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Original Article

Microbiology Section

Evaluation of Antimicrobial Potency of *Tinospora cordifolia* on Subgingival Microbiota: An In-vitro Study

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ABSTRACT

Introduction: *Tinospora cordifolia* (Tc), a well-known ayurvedic herb commonly known as giloy, has demonstrated multifaceted benefits, such as anti-inflammatory, analgesic, antibacterial and antioxidant properties, in animal, as well as, in-vitro studies. All these are properties which can be collectively applied in the management of periodontal conditions which demonstrate infective as well as inflammatory facets. But prior to its applications as such, it is necessary to definitively determine the optimal concentration and mode of application of the medication.

Aim: To evaluate the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of Tc on general subgingival microbiota.

Materials and Methods: The in-vitro, analytical study was conducted in Krishnadevaraya Dental College and Hospital, Bengaluru, Karnataka, India for duration of two and half months starting from November 2021 to January 2022. Ethanolic extracts of Tc were prepared in serial dilutions. Subgingival plaque samples of 12 chronic periodontitis patients were collected, cultured on Brain Heart Infusion (BHI) agar and subject to serial dilutions of Tc. In-vitro antibacterial activity of the ethanolic extracts was assessed using agar well diffusion method with Chlorhexidine (CHX) as positive control and Dimethyl sulfoxide (DMSO) as negative control, MIC and MBC were determined based on the zones of inhibition after 48 hours of anaerobic incubation. The Statistical Package for Social Sciences (SPSS) for windows version 22.0 was used to perform statistical analyses.

Results: The MIC of Tc was noted to be 100 mg/mL where the maximum zone of inhibition was 11.42 mm. MBC was noted at a concentration of 25 mg/mL. The zones of inhibition at these concentrations was comparable to the CHX. The difference in the diameter of the zones produced by Tc, DMSO/Saline and CHX were statistically significant.

Conclusion: Although CHX remains the gold standard, Tc extract has significant antimicrobial activities and can be considered for further clinical trials.

Keywords: Amritha, Antimicrobial efficacy, Chronic periodontitis, Chlorhexidine

INTRODUCTION

Chronic Periodontitis (CP), an inflammatory disease of the supporting tissues of the teeth, results in attachment loss and alveolar bone resorption through host inflammatory response. Although multifactorial, it is primarily driven by the anaerobic bacterial species of the subgingival biofilm, consisting mainly of anaerobic bacterial species [1].

Scientific literature has indicated that certain periodontopathogens such as *Aggregatibacter actinomycetemcomitans, Tannerella forsythia* and *Porphyromonas gingivalis*, when present even in very small proportions can affect the integrity of the periodontium [2,3]. Apart from the bacteria themselves, the collective noxious products of plaque also reach the subgingival tissues resulting in inflammatory responses such as increased vascularity and leukocyte diapedesis. The effects of these are compounded by the products of the host immunity, attempting to combat the persistent bacteria challenge. The initial reaction to this is gingivitis which may progress to periodontitis, clinically evidenced by deep periodontal pockets, mobility etc., if left untreated [4].

Complete elimination of plaque microorganisms, is therefore the first step in the treatment of periodontal diseases [1]. Comprehensive mechanical therapy, although considered the gold standard in plaque removal, is bound by limitations such as difficulty in accessing certain areas, very deep periodontal pockets, dentinal tubules, root surface irregularities, root concavities, bifurcations, and large invaginations and addressing the tissue invasive microorganisms. The periodontal pocket is one such microniche, wherein the environment is partially sheltered from the physical shear forces in the oral cavity allowing the microorganisms to thrive [5]. These are the reasons necessitating adjunctive measures for periodontal therapy like antimicrobial therapy [6], lasers, photodynamic therapy [7,8] and host modulation [9,10]. The most commonly used of these is antimicrobial therapy, either in systemic or local formulations [11].

But indiscriminate use of antimicrobials in dentistry has led to antimicrobial resistance [12], a global concern, resulting in increased morbidity, mortality, healthcare costs. These adverse effects and high dosage of systemic medication needed to influence the plaque bacteria prompted the research towards alternative sources such as herbal medication [13].

