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In vitro Antibacterial activity of Plants extracts against *Porphyromonas gingivalis, Prevotella intermedia* and Aggregatibacter actinomycetemcomitans *Streptococcus mutanus*, Isolated from Periodontitis Patients in Babylon province, Iraq

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Abstract : Background: Human periodontitis has been related to a posh microbiota. Gram positive circular bacterium is associated with dentistry health, whereas periodontitis was related to Gram negative rods.
Theaim of his study was to detect the antimicrobioal activity of extract against tested bacteria.
Methods: Swabs were taken from dentistry pockets of twenty eight patients (periodontic Department, teaching clinics of oral and dental medicine in Hilla town, in 2016), porphyromonas gingivalis, Prevotella intermedia, Actinobacillus actinomycetemcomitans and Strep mutanus was known in step with the cultural properties, microscopic examination and organic chemistry tests. Antibacterial drug action was evaluated on this isolate by exploitation seven totally different extracts.
Results: Antimicrobial activity of propolis, alum and plant extracts at 50% concentration by well-diffusion technique was characterized by inhibition zones. At this concentration, the most inhibition zone diameters 35mm, forty mm were found in toothbrush tree and alum, severally, for propolis the inhibition zone was thirty mm, whereas tea and clove offer an equivalent inhibition zone twenty mm, the minimum inhibition zone ten mm was found in tea.
Conclusions: They showed a wide spectrum antibacterial drug activity of each extracts against black pigmented and S. mutanus and will be used for the treatment and prevention against dentistry diseases.
Key words : Porphyromonas gingivalis, Prevotella intermed*ia*, Actinobacillus actinomycetemcomitans and Streptococcus mutanus, plant extract, antibacterial activity.

Introduction:

Human periodontitis has been related to a fancy microbiota, the event of damaging periodontal disease appears to be the results of a particular infection. Gram positive round bacterium is associated with dental medicine health, whereas periodontitis was related to Gram negative rods. Human periodontal disease is initiated and perpetuated by a tiny low cluster of organism that colonize the subgingival region, in the main Gram negative, anaerobic or microaerophilic bacterium[1]. The black pigmented P. gingivalis, P. intermedia and Gram positive (S. mutanus), especially, possess virulence factors that measure relevant to the pathologic process of oral infections. It is shown to induce progression of periodontal disease in Laboratory animals[2-4]. Oral infections together with the varied clinical stages of dental medicine diseases are rumored among patients

of all age teams[5]. However, there's no study up to now that has established the prevalence or characteristics of dark pigmented Bacteroides isolated from healthy or from oro-dental infections and diseases in Hilla town.

The clinical use of antibiotics and alternative antimicrobial agents, as adjutants for the treatment of periodontal disease, has been extensively investigated within the past decade[6, 42]. Recently, special attention has been paid to natural extract, and propolis has been reported to possess sure medicative properties[7, 43]. Propolis may be a natural composite balsam, created by honey bees (Apis melifera) from the gum of varied plants. Bees collect vegetal exudates and kind pellets with their mandibles, compounding the exudates with wax and product of their secretion glands. The ensuing material is employed to strengthen the nest, give protection from microorganisms, and as an embalming substance to cover the body of a hive trespasser[8,44]. The medicative properties of propolis are wide investigated. Antimicrobial action of propolis has already been shown, varied studies report on medication, antifungic and antiparasitic actions [9,44].

Rosalen et al., (1998)[9] reported the propolis antimicrobial effect against oral organism, also as its action in inhibiting the assembly of polysaccharides, the appliance of propolis extract on rat molars reduced the severity of unhealthy lesions in these animals.

