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Original Article

Dentistry Section

Evaluation of Antibacterial Activity of Ethanolic Extract of *Portulaca Oleracea* against Periodontal Pathogens: An In-vitro Study

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ABSTRACT

Introduction: The need of the hour is to search for new antimicrobial agents of herbal origin to combat the global problem of drug resistance. *Portulaca oleracea* (common purslane) is an agricultural weed with a long history of medicinal use. In the present study, the authors intend to test its antibacterial efficacy against *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis* and *Tannerella forsythia*, which are the main pathogens of periodontal disease in children and adults.

Aim: To evaluate the antibacterial activity of the ethanolic extract of *Portulaca oleracea*.

Materials and Methods: The in-vitro study was conducted in the Department of Paediatric and Preventive Dentistry and the Central Research Laboratory at Maratha Mandal's NGHIDS and Research Centre, Belagavi, Karnataka, India, from November 2023 to January 2024. A crude extract from the aerial parts of *Portulaca oleracea* was prepared by the Soxhlet extraction method using ethanol as a solvent at KAHER's Shri BM Kankanawadi Ayurveda Mahavidyalaya Post Graduate Studies and Research Centre, Belagavi, Karnataka. The extract's Minimum Inhibitory Concentration (MIC) was assessed against the standard strains *A. actinomycetemcomitans* (ATCC 29523), P. gingivalis (ATCC 33277) and T. forsythia (ATCC 43037) and compared with that of chlorhexidine gluconate. A total of 10 serial dilutions of the extract were prepared using the double broth dilution method. After incubation, turbidity was noted at the lowest dilution. The procedure was performed three times and the mean value was calculated.

Results: The MIC of the ethanolic extract of *Portulaca oleracea* for *P. gingivalis*, *T. forsythia* and *A. actinomycetemcomitans* was reported at 9.3, 0.5 and 4.7 µL/mL, respectively. Chlorhexidine showed consistent microbial resistance at 0.6 µL/mL for all the three periodontal pathogens.

Conclusion: The ethanolic extract of *Portulaca oleracea* can be used as an effective antimicrobial agent against periodontal pathogens, since as it has been found to possess antibacterial properties.

Keywords: *A. actinomycetemcomitans*, Antimicrobial agent, Chlorhexidine, *Porphyromonas gingivalis*, *Tannerella forsythia*

INTRODUCTION

Antibiotic resistance has become a major problem in today's world due to its inadvertent use. Hence, there is an urgent need to search for new antimicrobial agents, especially those of herbal origin, to combat this problem [1].

Portulaca oleracea [Table/Fig-1] is a warm-climate, herbaceous, succulent annual plant with a cosmopolitan distribution, belonging to the Portulacaceae family and has a long history of medicinal use. It exhibits a wide range of pharmacological effects, including antibacterial, antiulcerogenic, anti-inflammatory, antioxidant and wound-healing properties [2-4]. The World Health Organisation (WHO) lists it as one of the most commonly used medicinal plants and it has been referred to as a "Global Panacea" [5].

[Table/Fig-1]: *Portulaca oleracea*.

Portulaca oleracea L. (common purslane) is an agricultural weed found worldwide and ranks among the eight most common plants growing on Earth [6].

Periodontal disease is one of the major dental diseases that affect human populations globally. It is mediated by a combination of periodontal pathogens and host defense systems [7]. More than 700 different bacterial species colonise the oral cavity, but only a few are considered potential periodontal pathogens, such as *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis* and *Tannerella forsythia*, which are the main causative agents of periodontal disease in children [1,8].

Scaling and Root Planing (SRP) is the gold standard treatment for periodontal diseases; however, it has limitations, such as the inability to access deeper areas of the gingival sulcus [1]. This has led researchers to investigate the local delivery of drugs capable of reaching these inaccessible areas of the gingival sulcus, such as chlorhexidine, which is recognised as the most effective antiplaque agent [9,10].

In the present study, chlorhexidine has been used as a positive control since it is considered the gold standard in dentistry [1].

The aim of the present study was to evaluate and compare the antibacterial activity of the ethanolic extract of *Portulaca oleracea* with that of chlorhexidine gluconate against *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis* and *Tannerella forsythia*.

MATERIALS AND METHODS

The in-vitro study was conducted in the Department of Paediatric and Preventive Dentistry and the Central Research Laboratory at Maratha Mandal's NGHIDS and Research Centre, Belagavi, Karnataka, India, from November 2023 to January 2024. The aerial parts of *P. oleracea* were collected from the Belagavi district, Karnataka, in November 2023. The plant was authenticated by Shri BMK Ayurvedic Mahavidyalaya, Belagavi (BMK/CRF/14/2024-25).

