ORIGINAL ARTICLE OPEN ACCESS

The Effect of Herbal Formulation of Centratherum Anthelmintic Seeds Extract on Salivary Microbial Count and Oral Health in Type-2 Diabetic and Non-Diabetic Adults

Hina Akram Mudassir[1], Arisha Sohail[2]* Sehar Afshan Naz[3], Maryam Shafique[3], Abdur Rahman[4], Qandeel Laraib[5], Musab Kamran[1]

[1]Biochemistry Department, Federal Urdu University of Arts, Science and Technology, [2]Biochemistry Department, Dow University of Health Sciences, [3]Microbiology Department, Federal Urdu University of Arts, Science and Technology, [4]Surani Dental Clinic, [5]Biosciences Department, Muhammad Ali Jinnah University, Karachi

ABSTRACT

Background: Diabetes can cause oral health issues like periodontal disease, dental caries, and delayed wound healing, requiring effective diabetes management and good oral hygiene. This study evaluated the efficacy of Centratherum anthelminticum (eCset) seed extract in herbal formulation against oral issues in adults with and without diabetes.

Methods: This randomized control trial study was conducted at Surani Dental Clinic, Karachi, for 4 weeks in January 2023 and included 18-60 years of type-2 diabetics on standard anti-diabetic therapy for at least 6 months, moderate to severe oral ailments, at least 24 teeth, and no recent antibiotic therapy. The antibacterial and antifungal activity of eCset seed extract was tested and compared with reference drugs Chloramphenicol and Nystatin by using the diffusion zone methods and eCset herbal formulation was prepared. The weight, height, and fasting blood sugar (FBS) were measured, oral health at baseline and after intervention was assessed, and saliva samples were collected before and after using eCset mouthwash for four weeks and cultured. SPSS v.21 was used with $p<0.05$ considered significant.

Results: The growth inhibition zones(mm) for Staphylococcus aureus were (Chloramphenicol:25 vs eCset:22), Micrococcus luteus (55 vs 22), Vibrio cholera (40 vs 29), Salmonella typhi (23 vs 16) and Aspergillus niger (eCset:18 vs Nystatin:22mm). The eCset herbal mouthwash improved plaque in healthy and diabetic participants and decreased the salivary CFU values ($p<0.01$) in both groups after using eCset mouthwash for four weeks.

Conclusion: The in-vitro antibacterial and antifungal activity of eCset seed extract supports its herbal mouthwash formulation used to maintain and improve oral hygiene.

Keywords: Anti-Microbial Agents, Diabetes Mellitus, Mouthwashes, Oral Health.

Corresponding Author:
Dr. Arisha Sohail
Biochemistry Department,
Dow Medical College,
Dow University of Health Sciences,Karachi, Pakistan.
Email: arisha.sohail@duhs.edu.pk
ORCID ID: https://orcid.org/0000-0001-5986-1786

Doi: 10.36283/PJMD13-3/012

How to cite: Mudassir H A,Sohail A,Naz S A,Shafique M,Rahman A,Laraib Q,Kamran M The Effect of Herbal Formulation of Centratherum anthelminticum Seeds Extract on Salivary Microbial Count and Oral Health in Type-2 Diabetic and Non-Diabetic Adults. Pak J Med Dent. 2024;13(3): 81-88. Doi: 10.36283/PJMD13-3/012

Received: Thu, Feb 15, 2024 **Accepted:** Thu, May 30, 2024 **Published:** Wed, 24 July 2024

