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Review Article

A systematic review of in vitro studies on the anti-microbial efficacy of tulsi against periodontal pathogens

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ABSTRACT:

Background: Ocimum tenuiflorum, more commonly known as Tulsi, is known for its anti-microbial effect and is used to treat various diseases. Periodontitis is an oral disease occurring due to the action of various periodontal pathogens, which eventually leads to the destruction of periodontal tissues. Aggregatibacter actinomycetemcomitans is the most common organism which is responsible for the initiation of Periodontitis. Many studies are going on to establish the efficiency of tulsi against periodontal pathogens. **Aim:** To determine the anti-microbial efficiency of tulsi against periodontal pathogens. **Method:** A systematic review of in-vitro studies used Tulsi as an effective agent against periodontal pathogens such as A.actinomycetemcomitans, P. gingivalis, E.faecalis, and F. nucleatum and P. intermedia were searched in Electronic databases, and a total of 182 articles were obtained, among which five studies were included in this study. **Result:** Five studies were included in the systematic review, of which all are in-vitro studies. Among which, four showed a good inhibition zone against periodontal pathogens and proved and supported the anti-microbial property of tulsi, while one showed resistance against P. gingivalis and P. intermedia, **Conclusion:** Tulsi extract shows anti-microbial properties, and periodontal application of tulsi can be as a mouthwash, dentifrice, gel and intracanal irrigant. Tulsi may be useful as an adjunctive to mechanical therapy in the prevention and treatment of periodontal diseases.

Keywords: Ayurveda, Dentistry, Tulsi, Periodontitis.

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INTRODUCTION

Ocimum tenuiflorum, more commonly known as Tulsi, is known for its anti-microbial effect, which has been tested and used extensively in Ayurvedic medicine to treat various diseases. Tulsi is easily available in abundance, is affordable and is widely accepted.[1,2]

Medicinal plants contain phytochemicals that can be used as medicines with little or no side effects like oil, Lauric acid makes up about 50% of the fatty acids in coconut oil. When the body breaks down lauric acid, it produces a substance called monolaurin. Both monolaurin and lauric acid help destroy harmful organisms such as fungi, bacteria and viruses. [4]

Tulsi furthermore has an effective role in the treatment of various oral infections and the maintenance of oral hygiene. Periodontitis is an oral disease occurring due to the action of various periodontal pathogens, which eventually leads to the destruction of periodontal tissues.[5]

Aggregatibacter actinomycetemcomitans is the most common microorganism which is found to be responsible for the initiation of Periodontitis, while levels of *Porphyromonas gingivalis* and *Prevotell intermedia* are found to have increased in cases of chronic periodontitis.[6,7]

Enterococcus faecalis is also detected in conditions like marginal Periodontitis and can easily survive in conditions deprived of nutrition. It is also found to be resistant to the action of most antibiotics.[8,9]

A complex interaction between pathogenic bacteria and the host's immune response results in the loss of connective tissue, formation of periodontal pockets and resorption of the alveolar bone, which are the characteristic features of Periodontitis. If left untreated, it leads to the destruction of the entire periodontal attachment apparatus of the affected teeth.[10]

OBJECTIVE

To determine the anti-microbial efficiency of tulsi

against periodontal pathogens.

MATERIAL AND METHODS

INCLUSION CRITERIA

1. Original articles
2. In-vitro studies
3. Articles on anti-microbial efficiency of tulsi against periodontal pathogens.

EXCLUSION CRITERIA

1. Review articles
2. Articles without open access
3. In-vivo studies
4. Randomized control trial
5. Articles in other languages.

