especially in the field

of periodontics. It clearly signifies that a whole lot of scientific exploration needs to be done on this wild berry which could give the lead for natural alternative for diverse diseases against synthetic drugs.

CONCLUSION

Nature holds the key to good health. Seabuckthorn is one such product of nature. It has been studied extensively in the field of medicine and found to be effective for many systemic conditions. The present study provides preliminary evidence for the antimicrobial activity of SBT pulp extract against periodontal pathogens such as Pg, Tf, and Aa. Traditional plants may represent new sources of antimicrobials, with SBT showing maximum effect against periodontal pathogens at a minimum concentration of 25 μg/ml within 2 h interval. It also shows a lot of promise in the field of alternative medicine, especially for applications in periodontitis and possibly other oral infections. Further studies are necessary to determine its clinical efficacy and potential to be included in daily home oral care products.

ACKNOWLEDGEMENT

We would like to thank Ishita Khanna, Founder and Director, Spiti Ecosphere and Takpa, Field guide, Spiti Ecosphere, Himachal Pradesh, for introducing Seabuckthorn (wonder berry) and also providing these berries for the present study.

REFERENCES

1. Goodson JM. Antimicrobial strategies for treatment of periodontal diseases. Periodontol 2000 1994;5:142-68.
2. Rousi A. The genus *Hippophae* L. A taxonomic study. In: Annales Botanici Fennici. Helsinki, Finlandia: Societas Biologica Fennica Vanamo; 1971. p. 177-227.
3. Beveridge T, Li TS, Oomah BD, Smith A. Sea buckthorn products: Manufacture and composition. J Agri Food Chem 1999;47:3480-8.
4. Zeb A. Important therapeutic uses of sea buckthorn (*Hippophae*): A review. J Biol Sci 2004;4:687-93.
5. Sharma A, Sharma S, Khattri S, Garg H. Role of sea buckthorn oil in management of chronic periodontitis: Follow-up study. Int J Dent Res 2016;4:33-7.
6. Singh B. Indian sea buckthorn. In: New Age Herbals. Singapore: Springer; 2018. p. 29-54.
7. Johansson AK, Korte H, Yang B, Stanley JC, Kallio HP. Sea buckthorn berry oil inhibits platelet aggregation. J Nutr Biochem 2000;11:491-5.
8. Süleyman H, Demirezer LÖ, Büyükokuroglu ME, Akcay MF, Gepdiremen A, Banoglu ZN, *et al.* Antiulcerogenic effect of *Hippophae rhamnoides* L. Phytother Res 2001;15:625-7.
9. Khajuria P, Raghuwanshi P, Rastogi A, Koul AL, Zargar R, Kour S. Hepatoprotective effect of Seabuckthorn leaf extract in streptozotocin induced diabetes mellitus in Wistar rats. Indian J Anim Res 2018;52:1745-50.
10. Smida I, Pentelescu C, Pentelescu O, Sweidan A, Oliviero N, Meuric V, *et al.* Benefits of sea buckthorn (*Hippophae rhamnoides*) pulp oil-based mouthwash on oral health. J Appl Microbiol 2019;126:1594-605.