Study Procedure

The aerial parts of *P. oleracea* were washed three times in distilled water and shade dried at room temperature. After complete drying, they were subjected to size reduction in a grinder and stored in an airtight glass jar.

The ethanolic extract was prepared using 1,000 mL of 99.9% ethanol as the solvent, at room temperature, using the Soxhlet extraction method with a Soxhlet apparatus [Table/Fig-2] [11]. The ground powder was placed in thimble-shaped filter paper and then positioned in a glass cylinder. This cylinder is equipped with a siphon tube and an inlet tube, while a water condenser is attached to the top of the cylinder. This entire assembly is fitted into the neck of a round-bottom flask that contains the solvent—ethanol in the present study.

[Table/Fig-2]: Soxhlet apparatus [11].

The flask was heated in a water bath or sand bath. The solvent vapours travel through the inlet tube into the cylinder and condense upon passing upward into the condenser. The condensed solvent comes into contact with the crude organic powder, dissolving it. Hence, a continuous supply of solvent vapours was maintained in the cylinder, allowing the dissolved organic compounds to flow back into the flask. Finally, the heating was stopped and the solution in the flask was distilled to recover the solvent, leaving the organic compound behind. The extract was prepared at Shri BMK Ayurvedic Mahavidyalaya, Belagavi.

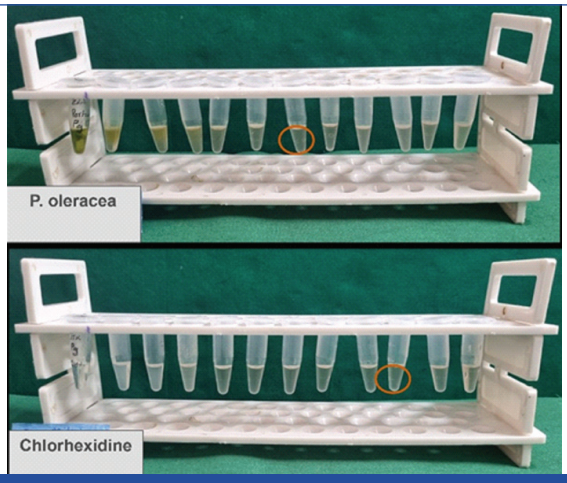
The standard strains of *A. actinomycetemcomitans* (ATCC 29523), *P. gingivalis* (ATCC 33277) and *T. forsythia* (ATCC 43037) were procured from the Central Research Laboratory at Maratha Mandal's Nathajirao G. Halgekar Institute of Dental Sciences and Research Centre, Belagavi.

Microbiological analysis: Microbiological analysis was conducted at the Central Research Laboratory at Maratha Mandal's Nathajirao G. Halgekar Institute of Dental Sciences and Research Centre, Belagavi.

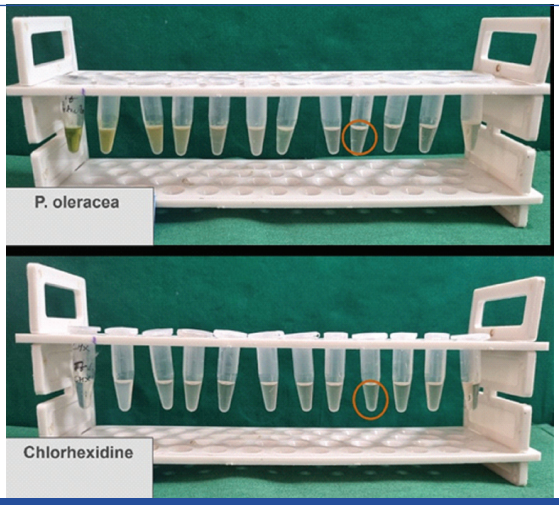
The Minimum Inhibitory Concentration (MIC) of the extract was assessed against *A. actinomycetemcomitans*, *P. gingivalis* and *T. forsythia* and compared with that of chlorhexidine gluconate.

A total of 10 dilutions of the extract were prepared using the double broth dilution method. After incubation, the MIC was determined by observing turbidity at the lowest dilution [Table/Fig-3-5]. The procedure was repeated three times for each group [Table/Fig-3].