SEARCH STRATEGY

Published clinical trials and original studies on anti-microbial efficiency of tulsi against periodontal pathogens in electronic databases such as Cochrane Central Register of Controlled Trials (CENTRAL), Wiley, Science Direct, PubMed, Ovid MedLine, Google scholar as collected for review from April 2021 to August 2021. Preferred Reporting Items for Systematic Review and Meta-analysis (PRISMA) was used to select the studies. Mesh keywords like tulsi, Periodontitis, Ayurveda and dentistry were used to search and collect relevant data. A total of 182 articles appeared with these keywords. After the removal of duplicates, 158 articles were obtained. These articles were screened, and 26 articles were selected to be research-related. Finally, after applying the exclusion criteria, five articles were chosen for review. Figure 1 shows the number of studies identified, screened, assessed for eligibility, excluded and included in the systematic review.

SEARCH ENGINES

1. PubMed
2. Ovid MedLine
3. Google Scholar
4. Wiley Online Library
5. Cochrane Central Register of Controlled Trials (CENTRAL)
6. Science Direct

Figure 1: Flow Diagram showing the numbers of studies identified, screened, assessed for eligibility, excluded and included in the systematic review

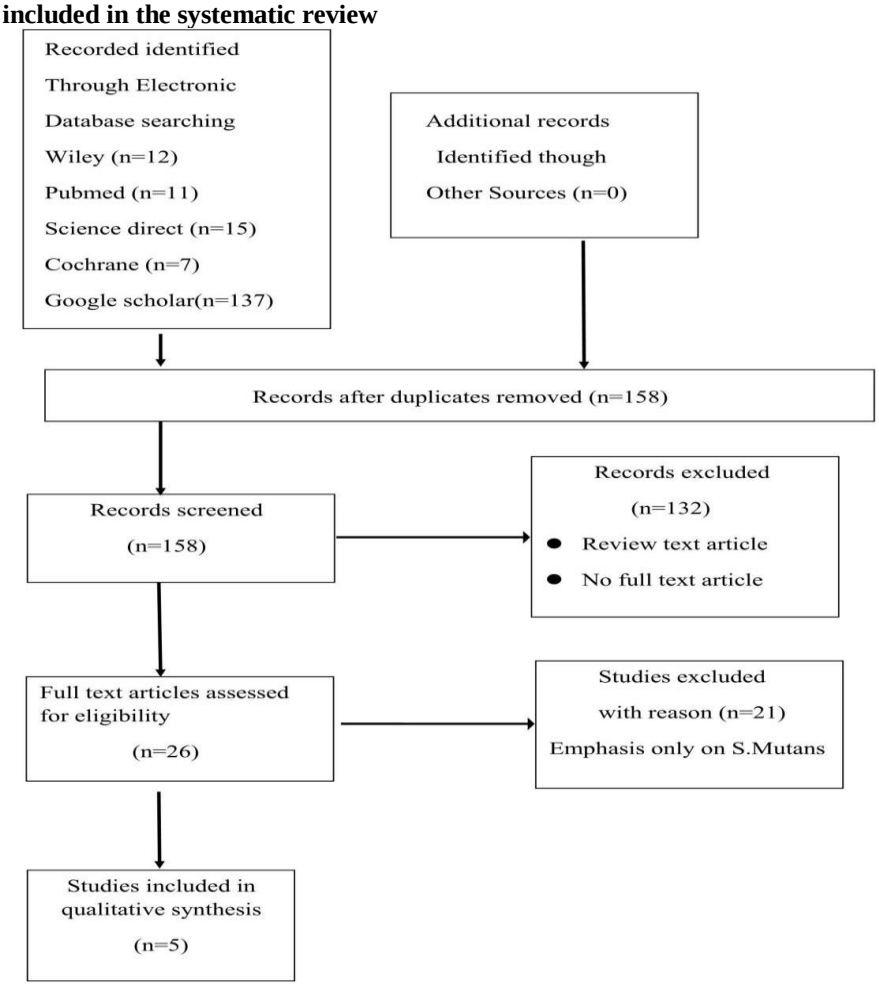

TABLE 1: CHARACTERISTICS OF THE INTERVENTIONS IN THE INCLUDED STUDIES

S.no	Author	Inoculation	Preparations used	Intervention	Incubation time
1	Ipsita Jayanti; Md jalaluddin[2]	Kanamycin blood agar was used to isolate Porphyromonas gingivalis. The main ingredients of Kanamycin blood agar are Trypticase blood agar base containing 5% of sheep's blood added to hemin, Vitamin K1, L-cysteine, yeast extract, and 100 mg / L Kanamycin. Aggregatibacter actinomycetemco and mutans was separation using Dentaid agar.	Tulsi extraction was prepared with ethanolusing a cold extraction method to develop an *in vitro* tutorial. Various concentrationssuch as 2, 4, 6, and 8% were obtained by diluting them with dimethylformamide.	0.2% chlorhexidine acted as a positive control, while the negative control was dimethylformamide. Prohibition areas were estimated, each *P.gingivalis* and *A. actinomycetemcomitans*. In-group comparisons and one-way variance analysis (ANOVA), and Tukey's *post hoc* analysis were used between study groups.	48 hours
2	Pranati Eswar;C.G Devara[9]	A plaque sample wascollected from 5 patients diagnosed with periodontal disease. Isolation of *Actinobacillus actinomycetemcomitans* from plaque samples was done using Tryptic Soy Serum Bacitracin Vancomycin agar. (TSBV) medium.	The cold extract*ion method* was used to prepare the alcoholic extract of10 different concentrationsof solution [1%, 2%, 3%, 4%, 5%] was prepared by diluting with dimethyl sulfoxide.	*Ocimum sanctum (Linn.)* extract has Anti-microbial activity tested by the agar well diffusion method. To compare the results, a positive control agent[0.2% chlorhexidine] and a negative control[dimethyl sulfoxide] were selected. The final zone of inhibition was measured using vernier calliper in millimetres.	24 hours.
