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COMPARISON OF ANTIMICROBIAL ACTIVITY OF FOUR HERBAL EXTRACTS AGAINST *STREPTOCOCCUS MUTANS*, ISOLATED FROM DENTAL DISEASES IN VITRO

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ABSTRACT

The present study aims to evaluation the antimicrobial effects of four herbal extracts *in Streptococcus. mutants*. These plants including *Rhuscoriaria*, *Cinnamomum zelanicum*, *zingiber officinale*, *Camellia sinensis*. Detection of antimicrobial activities of four methanolic herbal plants performed using agar diffusion method. After 24h of incubation the diameters of halos indicative of lack of growth in each well. The zone diameters around each disc were compared with chlorhexidine which is used as positive control.

The study indicates methanolic concentration at (20%) have good potential activity on inhibition growth in *Streptococcus mutants* for *C. zelanicum*, *Rcoriaria*, *Z. officinale*, *C. sinensis.* (20.33, 17, 9.3, 8.2) respectively.

Key wards: *Streptococcus mutants*, Methanolic aqueous extract, *chlorhexidine, Rhuscoria- ria, Cinnamomum zelanicum, zingiber officinale, Camellia sinensis.*

INTRODUCTION

Dental plaque, caries and pyorrhea continue to be a major health problem worldwide, (Marsh, 2003, Petersen, 2005, Allaker, 2009). *Streptococcus mutants* is the most cariogenic pathogen (Loesche *et al*., 1986) produces glycosyltransferases (GTFs), exopolysaccharide synthesis, adherence to tooth surface and biofilm formation (Koo *et al*., 2002 Marsh, 2005, Nisaa, *et al.*, 2017)

Many side effects have been reported in chemical mouth rinses like Chlorhexidine such as undesirable tooth discoloration, unpleasant taste, dryness and burning sensation in the mouth (Jones, 2000, Mathur, 2011). Side effects observed such as oral mucosal ulceration, unilateral/bilateral parotid swelling and enhanced supra gingival calculus formation for long time using of chlorhexidine (Ernst *et al*., 1998, Parwani*et al*., 2013)

Since ancient times, spices and herbs have also been used in traditional treatment of some diseases. Now-a-days, several experimental studies and to a lesser extent, clinical trials have also emphasized the role of herbs in the treatment of a variety of disorders. *Rhuscoriaria* L. was provided renewable bio-products with the following reported desirable bioactivities: Anti-fibrogenic, antifungal, anti-inflammatory, anti-malarial, antimicrobial, anti-mutagenic, antioxidant, anti-thrombin, anti-tumorigenic, anti-viral, cytotoxic, hypoglycemic, and leukopenic. (Loesche, 1986, Chopra *et al.*, 1986, Grieve., 1984, Ahmad *et al*., 2017). For example, in plant herbs the tea has been demonstrated to inhibit tumor genesis in many animal models for different organ sites, including the lung, oral cavity, esophagus, stomach, small intestine, colon, skin, liver, pancreas, bladder, prostate, and mammary glands. Also shows effective inhibitory activity in the lung and skin (Yuan., 2011). While *Cinnamomum zeylanicum* exhibited various bio-regulatory activities such as antibacterial (Baratta *et al*., 1998, Bayoub, 2010), antifungal (Bhatia, 2012; Carmo *et al*., 2008, Jantan *et al*., 2008, Khan *et al*., 2009), Also *Cinnamomum zeylanicum* reduce incidence of various pathological conditions including reduction of Fasting Blood Glucose, increasing circulating insulin levels (Ranasinghe *et al*., 2012) reduced total cholesterol and triglycerides (Hassan *et al.*, 2012) anti-aging treatment (Tsuji-Naito, 2008). Moreover, it affords significant protection against Alzheimer's disease (Senhaji, 2007). *Zingiber officinale* (Ginger) is like an antibiotic, antibacterial, antifungal (Ali *et al*., 2007, Girirajuand Yunus *et al*., 2013) antioxidants, antimicrobial and other various medicinal values (Samir and Amrit 2003, Jalal and Nasroallah, 2014).

This study was conducted to show the antimicrobial effects of Methanolic- aqueous of four herbal plants against *Streptococcus mutants*.

MATERIALS AND METHODS

Microorganisms tested: Samples were collected from 50 samples, 20 males and 30 females between the age group of 18-50 years. Samples were collected from different private dental clinics. Samples collected by transported media (Amies transport medium). The sample was streaked on sterile Mitis – Salivarius Bacitracin agar **(**MSB Agar), the selective media for *Streptococcus mutants***.** The plates were incubated under anaerobic conditions at 37 C^0 for 24- 48 hours.

Samples collected for the isolation of bacteria from Supraging val (n=35), Dental plaque caries (n= 15).