Earlier in 20th century herbal medication such as morphine, aspirin, ergot alkaloids were primarily in use, as synthetic analgesics and antibiotics were not available. But due to the faster therapeutic action of allopathic medication, its counterpart gradually lost ground to the former. Despite this, approximately 70-80% of population still use herbal medication as primary healthcare, as it is deemed to have fewer side effects and to be more compatible with the human body. (WHO 2003) [14]. But most of the evidence in support of herbal medication is anecdotal, without much scientific justification or well -designed studies to support or validate their modes of action. This statement holds true in the field of periodontia as well.

A wide range of herbal extracts, *Acacia catechu* (Cutch tree), *Aloe vera*, *Azadirachta indica* (Neem), *Glycyrrhiza glabra* (Licorice), *Ocimum sanctum L* (holy basil), *Curcuma longa* (turmeric), and *Matricaria chamomilla* (Chamomile), to name a few, have demonstrated potent anti-inflammatory, antibacterial, antioxidant, and astringent properties when used to treat periodontal conditions [15]. Some are currently incorporated in popular dentrifices and chemical plaque control aids like Neemayu neem™ tooth paste (Neem extract), Herbodent™, Dantkanth™ (Eucalyptus, lemon, giloy, clove and cinnamon extracts), Meswak™, Colgate Herbal™

(Myrrh, sage, eucalyptus, tea tree oil and chamomile), Hi Ora mouthwash™ (Betel leaves), Cur Q fresh mouth rinse™ (Mint, tulasi, cloveoil and curcumin) Periowash mouthwash™ (Oregano, Cinnamon and Clove) etc.

The Tc, otherwise known as Guduchi, Amritha, Giloya, is a deciduous climbing shrub reported to possess antimicrobial [16], anti-inflammatory [17], immunosuppressive [18], antiallergic, antidiabetic [19] and antispasmodic properties and to increase antibody production in-vivo in animal studies [20]. Despite this, studies regarding this herb in periodontal conditions are few. Since periodontitis is a multifactorial disease, usage of herbal products with multiple effects would be beneficial. No allopathic drug has shown multifaceted actions similar to Tc. Furthermore, the acquisitions of resistance of microorganisms for herbal products are reported to be minimal.

Numerous individual periodontopathogens have been tested against Tc, and its Minimum Inhibitory Concentration (MIC) for the same has been determined, but there are no studies which have assessed its effect against collective subgingival microbiota. This would be more relevant to the application of this herbal medication against periodontitis which is known to be caused by plaque bacteria as a whole. This in-vitro study was the first to determine the MIC as well as MBC of Tc against the aforementioned periodontal pathogens.

MATERIALS AND METHODS

The present study was an in-vitro, analytical study done in Department of Periodontics, Krishnadevaraya College of Dental Sciences and Hospital, Bengaluru, Karnataka, India for two and half months from November 2021 to January 2022. The study was done in accordance to Helsinki declaration, after obtaining the ethical clearance from the Institutional Ethical Board (Ethical clearance no. KCDS/Ethical Comm/SS/02/2020-21). The subjects for this study were selected from the outpatient department of the same, upon meeting inclusion and exclusion criteria. Informed consent was obtained from all the patients for sample collection.

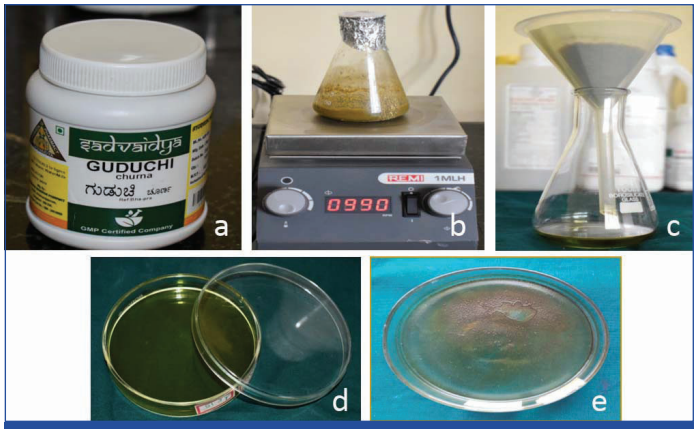
Sample size calculation: A total of 12 patients with chronic periodontitis were selected for the purpose of the study based on sample size analysis done using G Power software v. 3.1.9.4 (Franz Faul, Universität Kiel, Germany), considering the effect size to be measured (dz) at 80% for two-tailed hypothesis, power of the study at 80% and the margin of the error at 5%.