INTRODUCTION

The estimated prevalence of diabetes among adults has increased more than threefold since 2000, growing from approximately 151 million individuals to 537.5 million individuals to the present [1]. In Pakistan, 27.4 million people have diabetes, according to the National Diabetes Survey[2]. Proper blood glucose regulation is essential, as uncontrolled hyperglycemia can harm organs such as the oral cavity. Oral manifestations of diabetes include periodontal disease, dental caries, oral infections, salivary and taste dysfunction, delayed wound healing, tongue abnormalities, halitosis, and lichen planus[3]. Poor blood sugar control and oral health are closely linked, emphasizing the importance of promoting oral and periodontal health as crucial components of diabetes management. Potential mechanisms associated with oral complications in diabetes may involve impaired neutrophil function, heightened collagenase activity, decreased collagen synthesis, microangiopathy, and neuropathy[4]. In the United States, diabetes impacts approximately 10% of adults, while periodontal disease affects around 40% of adults, and these conditions are interconnected[5]. Those with diabetes have an increased risk of developing periodontal disease, which in turn can influence glycemic control and exacerbate diabetes-related complications[5]. Longitudinal studies indicate that diabetes frequently precedes periodontitis, suggesting that diabetes may play a role in the development of periodontitis. Additionally, diabetes is associated with a heightened risk of oral fungal infections. Individuals with diabetes often experience decreased salivary flow, which can be attributed to diabetes medications and neuropathy affecting the salivary glands, potentially leading to an increased incidence of dental caries. Moreover, diabetic neuropathy can cause burning mouth syndrome and taste impairment[6]. The oral microbiome, including Staphylococcus, Streptococcus, Bacillus species, and E. Coli, among others, significantly impacts oral diseases such as dental caries and periodontal diseases and should be closely monitored. Leptotrichia, Staphylococcus, Catonella, and Bulleidia genera were reported markedly abundant in patients with diabetes exhibiting extremely elevated glucose levels, indicating that an imbalance in the oral microbiota might be characteristic of hyperglycemia and could potentially contribute to its progression. Nonetheless, the specific oral microbial markers involved in the advancement of hyperglycemia remain unidentified[7].

Bacteria residing in biofilms typically display modified phenotypes, including enhanced resistance to antimicrobial agents. The stable structure of biofilms and the proximity of bacterial cells within them facilitate the horizontal transfer of resistance genes, thereby potentially elevating antibiotic resistance rates[8]. The ineffectiveness of antimicrobial agents may be attributed to two main factors: the biofilm scaffold not only facilitates an environment conducive to gene mutation but also acts as a physical barrier against these agents. This dual role appears to be a significant factor in the development and progression of microbial diseases[9]. Due to the increasing resistance of biofilm bacteria to antimicrobials, there is a need for new antimicrobial agents.

Therefore, herbal agents have been increasingly incorporated into oral care products in addition to traditional treatment methods in recent years. These substances possess notable medical and physicochemical properties. Currently, many manufacturers include herbal ingredients in their products to enhance their therapeutic benefits. Centratherum anthelminticum is a herb that has been used in traditional medicine to treat various health issues, including diabetes, skin conditions, gastrointestinal diseases, and oral health issues. Antimicrobial and antifungal activity of the ethanolic extract of Centratherum anthelminticum has also been reported with promising results in treating oral pathogens. The phytochemical studies reveal that Centratherum anthelminticum contains terpenes, carbohydrates, flavonoids, steroids, fatty acids, and sesquiterpene lactones[12].

However, a limited number of compounds have undergone pharmacological investigations and data linking the pharmacological properties of this plant to its traditional uses and safety profile is inadequate. This study aimed to explore the potential of Centratherum anthelminticum seed extract in herbal formulation against oral issues in adults with and without diabetes.

METHODS

The study was a randomized control trial conducted at Surani Dental Clinic, Karachi for 4 weeks in January 2023. The study adhered to the declaration of Helsinki and was approved by the Institutional Review Board

FUASST (Ref No.: 2030). Participants at Surani Dental Clinic, Karachi, aged 18 to 60, diagnosed with type-2 diabetes on standard anti-diabetic therapy for at least 6 months, and experiencing moderate to severe oral ailments were included after providing verbal and written consent. Criteria included having at least 24 teeth, six in each quadrant, and no recent (previous 4 weeks) antibiotic therapy. Exclusions comprised individuals with physical or mental ailments, current smokers, and those using any mouthwash. Participants used eCset mouthwash twice daily for four weeks. Oral health assessments were conducted at baseline and after the intervention by the dentist at each visit. Unstimulated saliva (2 ml) from each participant was collected in a sterile container over 15 minutes, transported to the lab within one hour, and refrigerated at 4°C. Bacterial load (Colony Forming Units) and fungal growth were assessed by plating 0.1 ml of saliva samples on nutrient agar plates[18].