3	Kush Kalra,Ramprasad Vasthare[10]	P. gingivalis, P. intermedia, F. nucleatum and S. aureus isolates and S. mutans was grown on were grown on 5% sheep blood agarand Brain Heart Infusion (BHI) agar respectively.	Essential oil of two varieties of Tulsi, i.e. Ocimum sanctum [PureHoly Basil (Tulsi) Essential Oil - OcimumSanctum, dève herbs, New Delhi, India] and Ocimum basilicum [Basil Tulsi Essential Oil (Ocimum basilicum), Kazima Perfumers, New Delhi, India] were	The anti-microbial susceptibility was determined by the agar well diffusion (punchwell diffusion) method. Vancomycin 30µg discs were used as a procedural control for S. aureus and S. mutans. Metronidazole 5µg discs were used as theprocedural control for anaerobic bacteria.After the	48 hours

			procured from market retailers.	desired incubation period was over, the zone of inhibitions for the extracts were measured using Verniercallipers.	
4	Sajjanshetty Mallikarjun Ashwini Rao[11]	Blood agar was used to culture *A. actinomycetemcomitans, P. gingivalis* and *P. intermedia*	the cold extraction method was used for making an alcoholic extract of tulsi. The extract was diluted with an inert solvent such as dimethylformamide to obtain five different concentrations (0.5%, 1%, 2%, 5%, and 10%).	The Anti-microbial activity of T*ulsi* extract was tested by the agar welldiffusion method. To compare the result, doxycycline and dimethylformamide were the control, respectively.	48 hours
5	Dr Raghavendra an m Shetty; Dr Anita ghoyal[12]	Mueller Hinton agarwas used to culture Streptococcus mutans and E. faecalis	The cold extraction method was used to get the alcoholic extract of tulsi. A default solution (30% concentration of the extract in normal saline) was selected, and five solutions were made out of it by doubling each of the extracts to make concentrations of 15%, 7.5%,3.75%, and 1.88%.	The good diffusion method was wont to determine the zone of inhibition. During this method, five circular wells that would incorporate five different concentrations of the test agent (Tulsi extract) were cut within the agar plates employing a template. Four plates were prepared and labelled for thefive different concentrations of Tulsi extract. The extract was cultured aerobically at 37°C for 48hours by transferring it to the respective agar plates .A vernier calliper was used to measure the zone of	48 hours

				inhibition in millimetres.	