Inclusion criteria: Patients between the ages of 30-50 years, diagnosed with moderate chronic periodontitis, presence of periodontal pockets (5-7 mm), clinical and radiographic evidence of bone loss were selected for the study. They were required to provide the written informed consent.

Exclusion criteria: Non compliant patients, patients who received any surgical or non surgical therapy six months before the start of the study, pregnant or lactating females, use of systemic antibiotics in the past six months and smokers/alcoholics were excluded from the study.

Preparations of Plant Extract [21,22]

Non destructive cold percolation method was employed using powder of Tc (procured from local ayurvedic store) with ethanol as per the method detailed by Rosenthaler (1930) [Table/Fig-1a]. The crushed powder of Tc was macerated in ethanol (procured from HiMedia Laboratories Pvt. Ltd.) in a ratio of 1:10 solute versus solvent in a 250 mL conical flask. The entire set up was kept on a rotary shaker [Table/Fig-1b] (990 rpm) at room temperature for 24 hours with intermittent shaking. After 24 hours, the mixture was filtered through Whattman No.1 filter paper [Table/Fig-1c], and the filtrate [Table/Fig-1d] was allowed to evaporate under room temperature [Table/Fig-1e]. The extract settled at the bottom was used for the experiment at varying concentration. The concentrated extracts were dissolved in ethanol and used for further tests. Filtrate was stored in a refrigerator until use.



[Table/Fig-1]: Preparation of plant extract. a) *Tinospora cordifolia*; b) macerated ethanol and Tc on rotary shaker; c) filtration; d) filtered mixture; e) dried Extract.
†Procured from HiMedia Laboratories Pvt. Ltd.
‡Procured from HiMedia Laboratories Pvt. Ltd.
Source of image: Krishnadevaraya College of Dental Sciences and Hospital

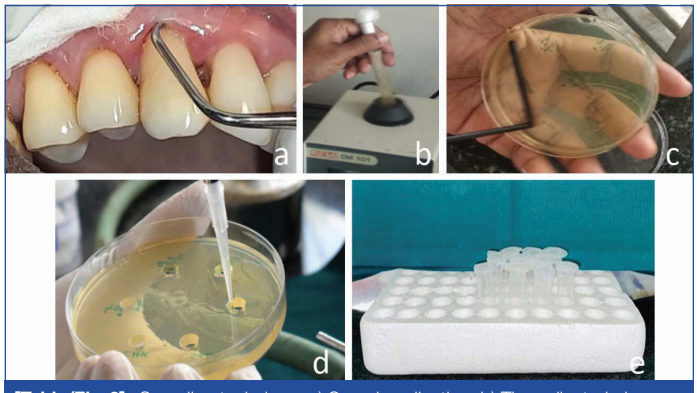
Procedure for MIC Estimation

Supragingival hand scaling was done in the quadrant with the deepest pocket ensuring not to disturb the subgingival plaque. Subgingival plaque sample of patients was collected with the help of Gracey curette number 2R/2L and 4R/4L (From Hu-Friedy Mfg. Co) [Table/Fig-2a], without blood or saliva contamination after thorough supragingival hand scaling [Table/Fig-2a]. The collected plaque sample was transferred to thioglycolate broth containing vitamin K and hemin (Procured from HiMedia Laboratories Pvt. Ltd.) without touching the sides of the test tube, and transported to the laboratory within an hour for microbiological analysis. The plaque samples were vortexed for 10 seconds [Table/Fig-2b]. A 20 µL/mL of the plaque sample was seeded on to the BHI agar with the help of the L spreader (Procured from HiMedia Laboratories Pvt. Ltd.).

[Table/Fig-2c] 6 mm diameter wells were cut on the BHI agar and subjected to three serial doubling dilutions of Tc extract ranging from concentration of 100 mg/mL, 50 mg/mL and 25 mg/mL and taking chlorhexidine (procured from Icpa Health Products Ltd) as positive control and Dimethyl sulfoxide (DMSO) (procured from Sigma -Aldrich®) as negative control [Table/Fig-2d] [14].