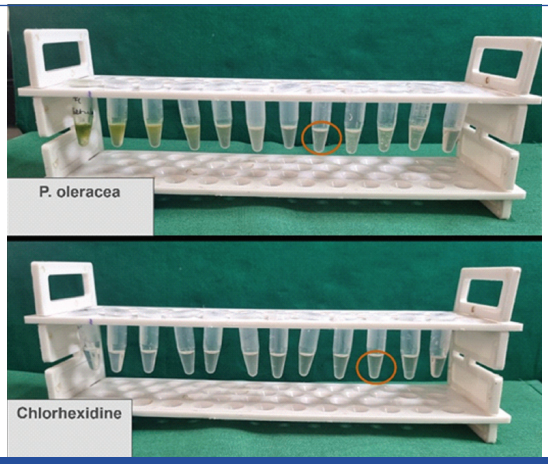
Thioglycolate broth composition used for MIC: Thioglycollate Medium with Hemin and Vit K (for 1 litre): Tryptose: 15 g; Yeast



[Table/Fig-3]: Microbiological analysis for *P. gingivalis*- MIC noted using turbidity seen at the lowest dilution indicated by red circles.



[Table/Fig-4]: Microbiological analysis for *T. forsythia*- MIC noted using turbidity seen at the lowest dilution indicated by red circles.



[Table/Fig-5]: Microbiological analysis for *A. actinomycetemcomitans*- MIC noted using turbidity seen at the lowest dilution indicated by red circles.

extract: 10 g; Sodium thioglycollate: 0.50 g; Sodium chloride: 2.5 g; L-cystine Hydrogen Chloride (HCl): 0.5 g; Sodium bicarbonate: 0.40 g; Resazurin: 0.001 g; Hemin: 0.005 g; Vitamin K: 0.0005 g.

MIC test (Anaerobes): A total of 10 dilutions of chlorhexidine and *P. oleracea* were prepared with thioglycollate broth for MIC testing. In the initial tube, 400 microlitres of the drug was added, which was considered as "neat." From the second to the 12th tube, 200 microlitres of thioglycollate broth was added. The tubes were then incubated in an anaerobic jar for 48-72 hours at 37°C and observed for turbidity at the end of the incubation period.

RESULTS

The antimicrobial activity of the ethanolic extract of *P. oleracea* was evaluated using the Minimum Inhibitory Concentration (MIC) method. Serial dilutions of the extract and standard chlorhexidine were prepared, ranging from 100 µL/mL to 0.2 µL/mL, for a total of nine dilutions.

The MIC values for *P. gingivalis*, *T. forsythia* and *A. actinomycetemcomitans* were reported at 9.3 µL/mL, 0.5 µL/mL and 4.7 µL/mL, respectively, as summarised in [Table/Fig-6]. Chlorhexidine demonstrated consistent antimicrobial resistance at 0.6 µL/mL for all three periodontal pathogens.

Periodontal pathogen	MIC in µL/mL	
	P. oleracea	Chlorhexidine
P. gingivalis	9.3	0.6
T. forsythia	0.5	0.6
A. actinomycetemcomitans	4.7	0.6

[Table/Fig-6]: MIC values of ethanolic extract of *P. oleracea* and chlorhexidine gluconate against three periodontal pathogens.

DISCUSSION

One of the main dental problems faced by children, adolescents, adults and the elderly is periodontal disease. This condition includes any inherited or acquired disorders of the tissues that invest in and support the teeth, namely the gingiva, cementum, Periodontal Ligament (PDL) and alveolar bone. It can also be defined as chronic infectious disorders caused primarily by bacteria [8]. *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis* and *Tannerella forsythia* are recognised as the main pathogens associated with periodontal disease in children [1,8].

Chlorhexidine is considered the most effective antiplaque agent and is regarded as the gold standard; therefore, it has been used as the positive control in the present study [9,10]. It has demonstrated consistent antimicrobial resistance at 0.6 µL/mL for all three periodontal pathogens (*P. gingivalis*, *T. forsythia* and *A. actinomycetemcomitans*). Chlorhexidine decreases pellicle formation, alters bacterial adhesion to the tooth surface and modifies the bacterial cell wall, ultimately leading to cell death [10].

However, chlorhexidine also presents some disadvantages, such as staining of teeth and altered taste sensation. In rare cases, serious adverse effects, including oral mucosal erosion, parotid swelling and enhanced supragingival calculus, have been reported [1,10,11].

Portulaca oleracea is highly medicinal and has been listed among the most widely used medicinal plants globally by the WHO [12]. It has been a part of folk medicine in several countries due to properties such as anti-asthmatic, antipyretic, anti-ascorbic, diuretic, antiseptic, antitussive and antispasmodic effects. It has been extensively used in Mediterranean and tropical Asian countries, as well as in America and Australia [13-15].