Table 1 shows the characteristics of the intervention in the included studies. In all the above studies, the anti-microbial efficiency of tulsi was evaluated.

TABLE 2: BIAS ANALYSIS OF INCLUDED STUDIES

S.NO	AUTHOR AND YEAR	RANDOM SEQUENCE GENERATION	ALLOCATION CONCEALMENT NT	SELECTE REPORTING	INCOMPLETEE OUTCOME DATA	BLINDING OF OUTCOME ASSESSMENT NT	BLINDING PARTICIPANTSAND PERSONNEL
1	Ipsita Jayanti; Md jalaluddin[2]	-	-	?	-	++	-
2	Pranati Eswar; C.G Devaraj[9]	++	-	?	-	-	-
3	Kush Kalra, Ramprasad Vasthare[10]	-	-	?	-	-	-
4	Sajjanshetty Mallikarjun Ashwini Rao[11]	-	-	?	-	-	-
5	Dr Raghavendra mShetty; Dr Anita ghoyal[12]	-	-	?	-	-	-

Table 2 shows the bias analysis of all the included studies. It is categorized as high-risk bias "-", low-risk bias "++" and unclear "?". Categorization was done according to the Cochrane risk of bias tools for controlled trials.

TABLE 3: OUTCOME DATA AS REPORTED IN INCLUDED STUDIES

SL.NO	AUTHOR	YEAR	OUTCOME	RESULT
1.	Ipsita Jayanti; Md jalaluddin[2]	2018	• For 2% of tulsi extract, a mean zone of inhibition against A.*actinomycetemcomitans* and *P. gingivalis* is 16.24 ± 1.01mm and14.32 ± 1.21mm, respectively. • For 4% of tulsi, a mean zone of inhibition against A.*actinomycetemcomitans* and *P. gingivalis* are 26.75 ± 2.57mm and 22.94 ± 1.52 mm, respectively. • For 6% of tulsi, a mean zone of inhibition against A.*actinomycetemcomitans* and *P. gingivalis* is 33.38 ± 1.86mm and 29.80 ± 1.60mm, respectively. • For 8% of tulsi, a mean zone of inhibition against A.*actinomycetemcomitans* and *P. gingivalis* is 40.10 ± 0.90	Therefore, It was concluded that 8% *of the output of O.* It is therefore recommended that this be useful as combining mechanical therapy wthe prevention and treatment of periodontal diseases.

			mm and 33.79 ± 1.82mm, respectively. • For 0.2% of chlorhexidine. a mean zone of inhibition against A.actinomycetemcomitans and P. gingivalis is 39.80 ± 1.24 mm and 32.28 ± 1.28mm, respectively. • For dimethylformamide mean zone of inhibition against A.*actinomycetemcomitans* and *P.* • *gingivalis* is 13.55 ± 1.92mm and 10.21 ± 2.16mm, respectively.	
2	Pranati Eswar; C.G Devaraj[9]	2016	• For 1% of the Ocimum sanctum, an inhibition zone of 09 mm was obtained. • For 2% of the Ocimum sanctum, an inhibition zone of 10 mm was obtained. • For 3% of the Ocimum sanctum, an inhibition zone of 10mm was obtained. • For 4% of the Ocimum sanctum, an inhibition zone of 12 mm was obtained. • For 5% of the Ocimum sanctum, an inhibition zone of 16 mm was obtained. • For 6% of the Ocimum sanctum, an inhibition zone of 22 mm was obtained. • For 7% of the Ocimum sanctum, an inhibition zone of 20 mm was obtained. • For 8% of the Ocimum sanctum, an inhibition zone of 20 mm was obtained. • For 9% of the Ocimum sanctum, an inhibition zone of 21 mm was obtained. • For 10% of the Ocimum sanctum, an inhibition zone of 21 mm was obtained.	In the light of 6% w / v of *Ocimum sanctum* (*Linn.*), A 22mm barrier was found. This was the most comprehensive blockade area seen in all ten different issues examined. Therefore, the minimum concentration of Minimum Inhibitory for cold ethanolic release of *Ocimum sanctum* (*Linn.*) Versus clini*nobacillus actinomycetemcomitans* isolated from 6% w / v., The good control area was 25mm, and no blocking area was detected near the wrong control. This indicates that tulsi has anti-bacterial properties and can be used to treat periodontal diseases.
3	Kush Kalra, Ramprasad Vasthare[10]	2019	• Zone of inhibition shown by Chlorhexidine against S. aureus S. mutans F. nucleatum P. intermedia • P. gingivalis are 25mm,21mm,36mm 36mm,33mm. • Zone of inhibition shown by Ocimum basilicum (Undiluted)against S. aureus S. mutans F. nucleatum P. intermedia • P. gingivalis are	Both of these oils have shown anti-bacterial anti-bacterial activity in all aspects of testing. The barrier area produced by Ocimum sanctum oil was high for Porphyromonas gingivalis (55 mm), followed by Prevotella intermedia (48 mm). Thus, the production area is much wider than