Procedure for MBC Estimation

The 10 different serial doubling dilutions of the Tc extract ranging from 100 mg/mL, 50 mg/mL, 25 mg/mL, 12.5 mg/mL, 6.25 mg/mL, 3.125 mg/mL, 1.625 mg/mL, 0.862 mg/mL, 0.413 mg/mL and 0.206 mg/mL [Table/Fig-2e] were prepared and incubated for two hours in the incubator at 37°C. Then they were streaked on to the brain heart infusion agar plates, with the L spreader, uniformly over the surface of the agar and incubated it at 37°C under strictly anaerobic conditions for 48 hours using anaerobic gas jar and gas pack for anaerobic obligate and facultative bacteria. The colonies were counted using digital colony counter.



[Table/Fig-2]: Sampling technique. a) Sample collection; b) The collected plaque sample was centrifuged; c) Plaque sample streaked on BHI agar; d) 6 mm wells subject to different dilutions of Tc extract for MIC; e) Ten dilutions of Tc for MBC.

STATISTICAL ANALYSIS

Statistical Package for Social Sciences (SPSS) for windows version 22.0 released 2013. Armonk, NY: IBM Corp was used to perform statistical analyses. Descriptive analysis of all the explanatory and outcome parameters were done using frequency and proportions for categorical variables, whereas in Mean and SD for continuous variables. Chi-square test was used to compare the presence of MBC values between 25 mg/mL and 12.5 mg/mL concentrations. One-way ANOVA (Analysis of variance) followed by Tukey's post hoc analysis was used to compare the mean MIC values between different groups. The level of significance was set at $p<0.05$.

RESULTS

The values obtained for the microbiological parameters of the 12 patients enrolled in the study were as follows. A total of seven males and five females were included in the study with 30-40 years aged patients significantly higher in number than other two groups. These demographic details are presented in [Table/Fig-3]. A total of three different concentrations of Tc extract along with positive and negative control were assessed for antimicrobial activity against the whole subgingival microorganisms. The MIC was assessed based on the zones of the inhibition produced by different concentrations of Tc on BHI agar plates. Simultaneously CHX and DMSO were used as positive and negative controls respectively. At 100 mg/mL concentration, the maximum zone of inhibition noted was 11.42 mm, whereas 50 mg/mL concentration showed 10.92±0.79 and 25 mg/mL concentration demonstrated 10.33 mm. Dilutions lesser than 25 mg/mL failed to produce a significant zone of inhibition and no inhibition zone was observed with negative control [Table/Fig-4]. The results with 0.2% CHX, which was used as positive control, showed a maximum zone of inhibition of 16.58±1.17 mm. The difference between the zones of inhibition at 100 mg/mL and 50 mg/mL was not statistically significant, whereas statistical significance was noted between 100 mg/mL and 25 mg/mL. The difference in the diameter of the zones produced by Tc, saline and CHX were statistically significant. 50 mg/mL was as effective as 100 mg/mL [Table/Fig-5,6,7a and 8].

Variables	Category	Frequency	Percentage
Age (mean±SD=38.17±5.13, range=31-47 years)	31-40 years	8	66.7%
	41-50 years	4	33.3%
Gender	Males	7	58.3%
	Females	5	41.7%

[Table/Fig-3]: Age and gender distribution among study samples.
n=12

Groups	No. of samples	Mean	SD	Min	Max	p-value
100 mg/mL	12	11.42	0.67	10	12	<0.001*
50 mg/mL	12	10.92	0.79	10	12	
25 mg/mL	12	10.33	0.89	9	12	
Saline	12	0.00	0.00	0	0	
CHX	12	16.58	1.17	15	18	