One of plants was *Salvadora persica* normally called Msiwak, that was used as a manduction stick. Prophet Mohammad (peace and prey upon him) is taken into account by muslims the primary dental professional within the oral hygiene. Since, he suggested them to use Siwak 5 times daily, as he aforesaid "if I had not found it exhausting for followers or the folks, i'd have ordered them to scrub their teeth with Miswak before every pray"[10]. Arak, a tree used for Miswak, is additionally called "tooth brush tree" and "mustard plant". Though the Miswak is sometimes obtained from the roots of the strong drink tree, some sticks ar made of its branches and bark[11]. The useful activities of Miswak in respect of oral hygiene and dental health are partially owing to its mechanical action and partially owing to pharmacologic actions. It was found that Salvadora persica was found that mustard tree root contain saponins beside tannins, silica, a little quantity of organic compound, trimethylamine and a reasonably great deal of organic compound constituents. Studies have indicated that mustard tree contain substances that possess plaque inhibiting and medicinal drug properties against many sorts of cariogenic bacterium that are of times found within the mouth. The expansion and acid production of that bacterium is therefore smothered[12, 45].

Natural merchandise is used for hundreds of years in treating human diseases and that they contain parts of therapeutic worth[46]. Natural merchandise is environmentally safer, simply accessible, and low cost [13]. Alum (Aluminum metal sulfate), the crystallized double sulphates with the formula $KAl(SO4)_2.12H2O$, are typically dour less, color less crystalline solids that flip white in air, that is employed as associate degree astringent and antisepsis in numerous food preparation processes[14]. The chemical material as alum has medicinal drug impact on Pseudomonas aeruginosa[15], within which most inhibition zone reached was thirty five mm once it used as an answer in eightieth concentration. Also, it's anti yeast impact that inhibits the expansion of Monilia albicans within which its impact on the budding method[16].

Green tea (Camellia sinensis) is wide consumed in China, Japan, Korea, and Morocco[17]. It's been thought of as a healthy liquid since precedent days. The standard Chinese medication has suggested this plant for headaches, body aches, general pain, digestion, depression, as an energizer, and normally to prolong life[18]. Tea additionally has several oral health benefits. it's thought of a healthful liquid owing to the biological activity of its polyphenols specifically catechins. Among the polyphenols, Epigallo Catechin three Gallate (EGCG) and Epicatechin three Gallate (ECG) square measure the foremost predominant catechins. The inhibitor, antimicrobial, anticollagenase, antimutagenic, and hemopreventive properties of those catechins tried to be useful within the treatment of chronic diseases like disease. Its psychological feature perform and positive impact on bone density, caries, disease, and polygenic disorder[19]. It's the second most consumed liquid within the world except for water, coffee, and effervescent soft drinks. Just about 76–78% of the tea made and consumed is black tea; 20–22% is tea. Tea is obtained by macerating and warmth drying this flush, whereas tea leaf comes by fermentation of flush before heat drying. Each kinds of tea share several pharmacologically active parts though at variable concentrations [20]. Clove (Syzygium aromaticum) is that the second most significant spice of the planet, as judged from the plane trade, being next solely to black pepper. In line with an ITC survey the overall world, the USA and India square measure the biggest importers of cloves. The dried clove bud contains free eugenol, eugenol acetate and caryophyllene. though these substances quantity to some ninety nine of the oil one in every of the most parts of essential oil obtained from the dried flower-buds of

Syzygium aromaticum is beta-caryophyllene, it's topical anaesthetic activity. The volatile oil of clove may be a colorless or light weight xanthous extract obtained from dried flower buds by steam distillation [21]. The volatile oils of clove of S. aromaticum were assessed for medicament activity against twenty five completely different genera of microorganism [22], that embodies animal and plant pathogens, illness and spoilage microorganism. A crude MeOH extract of clove (S. aromaticum) exhibited advantageous growth repressive activity against gram-negative anaerobic periodontic oral pathogens, as well as Porphyromonas gingivalis, Prevetolla intermedia, Aggregatibacter actinomycetemcomitans (Actinobacillus actinomycetemcomitans) and S. mutanus and it has been according that the volatile oil of Syzygium aromaticum conferred the biggest antimicrobial activity [23, 24].