The C. anthelminticum seeds were procured from the Hamdard Matab Karachi and after washing and drying were finely ground using a blender and macerated in 95% ethanol for two to three days. The mixture was twice filtered through Whatmann No. 42 (125mm) filter paper and concentrated paste extract was obtained by evaporating the solution using a Rotary Evaporator. The ethanol extract was kept at 4°C. The ethanol extract of C. anthelminticum seeds was dissolved in 100% DMSO to achieve a concentration of 40.000 ppm for the stock solution [13]. The ethanolic extract was prepared at the 25% concentration (25%= 25mg in 1 ml).

The antibacterial properties of eCset seed extract were tested by using the Disk Diffusion technique given by the Kerby-Bauer Disk Diffusion Susceptibility Test [14]. The gram-positive bacteria including Bacillus subtilis, Staphylococcus aureus, Staphylococcus epidermidis, Micrococcus luteus, Enterococcus species, and gram-negative bacteria including Pseudomonas aeruginosa, Salmonella typhi, Escherichia coli, Proteus mirabilis, Acinetobacter species, Vibrio cholera were sourced from the laboratory of applied Microbiology and clinical Mycology Federal Urdu University of Arts and Science, Karachi, Pakistan. The antibacterial activities of eCset seed extract were performed by using a nutrient agar broth medium. Eleven separate test tubes were filled with nutrient broth prepared by mixing 1.3g of nutrients with 100ml of double-distilled water and bacterial strains were then added to each test tube. After the nutrient agar medium had solidified, it was produced, placed into Petri dishes, swabbed with bacterial cultures, and left to incubate for 24 hours at 37°C[15]. Twenty µL negative control ethanol and positive control Chloramphenicol (5mg/ml) were added to the wells (diameter = 06 mm) in an agar plate and the zone of growth inhibition was recorded after incubation for 24 hours at 37˚C.

The Agar well diffusion method was employed to assess the antifungal activity of eCset seed extract the National Committee for Clinical Laboratory Standards (NCCLS). A. niger and C. albicans isolates were acquired from the Laboratory of Applied Microbiology and Clinical Mycology Federal Urdu University of Arts and Science, Karachi, Pakistan. Potato dextrose agar (PDA) was the culture medium and sodium phosphate buffer was used to bring the medium's final pH to 5.2 ± 0.2. Plates of C. albicans and A. niger were incubated for three and seven days, respectively. These culture plates were utilized to create A. niger and C. albicans inoculums. Using a sterile wire loop, two fungal colonies were transferred to 5 milliliters of 0.9% normal saline to achieve equal turbidity for 0.5 McFarland standards (10−6 cfu/mL). By using a sterile swab wet with the fungal solution, each fungal culture to be examined was spread out on agar plates. The necessary quantity of wells was then punched using a sterile pipette tip. Twenty microliters (µL) of the positive control, Nystatin (5 mg/mL), and negative control ethanol were placed in the well. After allowing the test samples to diffuse for an hour at room temperature, the plates were incubated for 24 to 48 hours at 37 °C for the growth of C. albicans, and A. niger zones of inhibition were recorded [16]. The herbal mouthwash containing eCset seeds extract was prepared following the method by Pedrazzi et al., 2015 [17]. The ingredients are detailed in Table 1, with the water and oil phases mixed uniformly to create the mouthwash. The water phase involved heating 100 ml water in a beaker, and adding specified ingredients, while the oil phase included mixing Mint oil, Menthol, and Eucalyptus oil in a separate beaker. The resultant solutions were mixed and labeled as eCset mouthwash in a glass bottle.

Table 1: Composition of herbal mouthwash formulation with *eCset* seeds extract

Ingredients	Function	Quantity (gm/L)
Water phase		
Sodium citrate	Conservative	2.00
Citric acid	Conservative	5.00
Sodium fluoride	Active	0.20
Glycerol	Cosurfactant	50.0

 DOI: https://doi.org/10.36283/PJMD13-3/012

Sorbitol	Cosurfactant	200.00
Cremophor	Conservative	40.0
Ethanol	Vehicle	216.00 (96%)
Benzoic acid	Conservative	1.00
Ethanolic extract of *C. anthelminticum* seeds	Main active component	1.00
Oil phase		
Mint oil	Corrective	20.00
Menthol	Freshness	0.40
Eucalyptus oil	Surfactant	0.90

Statistical analysis was performed using SPSS version 24.0 and the mean (SD) values of continuous variables were calculated and the unpaired T-test was used to compare the two groups with the significant value set at $p < 0.05$.