			13mm,13mm,28mm,40mm,20 mm. • Zone of inhibition shown by Ocimum sanctum (Undiluted)against • S. aureus S. mutans F. nucleatum P. intermedia P. gingivalis are 20mm,18mm,36mm,48mm,55 mm. • Zone of inhibition shown by Ocimum basilicum (1 in 10 dilutions) against S. aureus S. mutans F. nucleatum P. intermedia P. gingivalis are 15mm,15mm,24mm,22mm,19 mm. • Zone of inhibition shown by Ocimum sanctum (1 in 10 dilutions)against S. aureus S. mutans • F. nucleatum P. intermedia P. • gingivalis are 27mm,20mm,27mm,30mm,23 mm.	chlorhexidine. In the Fusobacterium nucleatum, the area was equal to the control. In aerobic bacteria, Ocimum sanctum showed similar activity to chlorhexidine, but the effect produced by Ocimum basilicum oil was under control. Conclusion: Essential oil of two types of Tulsi has shown excellent anti-bacterial activity against common anaerobic and aerobic oral products. This activity was highly specific in combating anaerobes and was found to be better than chlorhexidine. In addition, Ocimum sanctum oil produced a broader inhibitory area than Ocimum basilicum for all types of tests.
4	Sajjanshetty Mallikarjun; Ashwini Rao[11]	2016	• Aa-*A.actinomycetemcomitans* Pi -*P. intermedia* • Pg- *P. gingivalis* • For 0.5% of Tulsi, a mean zone of inhibition against Aa, Pi, and Pg are 15.50mm,12.25 mm,12 mm, respectively. • For 1% of Tulsi, a mean zone of inhibition against Aa, Pi, and Pg are 22.25mm,14.75 mm,16.75 mm, respectively. • For 2% of Tulsi, a mean zone of inhibition against Aa, Pi and Pg are 29mm,18.25 mm,16.25mm, respectively. • For 5% of Tulsi, a mean zone of inhibition against Aa, Pi, and Pg are 38.25mm,18.25 mm,18 mm, respectively. • For 10%of Tulsi, a mean zone of inhibition against Aa, Pi and Pg are 41mm,22.75 mm,21 mm, respectively. • For doxycycline, a mean zone of inhibition against Aa, Pi and Pg are 40.50mm,34.75mm,32.25mm, respectively. • For dimethylformamide, a mean zone of inhibition against Aa, Pi and Pg are 9.75mm,12.50 mm,16.50mm respectively.	In concentrations of 5% and 10%, the Tulsi extract showed anti-microbial activity against *A. actinomycetemcomitans*, similar to doxycycline with similar inhibitory properties ($P > 0.05$). *However, P. gingivalis* and P. *P. intermedia* showed resistance to Tulsi discharge, indicating very small areas of inhibition ($P < 0.05$). Tulsi shows anti-microbial properties against *A. actinomycetemcomitans* while showing resistance to *P. gingivalis* and *P. intermedia.*