The aim of this study was to detect the antimicrobial "invitro" action of extract for propolis, fruit tree, alum, inexperienced and tea leaf, cloves against P.gingivalis, P. intermedia, Aggregatibacter actinomycetemcomitans (Actinobacillus actinomycetemcomitans) and S. mutanus.

Methods:

Twenty eight Samples were taken from pockets of patients with periodontic; twenty male and eight female, the people from twenty to sixty six years recent (periodontic Department, teaching clinics of oral and dental surgery), thereafter, cultivated on blood agar plates now incubated aerobically and anaerobically (within the anaerobic jar) at 37^{o}C for twenty four to seventy two hours and 10% CO_2. Thensubjected to identification per the cultural properties such as black pigmented colonies, microscopic examination such as capsule and organic chemistry tests enzyme, biochemical tests catalase, Indole, antibiotic (vancomycin) sensitivity(30µg) [25].

Preparation of extracts:

Propolis extract:

Propolis samples were collected from of Al- Museiab hives , Iraqi throughout spring and summer seasons of 2015. Propolis samples were cleansed, freed from wax, paint, wood, dig little items, and placed in clean instrumentation. fifty gram of propolis were mixed with a hundred cubic centimeter of double D.W. in dark brown instrumentation and left for seven to fourteen days at temperature in dark place. for two weeks, the instrumentation was shacked two or three times per day and came back to heat dark place. The liquid was filtered through Whatman No.1 filter paper and the water was evaporated by oven at 45°C, then the extract was weighed and hold on in dark clean instrumentation for any victimisation. Water or binary compound extract was dissolved by distillated water, sterilized by filtration (using Millipore 0.45 filter paper), and therefore the requisite dilutions were ready.

Alum extract:

Fifty gram of alum material was purchased from the native market at Hilla town, Iraq 2016, and was known within the faculty of drugs, Department of Chemistry, and urban center University. Crystals of alum $KAl(SO_4)^2$•$12(H_2O)$.Dissolved fifty gramme by 100ml H_2O fully in hot (distilled) water at ninety two ºC, to get a final concentration of fifty % at pH scale 3.6.

Plants extract: (Kalpm Eucalyptus camaldulensis), Licorice (Glacyrrhiza glabra)

Dried plants inexperienced and tea leaf, cloves and toothbrush tree (Miswak) employed in this study were obtained from the native market at Hilla town, Iraq, 2016. H_2O was boiled, dried plants were added to the water and left to cool. Later on, these contents were mixed by the liquidizer and filtered to get rid of the big, international organisation homogenized particles to urge clear binary compound extracts. The extracts were kept at 4°C till to be use[47].

***In vitro* Antimicrobial activity testing using Agar well diffusion assayNCCLS,** 2002[26].

The agar well diffusion methodology was used for the determination of antibacterial drug activity of propolis, toothbrush tree (Miswak), (alum) (Eucalyptus camaldulensis), Licorice (Glacyrrhiza glabra), green, black tea leaf, and cloves extracts by using microorganism isolates taken from periodontic pockets to evaluted its effects on the isolated microorganism. Loopfull growth from microorganism isolate was inoculated into liquid media incubated at 37 °C for eighteen hours. The microorganism suspensions were diluted with normal

saline. Adjust the turbidity and compare with standered tube (McFarland 0.5) to yield a regular suspension containing 1.5×10^8 CFU / cubic centimeter. Muller- Hinton agar was inoculated with 0.1ml of microorganism matter .Using cork borer, wells were created on the culture media. The extracts were thought of because the fiffty percent concentration. Then, 0.1ml of extracts were added to wells, they were incubated at 37˚C for twenty-four hrs. The activities of extracts were determined by measure the diameter of inhibition zone in millimetre [27].

Antimicrobial susceptibility testing:

Susceptibility to antibacterial agents for all isolates was determined by the standard disk diffusion method on Muller-Hinton agar incubated for 18 hours at 37 ºC. The selection of antibiotic discs (ciprofloxacin) was performed according to the guidelines recommended by the Clinical and Laboratory Standards Institute [26]. After incubation, the diameter of each inhibition zone was measured with a pair of calipers, and recorded in mm. The results then interpreted according to CLSI documentation [28].