The eCset seeds extract demonstrated significant antibacterial activity against S.aureus, M. luteus, Vibrio cholera, and S. typhi while Bacillus subtilis, enterococcus spp., S. epidermidis, P. aeruginosa, E. Coli, Proteus mirabilis, and Acinetobacter spp showed resistance. The extract of eCset demonstrated significant antifungal activity against A. niger while showing resistance against other fungal strains (Table 2).

Table 2: In vitro analysis of the antimicrobial and antifungal activity of *eCset* seed extract using agar well diffusion method

Bacterial Strains	Diameter of zone of growth inhibition(mm)		
	***eCset* seed extract**	**Ethanol (Control)**	**Chloramphenicol (Positive Control)**
Gram-positive			
Bacillus subtilis	_	_	25
Staphylococcus aureus	22	_	25
Staphylococcus epidermidis	_	_	22
Micrococcus luteus	22	_	55
Enterococcus spp	_	_	18
Gram-negative			
Salmonella typhi	16	15	23
Pseudomonas aeruginosa	_	_	25
Escherichia coli	_	_	22
Proteus mirabilis	_	_	21
Acinetobacter spp	_	_	8
Vibrio cholerae	29	12	40
Fungal strains			**Nystatin (positive control)**
Aspergillus niger	22	_	18
Candida albicans	_	_	18

(-) = no zone of growth inhibition

The zone of growth inhibition of selected bacterial and fungal strains is shown in (Figure 1).

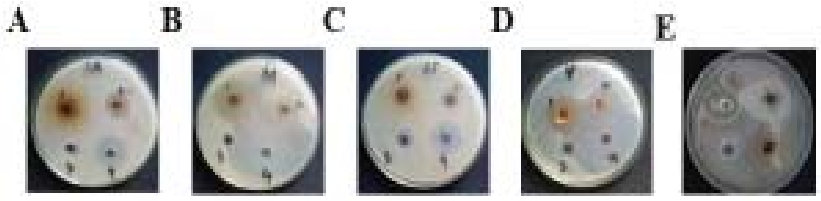

Figure 1: The zones of growth inhibition compared with negative control (ethanol) and positive control (chloramphenicol for bacterial strains and Nystatin for fungal strain) A) *Staphylococcus aureus* B) *Micrococcus luteus* C) *Salmonella typhi*, D) Vibrio *cholera* E) *Aspergillus niger* after administration of *eCset* seed extract.

The initial oral health status of both non-diabetic and diabetic participants was evaluated before the administration of eCset seeds extracts herbal mouth-wash. Diabetic participants exhibited a higher frequency and severity of plaque, gingivitis, and periodontitis compared to non-diabetic participants (Table 3). All diabetic participants displayed varying degrees of gingivitis and periodontitis. Specifically, severe gingivitis was observed in 7 out of 10 diabetic participants, whereas moderate gingivitis was found in 5 out of 10 non-diabetic participants. Moderate plaque was identified in six diabetic participants, with one participant showing severe plaque. Additionally, severe periodontitis was present in 5 diabetic partici-pants, while non-diabetic participants exhibited only moderate periodontitis.

Table 3: Baseline oral health assessment in study participants before administration of *eCest* seeds extract herbal mouthwash.

Parameters	Non-diabetic participants (n=10)										Type 2 diabetic participants (n=10)									
Oral ailments	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*
Plaque	+	-	+	+	++	-	++	+	-	++	-	++	+	++	++	++	+	++	+++	++
Gingivitis	++	+	++	++	+++	-	++	++	+	+	++	+++	+++	+++	+++	+++	++	++	+++	+++
Periodontitis	++	-	++	++	+++	-	++	+	+	++	++	+++	+++	++	++	+++	+	+++	++	+++

Following a 4-week regimen of eCset herbal mouth-wash, an oral health assessment was conducted. There was a notable reduction in the frequency and severity of plaque, gingivitis, and periodontitis in both diabetic and non-diabetic patients (Table 4). Among these, the most significant reduction was observed in the frequency and severity of plaque. In the diabetic group, 5 patients exhibited no plaque, while 4 had only a mild form. Similarly, in the non-diabetic group, 7 patients showed no plaque, with the remainder presenting only mild plaque.