5	Dr Raghavendra mShetty; Dr Anita ghoyal[12]	2015	• The zone of inhibition shown by 30% of Tulsi against S.mutans and E.faecalis is 16mm and 12mm, respectively. • Zone of inhibition shown by 15% of Tulsi against S.mutans and E.faecalis are 12mm and 10mm, respectively. • Zone of inhibition shown by 7.5% of Tulsi against S.mutans and E.faecalis are 10mm and 8mm, respectively. • The zone of inhibition shown by 3.75% of Tulsi against S.mutans and E.faecalis are 8mm and 4mmrespectively. • Zone of inhibition shown by 1.87% of Tulsi against S.mutans and E.faecalis is 0mm and 0mm • respectively.	Tulsi wasn't effective against S.mutans and E. faecalis at 1.87% concentration. Increasing the concentration further produced a bigger zone of inhibition. A maximumzone of inhibition of 16mm was achieved in Tulsi at the 30% concentration. This shows that tulsi has anti-microbial efficiency and maybe won't treat periodontal diseases.

Table 3: shows the outcome and result of the effectiveness of tulsi against different periodontal pathogens in the studies mentioned above. The outcome and results were positive in the above studies showing Tulsi as a potent adjunct for the treatment of Periodontitis.

DISCUSSION

This systematic review mainly focuses on the anti-bacterial properties of Tulsi against periodontal pathogen and their potential use in the treatment of periodontal diseases. In this study, a total of 182 articles were obtained. After careful assessment, five in-vitro studies were selected for further evaluation. Among the five articles, four yielded positive results, while in 1 article, tulsi showed resistance against P. gingivalis and P. intermedia.

1n 2018, Ipsita Jayanti[2] and colleagues conducted an in-vitro study on Antimicrobial Activity of Ocimum sanctum (Tulsi) Extract on Aggregatibacter actinomycetemcomitans and Porphyromonas gingivalis. Research into the extraction of tulsi from ethanol was prepared using a cold extraction method in the current in vitro study. Various concentrations (2, 4, 6, and 8%) were then obtained by dilution with dimethylformamide. 0.2% chlorhexidine acted as a positive control, while the negative control was dimethylformamide and was administered for 48 hours. Prevention sites were evaluated, each with A.actinomycetemcomitans and P. gingivalis. In-group comparisons and one-way variance analysis (ANOVA), and Tukey's post hoc analysis were used between study groups. It was concluded that 8% of the output of O. It is therefore recommended that this be useful as combining mechanical therapy with the prevention and treatment of periodontal diseases.

In 2016, Pranati Eswar et al[11] conducted an in vitro study on the Anti-microbial Activity of the Tulsi Ocimum Sanctum (Linn.) Which was prepared by a cold extraction method. The extract was purified with an inert solvent, dimethyl sulfoxide, to obtain ten different chemicals (1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%). The anti-microbial activity of Ocimum sanctum (Linn.) The compound was tested in agar well diffusion applied 24 hours against 0.2% chlorhexidine as a positive control and dimethyl sulfoxide as a negative control. In the light of 6% w / v of Ocimum sanctum (Linn.), A 22mm barrier was found. This was the largest defensive point identified in all ten issues examined. Therefore, the Minimum Inhibitory Concentration for the cold ethanolic release of Ocimum sanctum (Linn.) Against the clinically active Actinobacillus actinomycetemcomitans was 6% w / v. The area of good control was 25mm, and no blocking area was detected near the negative controls. So the tulsi acted against the periodontal pathogen to show inhibition, thus showing its usefulness in treating Periodontitis.

