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Effect of water extract on leaves of the plant in microorganisms causing gum inflammation and teeth and diagnosed PCR in the holy province of Karbala

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Abstract:

Study was carried out to investigate the bacteria that cause gum and tooth infections. The samples were collected from infected teeth and gums after the diagnosis of PCR scans for the specialists of the general dental health center in Karbala governorate for the period from February 2015 to December 2016. 120 samples were examined. The tests were conducted on the PCR examination and examined the samples of the teeth used a direct method of wiping and isolate the bacteria causing inflammation of the gums and teeth by the circles of plantation. The study revealed the presence of bacteria isolated from the gum and teeth, as follows: The types of bacteria and parasites, which were found include: Streptococcus pneumoniae (pSpnP1_p2 replication protein) ,Staphylococcus aureas (ORF28 ~ similar to putative transposase. The study showed that the extract of the leaves of Gujarat tea and leaves leaves affected the bacteria causing inflammation of the gums and teeth and showed a concentration of 1000 mg of the mixture the highest effect in the isolated bacteria (4.6.4.8) followed by the water extract of the leaves and the extract of tea leaves as less effective agglutin in isolated bacteria and causative For inflammation of gums and teeth . The results showed that there were significant differences (below 0.01 and 0.05) in the diameter of the inhibitory region of the isolated bacteria that caused gum and tooth inflammation with different concentrations of plant water extracts

Key word:- leaves of plant Hibiscu, plant Nerium oleander, inflammation and teeth

INTRODUCTION

Roselle (*Hibiscus sabdariffa*) is a species of *Hibiscus* probably native to West Africa,[1] used for the production of bast fibre and as an infusion, in which it may be known as carcade. It is an annual or perennial herb or woody-based subshrub, growing to 2–2.5 m (7–8 ft) tall. The leaves are deeply three- to five-lobed, 8–15 cm (3–6 in) long, arranged alternately on the stems.

The flowers are 8–10 cm (3–4 in) in diameter, white to pale yellow with a dark red spot at the base of each petal, and have a stout fleshy calyx at the base, 1–2 cm (0.39–0.79 in) wide, enlarging to 3–3.5 cm (1.2–1.4 in), fleshy and bright red as the fruit matures. They take about six months to mature. The *Hibiscus* leaves are a good source of polyphenolic compounds. The major identified compounds include neochlorogenic acid, chlorogenic acid, cryptochlorogenic acid, caffeoylshikimic acid and flavonoid compounds such as quercetin, kaempferol and their derivatives. The flowers are rich in anthocyanins, as well as protocatechuic acid. The dried calyces contain the flavonoids gossypetin, hibiscetine and sabdaretine. The major pigment, formerly reported as hibiscin, has been identified as daphniphylline. Small amounts of myrtillin (delphinidin 3-monoglucoside), chrysanthenin (cyanidin 3-monoglucoside), and delphinidin are present. Roselle seeds are a good source of lipid-soluble antioxidants, particularly gamma-tocopherol.[,2,3]

Nerium oleander is a shrub or small tree in the dogbane family Apocynaceae, toxic in all its parts. It is the only species currently classified in the genus *Nerium*. It is most commonly known as nerium or oleander, from its superficial resemblance to the unrelated olive *Olea*. It is so widely cultivated that no precise region of origin has been identified, though southwest Asia has been suggested. The ancient city of Volubilis in Morocco may have taken its name from the Berber name *oualilt* for the flower. Oleander is one of the most poisonous commonly grown garden plants(4)

Some invertebrates are known to be unaffected by oleander toxins, and feed on the plants. Caterpillars of the polka-dot wasp moth (*Syntomeida epilais*) feed specifically on oleanders and survive by eating only the pulp surrounding the leaf-veins, avoiding the fibers. Larvae of the common crow butterfly (*Euploea core*) also feed on oleanders, and they retain or modify toxins, making them unpalatable to potential predators such as birds, but not to other invertebrates such as spiders and wasps

The flowers require insect visits to set seed, and seem to be pollinated through a deception mechanism. The showy corolla acts as a potent advertisement to attract pollinators from a distance, but the flowers are nectarless and offer no reward to their visitors. They therefore receive very few visits, as typical of many rewardless flower species. Fears of honey contamination with toxic oleander nectar are therefore unsubstantiated.(5)

Several studies have been conducted on the effect of microscopic microbiology on teeth and gums. The study of the researchers (6) showed that Streptococcus sp has been responsible for inflammation and tooth decay. Some studies have also found that cranberry and saccharin use helps the porcupine stick to age and gingival wall.