P. oleracea belongs to the family *Portulacaceae* and is commonly known as purslane. It grows as a wild weed and is locally referred to as pigweed, kaalashakam, nadika and red root. This persistent plant is universally distributed, thriving in both tropical and temperate zones. It is a cosmopolitan species and the family *Portulacaceae* consists of 21 genera and 580 species [15].

In traditional Chinese medicine, *P. oleracea* extract has been used empirically for the treatment of infectious diseases, particularly intestinal infections, including dysentery, cholera and acute infectious gastroenteritis [15].

Different extracts of the plant have demonstrated bronchodilatory, neuropharmacological, wound healing, analgesic, hypolipidemic, anti-inflammatory, anti-aging, antibacterial, hypoglycaemic, antioxidant and skeletal muscle relaxant properties [16,17]. However, the ethanolic extract has shown the highest antibacterial activity; hence, we decided to test the ethanolic extract in the present study [4].

The phytochemical screening of the crude extract of *Portulaca oleracea* L. indicated that the ethanol extracts contained alkaloids, glycosides, proteins, saponins, flavonoids, tannins, gum and mucilage, steroids, phenols, oils and fats. The isolated bioactive compound apigenin also exhibits potential antibacterial activity, suggesting its potential use in the development of antibacterial drugs for the treatment of diseases associated with these pathogenic bacteria [18].

Mousavi SM et al., in their study on hydroalcoholic extract, found that this extract possessed antimicrobial properties against nine bacterial strains (both gram-positive and gram-negative) that were resistant to standard antibiotics. These organisms included *Enterococcus faecalis* and the hydroalcoholic extract made from the leaves and seeds of *P. oleracea* demonstrated antimicrobial efficacy against *E. faecalis* [19].

A study conducted by Khursheed A and Jain V in the Kashmir Valley of India showed that the ethanolic extract of *P. oleracea* exhibited the highest antifungal activity against *Fusarium oxysporum* and *Aspergillus flavus*, as well as antimicrobial activity against both gram-negative and gram-positive strains [17].

Studies have reported that the flavonoid apigenin, isolated from the ethanol extract of the above-ground parts of Portulaca oleracea, exhibited antibacterial activity against five pathogenic bacterial strains (*Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Proteus mirabilis*, *Klebsiella pneumoniae* and *Enterobacter aerogenes*) in in-vitro experiments [17,18].

However, the ethanolic extract of the aerial parts of *P. oleracea* has not been tested against potential periodontal pathogens. To the best of the present knowledge, based on current literature, this is the first report providing evidence that the ethanolic extract of the aerial parts of *P. oleracea* has antibacterial activity against potential periodontal microorganisms, with MIC values for *P. gingivalis*, *T. forsythia* and *A. actinomycetemcomitans* recorded at 9.3, 0.5 and 4.7 µL/mL, respectively.

P. gingivalis exhibited more resistance to the *P. oleracea* extract, which can be attributed to the potent virulence factors it possesses, such as lipopolysaccharides, gingipains and pili [20].

T. forsythia, on the other hand, demonstrated better antimicrobial properties against *P. oleracea* compared to the standard chlorhexidine, potentially because it is known to express these properties when the organism comes into direct contact with partner community bacteria [21].

Limitation(s)

Further work and in-vivo studies are required to evaluate the chemical compounds, their mechanisms and their effects on patients. This is a limitation of the present study, as it has been conducted in-vitro.

CONCLUSION(S)

The antimicrobial activity of the ethanolic extract of *P. oleracea* was evaluated using the Minimum Inhibitory Concentration (MIC) method. The extract demonstrated antimicrobial effects against *P. gingivalis*, *T. forsythia* and *A. actinomycetemcomitans*. Notably, the ethanolic extract of *P. oleracea* exhibited the highest antimicrobial effect against *T. forsythia* when compared to the standard chlorhexidine.

Therefore, it can be effectively used as an antimicrobial agent against periodontal pathogens.