Table 4: Oral health assessment in study participants after administration of *eCset* seeds extract herbal mouthwash for four weeks.

Parameters	Non-diabetic participants (n=10)										Type 2 diabetic participants (n=10)									
Oral ailments	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*
Plaque	-	-	-	-	+	-	+	-	-	+	-	+	-	+	-	-	-	+	++	+
Gingivitis	++	-	++	++	++	-	++	++	+	+	++	++	+++	+++	++	+++	++	++	+++	+++
Periodontitis	++	-	++	++	++	-	+	+	+	++	++	+++	+++	++	++	++	+	+++	++	+++

 DOI: https://doi.org/10.36283/PJMD13-3/012

When comparing the physical characteristics of healthy participants with diabetes at baseline, like age, weight, and fasting blood sugar (FBS), the age and FBS were significantly high ($p<0.01$) in the diabetic group. The salivary colony-forming units of S. aureus were significantly reduced in both the control and diabetic groups after rinsing with eCset mouthwash twice over four weeks (Figure 2).

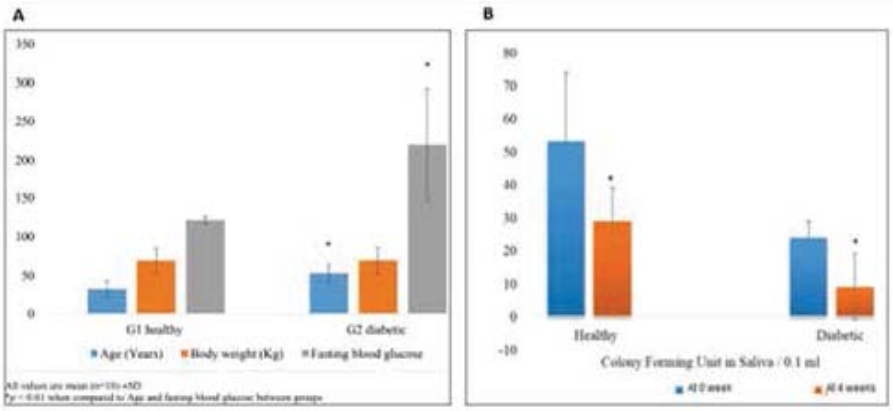

Figure 2: : Comparison between healthy and Gdiabetic groups (**A**) physical parameters (Age, Body weight and Fasting blood glucose) (**B**) Colony Forming Units in 0.1 ml saliva before and after using eCset Mouth wash

DISCUSSION

The present study aimed to assess the effectiveness of an herbal mouthwash formulation containing eCset seed extract on salivary microbial species. The results demonstrated that the mouthwash was effective in improving oral health for both diabetic and non-diabetic individuals. After four weeks of using the mouthwash, both groups particularly demonstrated better plaque control. These outcomes were supported by a significant decrease in the colony-forming units of S. aureus, in the saliva of diabetic patients compared to healthy adults. C. anthelminticum may have an impact on oral health due to its hypoglycemic properties, as diabetes is a risk factor for periodontal disease and other oral health issues. The antidiabetic effects of C. anthelminticum may arise from two potential mechanisms: reducing glucose absorption in the gastrointestinal tract by inhibiting carbohydrate hydrolyzing enzymes, such as α-amylase and α-glucosidase. This inhibition is a key therapeutic approach for managing type 2 diabetes. Additionally, C. anthelminticum may enhance insulin secretion from functional pancreatic beta cells by preventing the breakdown of triglycerides, redistributing fatty acids within adipose tissues, and removing them from circulation, insulin resistance is reduced, enhancing the availability of receptors for insulin binding or action. This impact can likely be due to the potent antioxidant properties of the phenolic compounds in eCset seed extracts, as demonstrated by a study that showed that C. anthelminticum can alleviate inflammation and oxidative stress by decreasing lipid peroxidation and enhancing the expression of antioxidant markers [19]. Given that periodontitis and gingivitis are linked to oral inflammation caused by bacteria, such as S. aureus, the anti-inflammatory activity of the eCset herbal mouthwash can be attributed to its anti-inflammatory activity. Studies have indicated that C. anthelminticum has anti-inflammatory activity through prostaglandin inhibition, reduced myeloperoxidase, and anti-transudation. The present study demonstrated that eCset seed extract exerted the highest zone of growth inhibition against S. aureus, Micrococcus luteus, Vibrio cholerae, Salmonella typhi, and Aspergillus niger. A study reported similar results in which the ethanolic extract of C. anthelminticum demonstrated the highest efficacy against all tested bacterial strains. likely attributable to the antimicrobial properties inherent in tannins and terpenoids [21]. Previous studies have shown that various natural compounds in C. anthelminticum seeds possess antimicrobial properties. Similar effects have been demonstrated in other species through the use of different solvent extracts, such as ethanol.