Preparation of plant extract: For preparation of plants extracts for all the four herbal plants were

prepared by dissolving 10 g of powder for each medicinal plant separately in 100 ml of (20 methanol +80 water). The contents were kept in orbitary shaker for 48 h. Then the extract was filtered, and it is dried in hot air oven at 40°C. Then the extract was stored under refrigeration at 4°C.

Determination of antibacterial activity: The antibacterial susceptibility of prepared herbal extract against bacteria were evaluated by Agar well diffusion method. Loop full of freshly grown bacterial isolates were inoculated in nutrient broth incubated at 37°C for 24hr. The bacterial suspensions were diluted with normal saline. Adjust the turbidity and compare with standard tube (McFarland number 0.5) to yield a uniform suspension containing 1.5×10^8 CFU/ml and spread the suspension by cotton swab, streaking on Muller Hinton agar Media were cut into four wells (5 mm diameter) by cork borer and add 100µ of suspension (different concentrations) (the plates were performed **i**n triplicates). All plates of the tested organisms were then allowed to incubate at 37°C for overnight. Reading the result by the measuring the distance / inhibition zones around the wells.

Concentrations of 10%, 15% and 20% were used to study the antibacterial activity of Methanolic aqueous herbal extracts.

RESULTS

The four-herbal extract showed some antibacterial activity against the selected *Streptococcus mutants* according to different concentration were measured by inhibition growth.

The antibacterial activity of Methanolic *C. zelanicum* were measured for six isolates of *S. mutants* showed inhibition zone at three concentrations (20.33mm) at concentration 20% compared to inhibition zone (17.7, 19.3) at concentration 10%, 15% respectively (table 1). Followed by the Methanolic-aqueous of *R.coriaria* showed antibacterial activity with inhibition zone (17mm) at concentration 20 %, While other inhibition zone (12.3mm, 15.3 mm) at concentration 10 %, 15% respectively (Table 2). Indeed, to Methanolic-aqueous *Z. officinale* and Methanolic aqueous *C. Sinensi* revealed just inhibition zone (9.3mm, 8.2 mm) at 20 % compared to no antibacterial activity were shown (0, 0% mm) at concentration 10 %, 15 % respectively (Table 3, and Table 4).

Table 1: Antibacterial activity of Methanolic-aqueous *C. zelanicum*

Plant extract	Diameter of zones of inhibition (mm) Mean values		
	10%	15%	20%
C zelanicum	**17.7**	**19.3**	**20.33**

Table 2: Antibacterial activity of Methanolic-aqueous *R. coriaria*

Plant extract	Diameter of zones of inhibition (mm) Mean values		
	10%	15%	20%
R coriaria a	**12.3**	**15.3**	**17**

Table 3: Antibacterial activity of Methanolic-aqueous *Z. officinaler*

Plant extract	Diameter of zones of inhibition (mm) Mean values		
	10%	15%	20%
z officinale	**0%**	**0%**	**9.3**

Table 4: Antibacterial activity of Methanolic-aqueous *C. sinensis*.

Plant extract	Diameter of zones of inhibition (mm) Mean values		
	10%	15%	20%
C sinensis	**0%**	**0%**	**8.2**

All methanolic aqueous extracts demonstrated antibacterial activity against *S. mutants* in the agar–well diffusion method (Fig. 1)

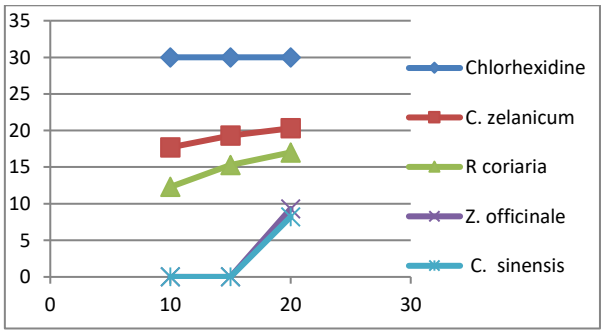

Fig. 1: Antibacterial activity of methanol aqueous of plants against *streptococcus mutants* (agar–well diffusion method) vertical column for concentration and horizontal column for inhibition diameter mean mm).

The inhibition zones that obtained by the four herbal extracts toward the bacterial isolates were measured as a determination of inhibitory effects of these plants (significance) table 5.

Table 5: Antibacterial activity of methanolic aqueous extract against Streptococcus mutants. Inhibition zone diameters in mm Mean winey significant difference in comparison with chlorhexidine.