In 2019, Kush Kalra et al[12] conducted an in vitro study on Antibacterial Efficacy of Essential Oil of Two Different Varieties of Ocimum (Tulsi) on Oral Microbiota. The study was conducted using commercially available essential oil of two varieties of Tulsi, i.e. Ocimum sanctum and Ocimum basilicum. The anti-microbial susceptibility was determined by the agar well diffusion (punch well diffusion) method. Vancomycin 30µg discs were used as a procedural control for S. aureus and S. mutans. Metronidazole 5µg discs were used as the procedural control for anaerobic bacteria. After the desired incubation period was over(48 hours), the zone of inhibitions for the extracts was measured using Vernier callipers. Both of these oils have shown anti-bacterial anti-bacterial activity in all aspects of testing. The barrier area produced by Ocimum sanctum oil was high for Porphyromonas gingivalis (55 mm), followed by Prevotella intermedia (48 mm). The production area is much wider than chlorhexidine. In the Fusobacterium nucleatum, the area was equal to the control. In

aerobic bacteria, Ocimum sanctum showed similar activity to chlorhexidine, but the effect produced by Ocimum basilicum oil was under control. Essential oil of two types of Tulsi has shown excellent anti-bacterial activity against common anaerobic and aerobic products. This activity was highly specific in combating anaerobes and was found to be better than chlorhexidine. Ocimum sanctum oil produced a broader inhibitory area compared to Ocimum basilicum for all types of tests.

In 2016, Sajjanshetty Mallikarjun et al[13] conducted an in-vitro study based on the anti-microbial activity of Tulsi leaf (Ocimum sanctum) on pathoidal pathogens. The study was performed using the Ethanolic extract of Tulsi, which was prepared in a cold extraction method. The extract was diluted with an inert solvent, dimethylformamide, to obtain five compounds (0.5%, 1%, 2%, 5%, and 10%). The anti-microbial activity of Tulsi extract has been tested by a 48-hour agar dispersion method. Doxycycline was used as a positive control with dimethylformamide as a negative control. In concentrations of 5% and 10%, the Tulsi extract showed anti-microbial activity against A. actinomycetemcomitans, similar to doxycycline with similar inhibitory properties (P> 0.05). However, P. gingivalis and P. P. intermedia showed resistance to Tulsi discharge, indicating very small areas of inhibition (P <0.05). Thus, Tulsi exhibits anti-microbial properties against A. actinomycetemcomitans while showing resistance to P. gingivalis and P. intermedia.

In 2015, Dr Raghavendra M Shetty et al[14] conducted an in vitro study on the anti-microbial activity of guava and tulsi against Streptococcus mutans and E. Faecalis. Stock solution (30% the absorption of extracts with normal salt) was taken and re-mixed twice as much as each extraction was performed to obtain 15%, 7.5%, 3.75%, and 1.88%. A good distribution method has been used to locate the blocking area. In this way, circular sources that can include five different combinations of test agents (Tulsi extract) were cut into agar plates using a template. Four plates were prepared and labelled, with five different focal points of Tulsi release. The extracts are transferred to agar plates, placed aerobically at 37 ° C for 48 h. Block areas were measured using a vernier dial. Tulsi failed to fight S. Mutans and E. Faecalis at 1.87%. Increased concentration continued to produce a large area of prevention. A large 16mm block area was found in Tulsi with a focus of 30%. This clearly shows that tulsi has the anti-bacterial ability and can be used to treat periodontal diseases.

Multiple researchers also conducted multiple animal studies and some randomized control trials to prove the efficiency of tulsi in treating periodontal diseases. But as these articles do not come under the inclusion criteria and are subsequently not discussed in detail.

CONCLUSION

Tulsi extract shows anti-microbial properties, and periodontal application of tulsi can be as a mouthwash, dentifrice, gel and intracanal irrigant. Its role in inhibiting and controlling the growth of various periodontal pathogens such as A.actinomycetemcomitans, P. gingivalis, E.faecalis, F. nucleatum and P. intermedia has been proved by various in-vitro studies. Furthermore, Tulsi has minimal side effects, is readily available on the market at affordable prices, and can help as a combination therapeutic agent in preventing and treating periodontal diseases.

CONFLICT OF INTERES

No conflict of Interest.

SOURCE OF FUNDING

None

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