Phytochemical analysis of C. anthelminticum seeds revealed 193 chemical components, including tanins, alkaloids, phenolic acids, chalcones, flavonoids, terpenes, fatty acids, steroids, and other compounds. The majority of these components have been isolated from seeds, with steroids being the most prevalent class, followed by terpenes. Tannins exhibit toxicity towards bacteria, yeast, and filamentous fungi. The identified chemicals are known for various pharmacological activities. For instance, alkaloids are commonly employed for their antibacterial, anti-malarial, cytotoxic, and anti-cancer properties [27]. Saponins play a crucial role due to their fungicidal, antibiotic, and

insecticidal effects 28 while flavonoids possess significant anti-inflammatory, antibacterial, antiviral, and antioxidant properties. Therefore, the potential of C. anthelminticum as a source of new, non-resistant antimicrobial agents is significant in light of growing antibiotic resistance. However, further research is needed to understand the appropriate human dosage and in vivo responses against different microbial strains. The results of this study showed that the use of C. anthelminticum seed extract in a herbal formulation as a mouthwash for oral health issues in both diabetic and healthy individuals has promising outcomes. The extract was effective in improving oral hygiene in both groups and significantly reduced plaque formation in patients with diabetes. The eCset herbal mouthwash contained sodium fluoride, which has bacteriostatic properties, eucalyptus oil and menthol with synergistic antimicrobial effects, and sorbitol, which improves anti-caries effects. The study participants reported no adverse effects of the extract, indicating its safety and tolerability. Commercial mouthwashes can have undesirable effects, such as tooth discoloration and loss of taste, but essential oils have low mammalian toxicity and do not cause these effects. The salivary analysis of diabetic and healthy controls after using the eCset mouthwash considered the effects of salivary dilution, intraoral pH, and dietary habits. Therefore, the current study suggests that the C. anthelminticum extract could be a safe and effective herbal remedy for oral health issues in both diabetic and healthy individuals. However, further research is needed to investigate the absorption and bioavailability of the extract, as well as its potential side effects or interactions with other medicinal plants, and toxicity studies on C. anthelminticum seed extract.

CONCLUSION

In this study, the herbal mouthwash eCset demonstrated preventative benefits in diabetic and healthy participants, as the formulation caused no irritation or staining of teeth and oral tissues. Nonetheless, further research is necessary to evaluate its effectiveness against dental caries, caused by S. mutans and other pathogens. The formulation and use of herbal mouthwashes for treating dental issues highlight the significance of medicinal plants in maintaining oral hygiene, as previously described.

ACKNOWLEDGMENTS

We are thankful to the dental staff of Surani Dental Clinic for technical support and the Microbiology department of FUAAST University for providing bacterial and fungal strains.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ETHICAL APPROVAL

The study was approved by the Ethics Committee of FUAAST University Letter no. (Ref No.: 2030).

AUTHORS CONTRIBUTION

HAM: Conceptualization & study design, AS: Manuscript writing & formal analysis, SAN: Experiment design and support, MS: data collection and curation, AR: Conceptualization & study design, QL: data collection and curation, MK: experiment conduction and data collection.