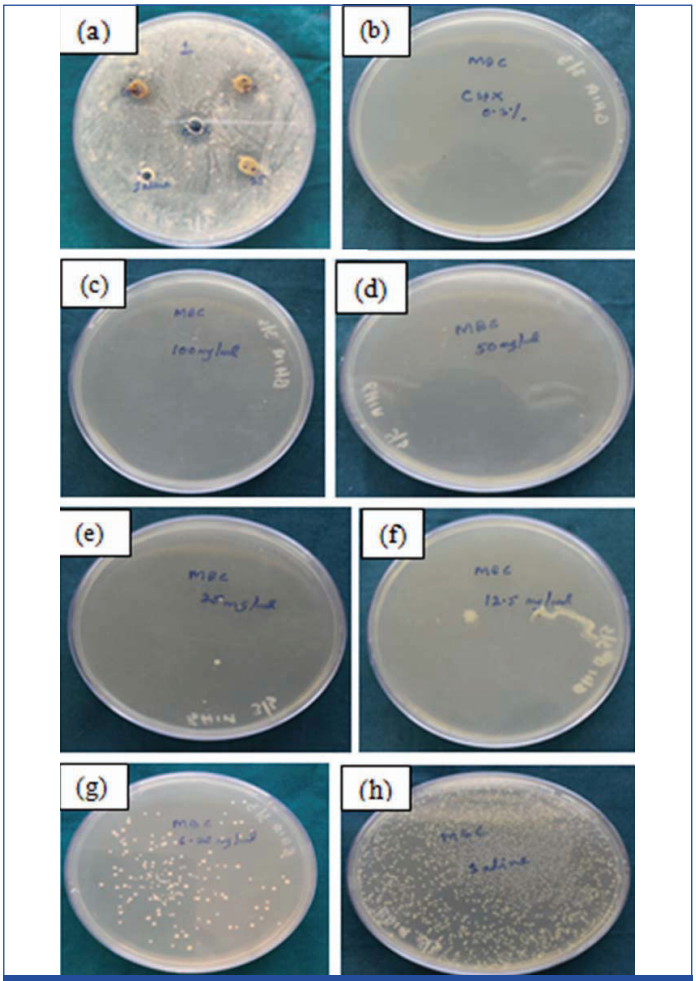
[Table/Fig-4]: Comparison of mean MIC values between different groups using One-way ANOVA Test.

(I) Groups	(J) Groups	Mean Diff. (I-J)	95% CI of the Diff.		p-value
			Lower	Upper	
100 mg/mL	50 mg/mL	0.50	-0.42	1.42	0.55
	25 mg/mL	1.08	0.16	2.01	0.01*
	Saline	11.42	10.49	12.34	<0.001*
	CHX	-5.17	-6.09	-4.24	<0.001*
50 mg/mL	25 mg/mL	0.58	-0.34	1.51	0.40
	Saline	10.92	9.99	11.84	<0.001*
	CHX	-5.67	-6.59	-4.74	<0.001*
25 mg/mL	Saline	10.33	9.41	11.26	<0.001*
	CHX	-6.25	-7.17	-5.33	<0.001*
Saline	CHX	-16.58	-17.51	-15.66	<0.001*

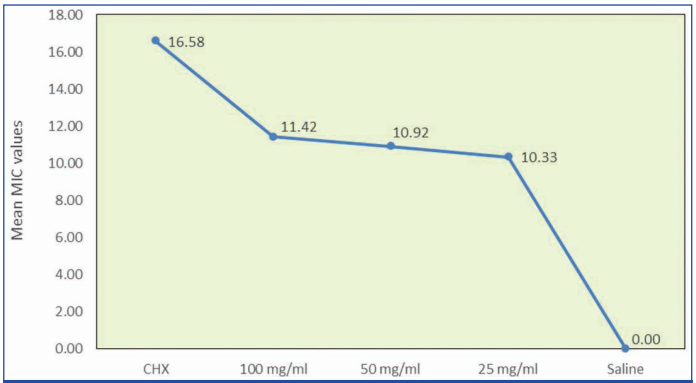
[Table/Fig-5]: Multiple comparison of mean difference in MIC b/w groups using Tukey's Post hoc Test.
*p-value <0.05

Variable	Category	25 mg/mL		12.5 mg/mL		χ^2 value	p-value
		n	%	n	%		
MBC	Present	9	75.0%	3	25.0%	6.000	0.01*
	Absent	3	25.0%	9	75.0%		

[Table/Fig-6]: Comparison of presence of MBC between 25 and 12.5 mg/mL concentration using Chi-square Test.
*p-value <0.05



[Table/Fig-7]: a) MIC of CHX, Tc and DMSO; b) MBC of CHX; c) MBC at 100 µg/mL; d) MBC at 50 µg/mL; e) MBC at 25 µg/mL; f) MBC at 12.5 µg/mL; g) MBC at 6.25 µg/mL; h) MBC of saline.