Result and disccusion:

The distribution of patients with peritonitis according to the Gender and age group were studied, the result showed that the incidence of age groups was highly frequency in (41-50) and (51-60) years old, while low frequency in age group (61-70).Male more susceptible to peritonitis than female with (71.5%)**(Table1)**, due to risk factors in these age groups such as; low hygiene, smoking, diabetes mellitus and immuncompromized patients.

Table (1) Distribution of patients with peritonitis according to the Gender and age group

%	NO.	Gender		Age group (year)
		Female	Male	
14.28%	4	2	2	21-30
17. 85%	5	2	3	31-40
28.57%	8	2	6	41-50
28.57%	8	2	6	51-60
10.7%	3	0	3	61-70
100%	28	8 (28.%)	20 (71.5%)	Total

Bacterial isolates

According to Forbes et al., (2007),the bacterial isolated identification per the cultural properties such as black pigmented colonies. microscopic examination such as capsule and, biochemical tests catalase, Indole,vancomycin sensitivity (30µg) **(Table2)** .

(Table2): **The character bacterial isolated**

vancomycin sensitivity	urease	lipase	Catalase test	Indole test	Appearance on blood agar	microscopic morphology	Bacteria
sensitivity	-	-	-	+	Characteristic after 72hr black pigmented colonies, convex, 1-2 µm in diameter (more mucoid)	Gram negative coccobacilli surrounded with a capsule or hallow	*Porphyromons gingivalis*
resistance	-	+	-	+	Characteristic after 72hr black pigmented colonies, convex, 1-2 µm in diameter	Gram negative coccobacilli surrounded with a capsule or hallow	*Prevotella intermedia*
resistance	-	-	+	-	Characteristic after 48hr Pinpoint,rough, stiky Adherent colony surrounedb by greenish tinge	Gram negative very short bacilli	http://en.wikipedia.org/wiki/Aggregatibacter_actinomycetemcomitans *Actinobacillus actinomycetem c-omitans*
resistance	-	-	-	-	Characteristic after 24hr white colony with alpha hemolytic	Gram positive diplococci	*Streptococcus mutanus*

Regarding, (Table 3 and 4) the distribution of bacterial isolates from patients with peritonitis according to the mixed and single isolates. The result showed that *Porphyromons gingivalis* was 3 in single isolates, while it was 4 in mixed isolates with *Prevotella intermedia* and *Streptococcus mutanus* for causes peritonitis.

Prevotella intermedia was 3 in single isolates, while it was 4 in mixed isolates with *Porphyromons gingivalis* and *Streptococcus mutanus* for causes peritonitis.

Streptococcus mutanus was 4 in single isolates, while it was 7 in mixed isolates with *Porphyromons gingivalis, Actinobacillus actinomycetemcomitans* and *Prevotella intermedia* for causes peritonitis.

Actinobacillus actinomycetemcomitans was 7 in mixed isolates only with *Porphyromons gingivalis, Streptococcus mutanus* and *Prevotella intermedia* for causes peritonitis.

Porphyromons gingivalis was predominant Gram negative bacteria causes' peritonitis, whereas *Streptococcus mutanus* was predominant Gram positive bacteria causes' peritonitis.

Bacteria have many virulence factors for causes peritonitis and dental plaque, the synergistic effect of virulence factors of mixed bacterial infecting for causes peritonitis.