REFERENCES

1. Kumar A, Gangwar R, Ahmad Zargar A, Kumar R, Sharma A. Prevalence of Diabetes in India: A Review of IDF Diabetes Atlas 10th Edition. CDR. 2024;20(1):e130423215752. https://doi.org/10.2174/1573399819666230413094200
2. Basit A, Fawwad A, Baqa K. Diabetes Registry of Pakistan: Diabetes Registry of Pakistan. Pak J Med Sci. 2020;36(3). https://doi.org/10.12669/pjms.36.3.1877
3. Mohan D, Bhuvaneshwar Y, Jeyaram RM, Saravanan S, Amutha A, Research Team. Dental caries and their relation to hba1c in adults with type 2 diabetes mellitus. Indian J Public Health. 2022;66(2):206–29. https://doi.org/10.4103/ijph.ijph_1935_21
4. Rohani B. Oral manifestations in patients with diabetes mellitus. WJD. 2019;10(9):485–489. https://doi.org/10.4239/wjd.v10.i9.485
5. Genco RJ, Borgnakke WS. Diabetes as a potential risk for periodontitis: association studies. Periodontology 2000. 2020;83(1):40–45. https://doi.org/10.1111/prd.12270
6. Ali T, Rumnaz A, Urmi UL, Nahar S, Rana M, Sultana F, et al. Type-2 Diabetes Mellitus Individuals Carry Different Periodontal Bacteria. Pesqui Bras Odontopediatria Clín Integr. 2021;21:e0107. https://doi.org/10.1590/pboci.2021.049
7. Wang RR, Xu YS, Ji MM, Zhang L, Li D, Lang Q, et al. Association of the oral microbiome with the progression of impaired fasting glucose in a Chinese elderly population. Journal of Oral Microbiology. 2019;11(1):1605789. https://doi.org/10.1080/20002297.2019.1605789
8. Roberts AP, Mullany P. Oral biofilms: a reservoir of transferable, bacterial, antimicrobial resistance. Expert Review of Anti-infective Therapy. 2010;8(12):1441–1450. https://doi.org/10.1586/eri.10.106
9. Vieira Colombo AP, Magalhães CB, Hartenbach FARR, Martins Do Souto R, Maciel Da Silva-Boghossian C. Periodontal-disease-associated biofilm: A reservoir for pathogens of medical importance. Microbial Pathogenesis. 2016; 94:27–34. https://doi.org/10.1016/j.micpath.2015.09.009
10. Moghadam ET, Yazdanian M, Tahmasebi E, Tebyanian H, Ranjbar R, Yazdanian A, et al. Current herbal medicine as an alternative treatment in dentistry: In vitro, in vivo and clinical studies. European Journal of Pharmacology. 2020; 889:173665. https://doi.org/10.1016/j.ejphar.2020.173665
11. Mudassir HA, Qureshi SA, Azmi MB, Ahsan M,