[Table/Fig-8]: Mean MIC values between different groups (In descending order).

The MBC was determined to assess the least concentration at which no bacterial growth is noted. The MBC of CHX showed absolute no growth [Table/Fig-7b] followed by 100 mg/mL [Table/Fig-7c] and

50 mg/mL [Table/Fig-7d]. The test results showed that the MBC was seen in 75% of the samples at 25 mg/mL [Table/Fig-7e] as compared to 25% of the samples at 12.5 mg/mL [Table/Fig-7f]. This difference in the MBC exhibited by the samples at two different concentrations was statistically significant at p=0.01. This gives the interpretation that concentrations less than 12.5 mg/mL, 6.25 mg/mL [Table/Fig-7g] and negative control, showed [Table/Fig-7h] no demonstrable effect on subgingival microbiota.

DISCUSSION

Anti-infective therapy in the management of periodontitis is not a novel concept but has seen significantly varied drugs being put to use. Most research articles predominantly feature allopathic drugs such as amoxicillin, ciprofloxacin, tetracycline and metronidazole to name a few as stand alone or in combination with each other. Significant adverse effects and antimicrobial resistance have been reported over the years. This has switched the focus towards herbal medication.

Studies on the antibacterial activity of medicinal plants against the microbial ecosystem of the oral cavity and often the cause of periodontal infections are fewer in comparison [22,23]. Due to the difficulty in isolation and identification of the complex microbiota of the oral cavity, in addition to the challenges of standardising techniques to evaluate the antibacterial efficacy of drugs, research articles regarding such medications are less common [24].

Presently multidrug resistance in microbial pathogens have become a serious health hazard to humans worldwide, owing to the indiscriminate, repetitive and at times unethical, use of antimicrobial drugs. There is an urgent need to develop new antimicrobials, which will overcome this as well as the other concerns posed by the existing drugs [8].

This vast, natural, time-tested medicinal resource is worth exploring as a possibility of developing efficient, economically viable, and clinically acceptable antimicrobials. It only remains to be harnessed in the right formulation, which is what has been attempted via this in-vitro study.

Scientifically, plants such as *Ocimum sanctum* (Tulsi) and *Stevia rebaudiana* (candy leaf) etc., have proven to be efficacious antimicrobials. Tc, a common tropical shrub, has shown anti-inflammatory [25-27], analgesic, antibacterial activity [28], antioxidant properties [29], and increase in antibody production in-vivo and immunosuppressive actions in animal studies [30,31].

In the present investigation ethanolic extracts of the different concentrations of Tc were evaluated to determine the one demonstrating optimal antimicrobial activity. Previous studies have applied the same study design for individual organisms [7,13,32] but present study was the first to assess its efficacy against the entire subgingival microbiota of the oral cavity.

In the present study, out of three different concentrations and dilutions tested against the whole subgingival microbiota, maximum inhibition zone of 11.42 mm was found with 100 mg/mL concentration of the extract, 50 mg/mL showing an insignificantly smaller inhibition zone of 10.92 mm, progressively smaller concentrations shown a proportionately smaller inhibition zones. MBC is the lowest concentration of an antibacterial agent required to kill the bacterium. The determination of the MBC of the ethanolic extract of Tc was found at 25 mg/mL for 75% of cases and for 25% of cases it was at 12.5 mg/mL.

The results of this study have given useful information about the inhibiting activity of medicinal plant extracts against periodontal pathogens. In fact, there is no information in the literature on the antibacterial properties of Tc against the subgingival pathogens responsible for periodontopathies.

The results of this study suggest that the use of the ethanolic extracts of Tc as topical medication in periodontal prophylactics or in the alteration of the microbic ecosystem, as mouthwashes or gels based wholly or partially on these plant extracts could be a valid aid to obtain a significant reduction of the total microbial population and, in particular, the most virulent microorganisms.

Limitation(s)

Small sample size and microbiological analysis is both time consuming and expensive.