Table (3) Distribution of bacterial isolates from patients with peritonitis according to the isolates

%	Total isolates	Mixed isolates	Single isolates	Bacteria
25%	7	4	3	*Porphyromons gingivalis*
25%	7	4	3	*Prevotella intermedia*
12%	3	3	0	http://en.wikipedia.org/wiki/Aggregatibacter_actinomycetemcomitans *Actinobacillus actinomycetemcomitans*
38%	11	7	4	*Streptococcus mutanus*
100%	28	18	10	Total

Table (4) Types of bacteria isolated from mixed growth

NO.	Mixed growth of Bacterial isolates
2	*Porphyromonas gingivalis+ Streptococcus mutanus*
4	*Porphyromonas gingivalis+Prevotella intermedia*
2	*Streptococcus mutanus+ Prevotella intermedia*
3	*Streptococcus mutanus+Actinobacillus actinomycetemcomitans*

Determination of antimicrobial activity:

The agar well diffusion assay is most typically accustomed confirm antimicrobial condition. During this study used extracts by agar well diffusion assay. In the present study at fifteen % concentration of the plants extracts was tested for their restrictive activity on gram-negative black pigmented P.gingivalis, P. intermedia, A. actinomycetemcomitans, and gram-positive S. mutanus isolates. All isolates were inhibited by extracts at fifteen %concentration. The most inhibition zone was ascertained in alum and Miswak binary compound extracts severally (40 millimetre, 35 millimetre), and the minimum was in black tea (10mm) (Figure 1, 2, 3, 4).

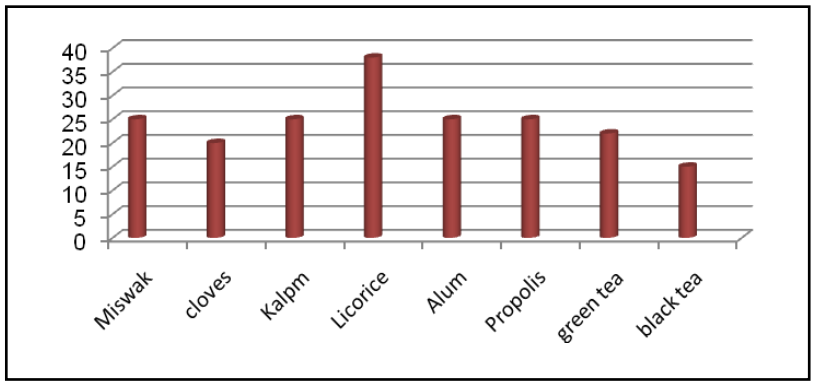

Figure (1) Antimicrobial activity of different aqueous extracts by agar well method for *Porphyromonas gingivalis*.

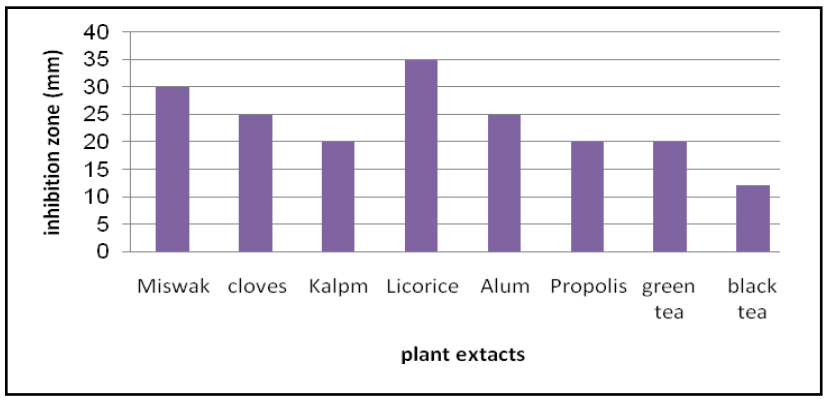

Figure (2) Antimicrobial activity of different aqueous extracts by agar well method for *Prevotella intermedia*