Kamran M, Jafar S. Hypoglycemic and hypolipidemic activities of crude seeds of Centratherum anthelminticum in healthy volunteers and type 2 diabetic patients. Pak J Pharm Sci. 2018;31(3(Supplementary)):1061–1065.
12. Khan J, Saleem A, Akram M, Afzal M, Jabeen F. Pharmacological evaluation & phytochemical screening of seed extracts of kaliziri (Centratherum anthelminticum). World Journal of Biology and Biotechnology.2024 9(2):53–58. https://-doi.org/10.33865/wjb.009.02.1286
13. Bashari MH, Arsydinilhuda FZ, Ilhamsyah RS, Nugrahani AD, Nurdin RA, Kartika A, et al. The Ethanol Extract of Marine Sponge Aaptos suberitoides Suppress Cell Viability, Cell Proliferation and Cell Migration in HER2-Positive Breast Cancer Cell Line. Asian Pac J Cancer Prev. 2021;22(S1):25–32. https://-doi.org/10.31557/apjcp.2021.22.s1.25
14. Hossain TJ. Methods for screening and evaluation of antimicrobial activity: A review of protocols, advantages, and limitations. Eur J Microbiol Immunol (Bp). 2024;14(2):97–115. https://-doi.org/10.1556/1886.2024.00035
15. Assiry AA, Ahmed N, Almuaddi A, Saif A, Alshahrani MA, Mohamed RN, et al. The antioxidant activity, preliminary phytochemical screening of Zingiber zerumbet, and antimicrobial efficacy against selective endodontic bacteria. Food Sci Nutr. 2023;11(8):4853–4860. https://-doi.org/10.1002/fsn3.3462
16. Reka L, Godage CM, Wijayabandara J, Siriwardhene A. Evaluation of Antifungal Activity of Languas galangal Rhizome and Development of a Topical Antifungal Cream. Medicines. 2023;10(6):34. https://-doi.org/10.3390/medicines10060034
17. Pedrazzi V, Leite MF, Tavares RC, Sato S, Do Nascimento GC, Issa JPM. Herbal Mouthwash Containing Extracts of Baccharis dracunculifolia as Agent for the Control of Biofilm: Clinical Evaluation in Humans. The Scientific World Journal. 2015;2015:1–6. https://-doi.org/10.1155/2015/712683
18. Mishra N, Trivedi A, Gajdhar SK, Bhagwat H, Khutwad GK, Mall PE, et al. Correlation of Blood Glucose Levels, Salivary Glucose Levels and Oral Colony Forming Units of Candida albicans in Type 2 Diabetes Mellitus Patients. J Contemp Dent Pract. 2019;20(4):494–498.
19. Baig N, Sultan R, Qureshi SA. Antioxidant and anti-inflammatory activities of Centratherum anthelminticum (L.) Kuntze seed oil in diabetic nephropathy via modulation of Nrf-2/HO-1 and NF-κB pathway. BMC Complement Med Ther. 2022;22(1):301. https://-doi.org/10.1186/s12906-022-03776-x
20. Patel V P, Hirpara M, Suthar M P. In vitro screening for antibacterial activity of various extract of Centratherum anthelminticum seeds. 2012;2(1):1–4.
21. Mahizan NA, Yang SK, Moo CL, Song AAL, Chong CM, Chong CW, et al. Terpene Derivatives as a Potential Agent against Antimicrobial Resistance (AMR) Pathogens. Molecules. 2019;24(14):2631. https://-doi.org/10.3390/molecules24142631
22. Farha AK, Yang QQ, Kim G, Li HB, Zhu F, Liu HY, et al. Tannins as an alternative to antibiotics. Food Bioscience. 2020;38:100751. https://doi.org/10.1016/j.f-bio.2020.100751
23. Ethiraj S, Balasundaram J. Phytochemical and Biological Activity of Cucurbita Seed Extract. JBT. 2016;6(1):813–821. https://doi.org/10.24297/-jbt.v6i1.4821
24. Thouri A, Chahdoura H, El Arem A, Omri Hichri A, Ben Hassin R, Achour L. Effect of solvents extraction on phytochemical components and biological activities of Tunisian date seeds (var. Korkobbi and Arechti). BMC Complement Altern Med. 2017;17(1):248. https://doi.org/10.1186/s12906-017-1751-y
25. Kavital A, Hiremath MB. Phytochemical screening and biological activities of Syzygium cumini seed extracts. Plant Biosystems - An International Journal Dealing with all Aspects of Plant Biology. 2023;157(6):1203–1210. https://-doi.org/10.1080/11263504.2023.2259375
26. Chung KT, Wong TY, Wei CI, Huang YW, Lin Y. Tannins and human health: a review. Crit Rev Food Sci Nutr. 1998;38(6):421–464. https://-doi.org/10.1080/10408699891274273
27. Wirasathien L, Boonarkart C, Pengsuparp T, Suttisri R. Biological Activities of Alkaloids from Pseuduvaria setosa . Pharmaceutical Biology. 2006;44(4):274–278. https://doi.org/10.1080/13880200600714111
28. Sparg SG, Light ME, van Staden J. Biological activities and distribution of plant saponins. J Ethnopharmacol. 2004;94(2–3):219–243. https://doi.org/10.1016/j.-jep.2004.05.016
29. Wen K, Fang X, Yang J, Yao Y, Nandakumar KS, Salem ML, et al. Recent Research on Flavonoids and their Biomedical Applications. CMC. 2021;28(5):1042–1066. https://-doi.org/10.2174/0929867327666200713184138