REFERENCES

[1] Gunjal S, Ankola AV, Bhat K. In-vitro antibacterial activity of ethanolic extract of *Morus alba* leaf against periodontal pathogens. Indian J Dent Res. 2015;26:533-36.
[2] Zhang XJ, Ji YB, Qu ZY, Xia JC, Wang L. Experimental studies on antibiotic functions of *Portulaca oleracea* L. in-vitro. Chinese Journal of Microbiology. 2002;14(6):277-80.
[3] Chen B, Zhou H, Zhao W, Zhou W, Yuan Q, Yang G. Effects of aqueous extract of *Portulaca oleracea* L. on oxidative stress and liver, spleen leptin, PARα and FAS mRNA expression in high-fat diet induced mice. Molecular Biology Reports. 2012;39(8):7981-88.
[4] Rashed AN, Afifi FU, Disi AM. Simple evaluation of the wound healing activity of a crude extract of *Portulaca oleracea* L. (growing in Jordan) in Mus musculus JVI-1. Journal of Ethnopharmacology. 2003;88(2-3):131-36.
[5] Xu X, Yu L, Chen G. Determination of flavonoids in Portulaca oleracea L. by capillary electrophoresis with electro-chemical detection. Journal of Pharmaceutical and Biomedical Analysis. 2006;41(2):493-99.
[6] Srivastava R, Srivastava V, Singh A. Multipurpose benefits of an underexplored species Purslane (Portulaca oleracea L.): A critical review. Environmental Management. 2021;01-12.
[7] Page RC, Offenbacher S, Schroeder HE, Seymour GJ, Kornman KS. Advances in the pathogenesis of periodontitis: Summary of developments, clinical implications and future directions. Periodontol 2000. 1997;14:216-48.
[8] Ghutaimel H, Riba H, Kahtani S, Al-Duhaimi S. Common periodontal diseases of children and adolescents. International Journal of Dentistry. 2014;2014:850674.
[9] Armitage GC. Periodontal diagnoses and classification of periodontal diseases. Periodontol 2000. 2004;34:09-21.
[10] Chauhan AS, Bains VK, Gupta V, Singh GP, Patil SS. Comparative analysis of hyaluronan gel and xanthan-based chlorhexidine gel, as adjunct to scaling and root planing with scaling and root planing alone in the treatment of chronic periodontitis: A preliminary study. Contemp Clin Dent. 2013;4:54-61.
[11] Gopalasatheeskumar K. Significant role of soxhlet extraction process in phytochemical research. Mintage Journal of Pharmaceutical and Medical Sciences. 2018:43-47.
[12] Sedaghati B; Haddad R; & Bandehpour M. Efficient plant regeneration and Agrobacterium-mediated transformation via somatic embryogenesis in purslane (Portulaca oleracea L.): An important medicinal plant. Plant Cell, Tissue and Organ Culture (PCTOC). 2019;136(2):231-45.
[13] Catap ES, Kho MJL, Jimenez MRR. In vivo nonspecific immunomodulatory and antispasmodic effects of common purslane (Portulaca oleracea Linn.) leaf extracts in ICR mice. Journal of Ethnopharmacology. 2018;215:191-98.
[14] Alu'datt MH, Rababah T, Alhamad MN, Al-Tawaha A, Al-Tawaha AR, Gammoh S, et al. Herbal yield, nutritive composition, phenolic contents and antioxidant activity of purslane (Portulaca oleracea L.) grown in different soilless media in a closed system. Industrial Crops and Products. 2019;141:111746.
[15] Zhou YX, Xin HL, Rahman K, Wang SJ, Peng C, Zhang H. *Portulaca oleracea* L. A review of phytochemistry and pharmacological effects. Biomed Res Int. 2015;2015:925631. Available from: http://dx.doi.org/10.1155/2015/925631.
[16] Kaveh M, Eidi A, Nemati A, Boskabady MH. Modulation of lung inflammation and immune markers in asthmatic rats treated by *Portulaca oleracea*. Avicenna Journal of Phytomedicine. 2017;7(5):409-16.
[17] Khursheed A, Jain V. Phytochemical screening, antioxidant, and antimicrobial activity of different *Portulaca oleracea* L. extracts growing in Kashmir Valley. J Biochem Technol. 2021;12(3):01-08.
[18] Londonkar R, Nayaka H. Phytochemical and antimicrobial activities of *Portulaca Oleracea* L. Journal of Pharmacy Research. 2011;4(10):3553-55.
[19] Mousavi SM, Bagheri G, Saeidi S. Antibacterial activities of the hydroalcoholic extract of *Portulaca oleracea* leaves and seeds in Sistan Region, Southeastern Iran. Int J Infect. 2015;2(2):e23214.
[20] Jia L, Han N, Du J, Guo L, Luo Z, Liu Y. Pathogenesis of important virulence factors of *Porphyromonas gingivalis* via toll-like receptors. Front Cell Infect Microbiol. 2019;9:262. Doi: 10.3389/fcimb.2019.00262.
[21] Sharma A. Virulence mechanisms of *Tannerella forsythia*. Periodontol 2000. 2010;54(1):106-16.

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