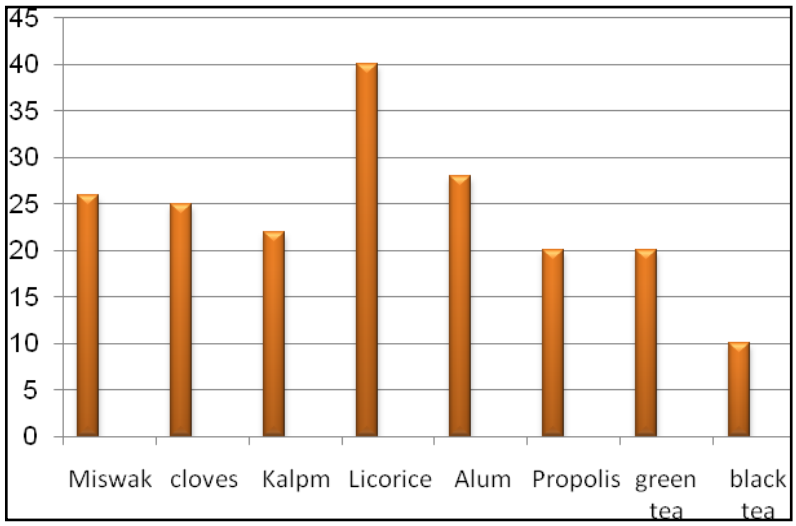

Figure (3) Antimicrobial activity of different aqueous extracts by agar well method for *Aggregatibacter actinomycetemcomitans.*

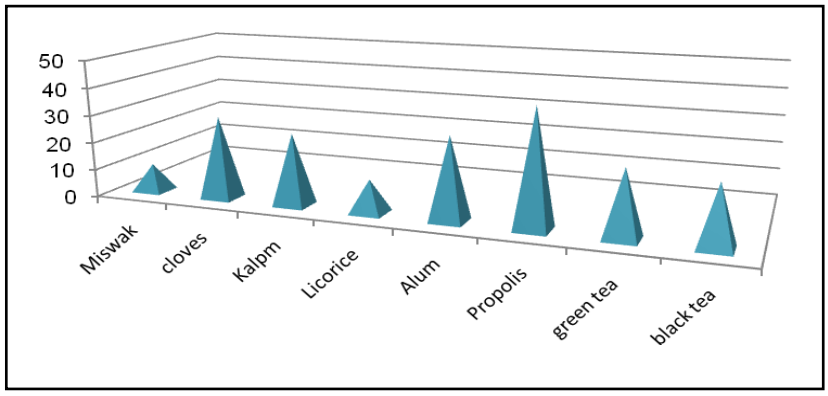

Figure (4) Antimicrobial activity at of different aqueous extracts by agar well method for *Streptococcus mutanus*

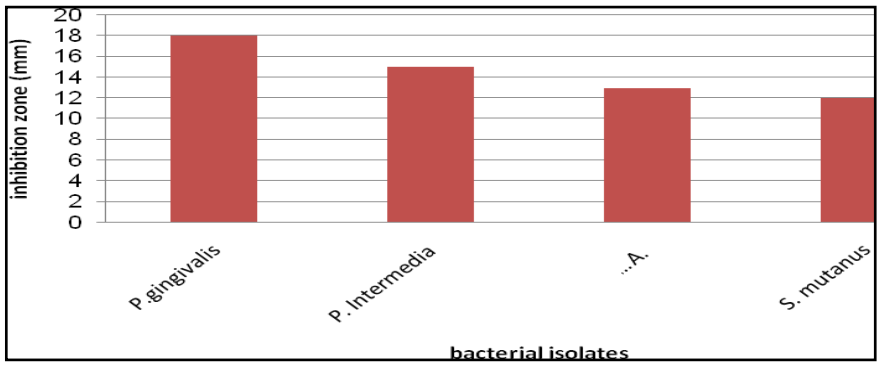

Figure (5) Antimicrobial activity ciprofloxacin by agar disc method against bacterial isolates

Moreover, In this study used ciprofloxacin to comparsion with aqueous extracts by disc well diffusion assay against gram-negative black pigmented *P.gingivalis,P. intermedia, A. actinomycetemcomitans*, and gram-positive *S. mutanus* isolates. These bacterial isolates were sensetive to ciprofloxacin but less than aqueous extracts (Figure 5).