Concentration %	Mean Inhibition zone (mm)				
	***C. zelanicu*m**	**R. coriaria**	***Z. officinale***	***C. sinensis***	**Chlorhexidine**
10	17.7 B, b	12.3 C, c	0.0 D, d	0.0 D, d	30.0 A, a
15	19.3 B, a	15.3 C, c	0.0 D, d	0.0 D, d	30.0 A, a
20	20.3 B, a	17.0 C, a	9.3 D, a	8.2 D, a	30.0 A, a

Capital letters for comparison among herbal extract within the same concentration; Small letters for comparison among different concentration for the same herbal extract; Similar letters = no significant difference; Different letters= significant difference (A, a being the highest value)

The results indicated there is significant differences between *C. zelanicu*m and Chlorhexidine at two cons. 15 % and 20 % and just in conc. 20% for *R. coriaria*. Where no significant difference in 10 %, and Chlorhexidine for *C. zelanicu*m, 10, 15 % for *R. coriaria.* Although these cons. gave the antibacterial activity. While the significant differences in cons. 20% and chlorahexidine for both *Z. officinale* and *C. sinensis.*

DISCUSSION

Some studies indicate the important role of activity several mouth washes against oral bacteria especially chlorhexidine (Ciancio, 2000, Mozaffari *et al*., 2005, Autio, 2008, Fard *et al*., 2011, Rathand Sing, 2013). But side effects on oral tissues like dryness, tooth discoloration, uncomfortable taste and burning sensation (Sorna *et al*., 2011). Recent studies suggested to investigate the bioactive compounds as alternative natural mouthwash extracted from herbs to prevention of oral diseases (dental caries and periodontal disease) (Taheri *et al*., 2010, Palombo, 2011, Taheri *et al*., 2011)

Antibacterial action of Methanolic aqueous extracts revealed different results may be due to differences in extract preparation methods

C. zelanicum have broad antimicrobial activities due to bioactive phytochemicals such as polyphenols and volatile phenols. Besides to flavors and aromas characteristics (Muchuweti *et al*., 2007, Seyed *et al*., 2015). Voukeng *et al*., 2011detected the presence the phenolic compounds in the methanol extract of *C. zelanicum* act as antibacterial against MDR Gram–negative bacteria. Similar study indicated that aqueous extract of *C. zelanicum* at low conc. (50 mg/ ml) have good antibacterial agents against oral bacteria (Ghada AL-Dubon*ietal*, 2013).

In recent study, Elahe *et al.,* (2016) suggested the effect of *C. zelanicum* as anti – biofilm formation by suppression genes which responsible for the initial adherence of *S. mutans* to tooth surfaces and formation of mature biofilm.

Secondly Sumac revealed antibacterial activity against *S.mutans:* Sumac extract was reported to be a source of natural antioxidants. A crude extract of *R. coriaria* exhibits interesting antioxidant properties, expressed by the capacity to either scavenge superoxide radical or uncompetitive inhibit xanthine oxidase (Candan and Sökmen, 2004). Sumac contains various substances, and exactly tannin and gallic acid may be responsible for the antioxidant ability of this plant (Hamid *et al*., 2014). OHara *et al*., (2004) carried out work on active components of ginger gingerols which one of characteristic of odor and flavor and antibacterial activity (Bisset, 1994, Marcello, 2001). Arash *et a*., (2015) showed that *Z. officinale* exhibit good antibacterial against *Streptococcus mutants* and *Streptococcus sanguinis*.

Shipra *et al.*, (2012) evaluated in the research to the phytochemical compounds which screened the presence of alkaloid, phlobotannins, flavanoids, glycolsides, saponins, tannin and terpenoids in methanolic extract which act as antibacterial against G-bacteria and G-bacteria. Purshotam and Pankaj (2011), study the active compound by phytochemical screening in methanol extract against Gram positive bacteria and Gram-negative bacteria. Several workers have revealed the antimicrobial action of black tea due to black tea contains high amounts of fluorides, 98 % of fluoride in the leaves (Cao *et al*., 2006, Malinowaska *et al*., 2008) which is regards the potent anti-caries agent (Ramsay *et al*., (1975), Suyama *et al*., (2011). Also, other Antibacterial action of black tea due to presence catechins (sub group of flavonids) (Kubo *et al*., 1992, Taylor *et al*., (2005), Groppo *et al*., (2007). Another study revealed the suppression level of amylase activity from *S. mutants* (Zang and Kashket, 1998, Hara and Honda, 1990). Finally, black tea protects from acid production from cariogenic bacteria due to bactericidal activity of EGCG (Song, 2007) which have influence effect on activity of lactate dehy drogenase (LDH) (Hirasawa *et al*., 2006).

Future studies to determine the effective components (aromatic or saturated organic compounds) which act as antimicrobial of these herbal extract.

Conclusion

The results exhibited Methanolic aqueous *C. zelanicum* exhibited highest inhibition zone especially with high concentration with three concentrations followed by *R. coriaria*than others herbal extracts. The study concludes that *C. zelanicum, R. coriari*a, *Z. officinale, C. sinensi* have anti-bacterial effects.

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