CONCLUSION(S)

The CHX showed maximum antibacterial activity against subgingival microbiota than Tc. The use of herbal alternatives in the treatment of chronic periodontitis might prove to be advantageous considering the undesirable characteristics of CHX. Current study aims to highlight the medicinal use of a new herbal product Tc which has shown to possess antimicrobial properties. The results of the study showed that the Tc could effectively inhibit the bacteria found in the subgingival plaque, combating the periodonto-pathogens and it further showed high efficacy in inhibiting the anaerobic bacteria. Hence, it can be used as a successful alternative to synthetic antimicrobials. The dosage and safety of Tc must be considered before it's possible in-vivo application for successful non surgical therapeutic modality of the management of chronic periodontitis.

REFERENCES

[1] Caton JG, Armitage G, Berglundh T, Chapple IL, Jepsen S, Kornman KS, et al. A new classification scheme for periodontal and peri-implant diseases and conditions-Introduction and key changes from the 1999 classification. Journal of periodontology. 2018;89(Suppl 1):S1-S8.

[2] Popova C, Dosseva-Panova V, Panov V. Microbiology of Periodontal Diseases. Biotechnology & Biotechnological Equipment. 2013;27(3):3754-59.

[3] da Silva-Boghossian CM, do Souto RM, Luiz RR, Colombo AP. Association of red complex, A. actinomycetemcomitans and non-oral bacteria with periodontal diseases. Arch Oral Biol. 2011;56(9):899-906.

[4] Khosla A, Gupta SJ, Jain A, Shetty DC, Sharma N. Evaluation and comparison of the antimicrobial activity of royal jelly-A holistic healer against periodontopathic bacteria: An in vitro study. Journal of Indian Society of Periodontology. 2020;24(3):221.

[5] Colombo AP, Teles RP, Torres MC, Rosalém W, Mendes MC, Souto RM, et al. Effects of non-surgical mechanical therapy on the subgingival microbiota of Brazilians with untreated chronic periodontitis: 9-month results. Journal of periodontology. 2005;76(5):778-84.

[6] Hung HC, Douglass CW. Meta analysis of the effect of scaling and root planing, surgical treatment and antibiotic therapies on periodontal probing depth and attachment loss. Journal of clinical periodontology. 2002;29(11):975-86.

[7] Azaripour A, Dittrich S, Van Noorden CJ, Willershausen B. Efficacy of photodynamic therapy as adjunct treatment of chronic periodontitis: A systematic review and meta-analysis. Lasers in medical science. 2018;33(2):407-23.

[8] Oktawati S, Rukmana A, Patimah, Wahab RF, Syafar IF, Wahab W. Photodynamic Therapy as Adjunctive Treatment of Chronic Periodontitis: A Systematic Review. Systematic Reviews in Pharmacy. 2020;11(6):492-98.

[9] Preshaw PM. Host modulation therapy with anti-inflammatory agents. Periodontology 2000. 2018;76(1):131-49.

[10] Hajishengallis G, Chavakis T, Lambris JD. Current understanding of periodontal disease pathogenesis and targets for host-modulation therapy. Periodontology 2000. 2020;84(1):14-34.

[11] Jepsen K, Jepsen S. Antibiotics/antimicrobials: systemic and local administration in the therapy of mild to moderately advanced periodontitis. Periodontology 2000. 2016;71(1):82-112.

[12] Ardila CM, Granada MI, Guzmán IC. Antibiotic resistance of subgingival species in chronic periodontitis patients. Journal of periodontal research. 2010;45(4):557-63.

[13] Bhatia M, Urolagin SS, Pentyala KB, Urolagin SB, KB M, Bhoi S. Novel therapeutic approach for the treatment of periodontitis by curcumin. Journal of clinical and diagnostic research: JCDR. 2014;8(12):ZC65.

[14] World Health Organization. Traditional medicine in asia. WHO Regional Office for South-East Asia; 2002.

[15] Sharma A, Batra A. In vivo antimicrobial activity of Indian medicinal plant Tinoscordifolia using serial tube dilution technique. Asian Journal of Pharmaceutical Research and Development. 2013;1(2):148-56.

[16] Singh S, Singh P. Effectiveness of Tinospora cardifolia stem extract on bacteria *Salmonella typhi, Pseudomonas aeruginosa, Staphylococcus aureus* and *Shigella dysenteriae*. International Journal of Pharmacy & Life Sciences. 2012;3(8):1923-25.