Mohammed, (2004) [29]found that different concentration of alum provides elevated inhibition zone against P. aeruginosa, Therefore, alum is employed in treatment of ulcers wound and burns. The antibacterial drug actions of fifty concentration of Salvadora persica (Miswak) extract on bacterial isolated from disease pockets discovered that Miswak is effective in oral medical aid and provides high inhibition zone (35mm). Burt

and Reinders (2003)[30] tested the antibacterial drug activity of Salvadora persica against some oral aerobic and anaerobic microorganism and reportable that the extract of those sticks had a forceful result on the expansion of Staph. aureus, and a variable result on different microorganism species. They commented that the chew sticks they used were harvested one month earlier, and steered that exploitation a lot of contemporary sticks can offer higher result, [31] tested contemporary. One- month-old Miswak extracts for antibacterial drug activity and located no distinction. A comparison of alcohol and aqueous extract of Miswak was made. it was found that alcoholic extract is simpler than extract for antibacterial drug activity[32].

In the present study has shown propolis antimicrobial activity against periodontic infectious agent, P.gingivalis with inhibition zone thirty mm. Antimicrobial activities demonstrated during this study, confirming previous results [33-35].The verification of the antimicrobial action of the propolis extract isn't shocking. The first perform of propolis within the hive is to act as a biocide, being active against invasive microorganism, fungi and even invading larvae. There are a unit variety of studies documenting the biocidal functions of propolis.

Marcucci (1995) [35] reportable that Kaempferol had antimicrobial activity against S. mutans and *Actinomyces viscosus* and incontestable that identical constituent inhibit the growth of P. intermedia and P. gingivalis.

In the present study both cloves and green tea aqueous extracts exhibit antibacterial drug activity with inhibition zone twenty millimeter for every. Extract of clove (S. aromaticum) exhibited discriminatory growth repressive activity against gram-negative anaerobic periodontic oral pathogens, as well as Porphyromonas gingivalis and Prevetolla intermedia [36]. The volatile oil is active against oral microorganism related to cavity and periodontitis and effective against an outsized range of different microorganism[37]. Cuman et al., (2009) [38]has been reportable antimicrobial, antifungal properties, the oil of S. aromaticum shows anti- inflammatory, cytotoxic and anesthetic activities and discovered the medicinal drug and antinociceptive activities of eugenol oil in experimental animal models. Chaieb et at., (2007)[36] have reportable the repressive impact of clove extract against staph aureus microorganism. Antibacterial drug compound of volatile oil is effective against food borne Gram positive microorganism (*Staphyococcus aureus, B.cereus, Enterococcus faecalis, listeria monocytogenes and gram-negative bacteria (Escherichia coli, Yersinia enterocolitica, Salmonella choleraesuis and genus Pseudomonas aeruginosa*[37].Babu et al., (2011)[39] found that a four-week regime of mouth washing with a dilute catechins answer reduced the mouth odor related to periodontitis. Tea catechins deodorizes methyle mercaptain, the most reason for mouth odor, reserved growth and adhesion of P. gingivalis to buccal animal tissue cells. tea catechins were reportable to be effective in preventing animal tissue and periodontic inflammation. Catechins inhibit IL- seventeen evoked production of human animal tissue fibroblasts [39]. Inhibition of extracellular signal regulated enzyme (ERK) decrease IL -17 evoked production of human animal tissue fibroblasts. Hosokawa et al., (2009)[40] demonstrated germicidal activity of tea catechins against prevotella and P. gingivalis at concentration of one mg/ml. They found vital reduction in markers of periodontal disease when the utilization of slow unharness buccal delivery system applied over a amount of eight weeks. More modern studies have shown that some virulence factors (toxic metabolites, supermolecule amino acid enzyme,& gingipains) & aetiological agents of periodontitis neutral by EGCG [41].

Conclusion:

The alum, *Salvadora persica* (Miswak), propolis, cloveand green tea have antibacterial actions against black pigmented *P.gingivalis,P. intermedia, Aggregatibacter actinomycetemcomitans(A. actinomycetemcomitans*) *and S. mutanus* (from periodontal pockets) and may be used for the treatment and prophylaxis against periodontal diseases.

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