[17] Pendse VK, Dadhich AP, Mathur PN, Bal MS, Madan BR. Antiinflammatory, immunosuppressive and some related pharmacological actions of the water extract of Neem Giloe (Tinospora cordifolia): A preliminary report. Indian journal of pharmacology. 1977;9(3):221-24.

[18] Prince PS, Menon VP. Antioxidant activity of Tinospora cordifolia roots in experimental diabetes. Journal of ethnopharmacology. 1999;65(3):277-81.

[19] Ranjith MS, Ranjitsingh AJ, Shankar SG, Vijayalaksmi GS, Deepa K, Sidhu HS. Enhanced Phagocytosis and Antibody Production by *Tinospora cordifolia*-A new dimension in Immunomodulation. African Journal of Biotechnology. 2008;7(2):81-85.
[20] Devi M, Sharma A, Navdeep, Saroea S, Kohli M, Bhatt AK. In vitro study of antimicrobial activity of Tinospora cordifolia (Thunb.) Miers plant extracts against selected clinical isolates. Annals of Phytomedicine. 2018;7(2):76-80.
[21] Gunes H, Gulen D, Mutlu R, Gumus A, Tas T, Topkaya AE. Antibacterial effects of curcumin: An in vitro minimum inhibitory concentration study. Toxicology and industrial health. 2016;32(2):246-50.
[22] Rosenthaler L. The chemical investigation of plants. F. L. Pvman. 1930;50(18):356. Doi: doi:10.1002/jctb.5000501802.
[23] Mishra S, Johnson L, Sangha KS, Gupta V, Agarwal S, Rajput S. An in vitro study to determine the physicochemical, mechanical, and antibacterial properties of a novel spirulina containing controlled release intrapocket drug delivery system. Journal of Pharmacy & Bioallied Sciences. 2021;13(2):178-87.
[24] Dib K, Ennibi O, Alaoui K, Cherrah Y, Filali-Maltouf A. Antibacterial activity of plant extracts against periodontal pathogens: A systematic review. Journal of Herbal Medicine. 2021. Doi: https://doi.org/10.1016/j.hermed.2021.100493.
[25] Philip S, Tom G, Vasumathi AV. Evaluation of the anti-inflammatory activity of Tinospora cordifolia (Willd.) Miers chloroform extract-a preclinical study. The Journal of Pharmacy and Pharmacology. 2018;70(8):1113-25.
[26] Birla H, Rai SN, Singh SS, Zahra W, Rawat A, Tiwari N, et al. Tinospora cordifolia suppresses neuroinflammation in parkinsonian mouse model. Neuromolecular medicine. 2019;21(1):42-53.
[27] Jacob J, Prakash BK. Dihydroxy berberine from *Tinospora cordifolia*: In silico evidences for the mechanism of anti-inflammatory action through dual inhibition of Lipoxygenase and Cyclooxygenase. Indian Journal of Biochemistry and Biophysics (IJBB). 2021;58(3):244-52.
[28] Bhardwaj A, Srivastava N, Rana V, Adlakha VK, Asthana AK. How efficacious are *Neem, Tulsi*, Guduchi extracts and chlorhexidine as intracanal disinfectants? A comparative *ex vivo* study. Ayu. 2017;38(1-2):70-75.
[29] Sharma R, Bolleddu R, Maji JK, Ruknuddin G, Prajapati PK. In-vitro α-amylase, α-glucosidase inhibitory activities and in-vivo anti-hyperglycemic potential of different dosage forms of Guduchi (Tinospora cordifolia [Willd.] Miers) prepared with Ayurvedic Bhavana process. Frontiers in Pharmacology. 2021;12:642300.
[30] Devi P. A review on Tinospora cordifolia: As an Immunomodulating agent. Himalayan Journal of Health Sciences. 2021;6(1):06-14.
[31] Panchabhai TS, Kulkarni UP, Rege NN. Validation of therapeutic claims of Tinospora cordifolia: A review. Phytother Res. 2008;22(4):425-41. Doi: 10.1002/ptr.2347.
[32] Vermani A, Navneet, Gautam SS. Screening of antibacterial activity of Tinospora cordifolia Miers extracts against dental pathogens. J Pharmacol Toxicol. 2013;8(1):28-34.

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