Some studies have shown that Perilla fructescent has an effect on Streptococcus (7).(8) showed the biological effect of some plant extracts in the growth of EPEC isolated from diarrheal cases in infants under the second year of age, 197 isolates were obtained from 243 faeces samples. The results showed that the water extract of tea (tannin)), Onion (volatile oils), rhizome rhubarb, leaves have a significant effect "inhibition of all isolated serotypes.

MATERIALS & METHODS:

Collection and examination of samples of inflamed teeth and gingivitis were collected at the General Specialized Dental Center in the holy city of Karbala in 2015 until December 2016. 120 samples were examined and the questionnaire was taken as to whether the infected person had an antibiotic or not, and included the microscopic examination and microscopy 120 extracted teeth were collected from patients who have been admitted to the local specialized central dental clinic in Kerbala/MOH during the period from 2015 to 2016. The collected samples were examined using compound microscope under X40 magnification.

Samples of biofilm were cultured in peptone water for 24 h temperature at 37 C after that culture in selected medium blood agar and MCchonckey agar Incubations were at 37 C in a humidified atmosphere supplemented with 5% CO2. Trypticase soy broth with 20% glycerol (BBL) was used for the storage of bacteria at 28C. Bacterial suspensions of freshly cultured bacertia were washed twice with 40 ml of sterile saline (0.9% NaCl) and recovered by centrifugation at 4,300 3 g for 10 min in an SS-34 rotor with a Sorvall RC centrifuge (duPont, Wilmington, Del.). The density of the bacteria was adjusted with saline to an

A420 of 0.200 as measured with a spectrophotometer , A series of 10-fold dilutions was prepared from this bacterial suspension and subsequently used for either quick lysis of the bacteria PCR sample preparation. (i) Bacterial DNA. The bacterial DNA used in this study was purified from freshly cultured bacteria by standard phenol-chloroform extraction methods (ii) Quick lysis of bacteria Portions (100 ml) of the bacterial suspensions were harvested by centrifugation at 2,800 3 g for 10 min in a microcentrifuge (Microspin 24S; Sorvall). After the supernatant was carefully removed, the pellet was resuspended in 40 ml of quick lysis buffer A (100 mM KCl, 10 mM Tris [pH 8.3], 2.5 mM MgCl2)–10 ml of lysozyme (20 mg/ml; Gibco BRL, Grand Island, N.Y.). After incubation for 30 min at room temperature, 40 ml of quick lysis buffer B (10 mM Tris [pH 8.3], 2.5 mM MgCl2, 1% Nonidet P-40) and 10 ml of proteinase K (5 mg/ml; Gibco BRL) were added. The proteinase K digestion was performed at 608C for 1 h. To inactivate the proteinase K and denature the DNA, the samples were incubated in a boiling water bath for 10 min. Samples were used immediately for PCR or stored at 2208C until used(9) .

The Streptococcus pneumoniae (pSpnP1_p2 replication protein) isolate was confirmed using the sense primer 5-TATTGAGTTGGCAAGTCAGT -3 and antisense primer 5- TACGTTCCCAATTCCATATT-3 with876 Bp ,Staphylococcus aureas (ORF28 ~ similar to putative transposase) isolate was confirmed using the sense primer 5-ATGGTACAGGAATATGCTCC-3 and antisense primer 5-TAACTAGCTAGCATGCATGC-3 561BP

PCR and sequencing: Universal primers for gene with primers (forward and reverse) were used for PCR amplification. PCR conditions were: initial denaturation at 94°C for 5 min, denaturation at 94°C for 1 min, annealing at 52°C for 1 min, extension at 72°C for 1.5 min and final extension at 72°C for 10 min. PCR reaction mixtures (20 µL total) contained 2 µL of 10x PCR buffer, 2 µL of dNTP mix (2.5 mM each), 1 µL of each primer (10 pmoles), 50 ng of DNA template, 0.5 µL of i-TaqTM DNA polymerase 5U µL, iNtRON Biotechnology, Seongnam). The resulting PCR product was examined by electrophoresis on a 1.5% agarose gel in order to see DNA sequencing.(10)

RESULTS:-

A study was conducted to investigate the microorganisms that cause inflammation of the gums and the teeth and the diagnosis, as samples of inflamed teeth and gums were collected after the diagnosis of PCR scans for the reviewers of the General Specialized Dental Health Center in the holy governorate of Karbala for the period from February 2015 to December 2016, If the infected person had an antibiotic or not, either the examination was a visual examination and a PCR examination. When the teeth samples were examined, the direct swab method was used to isolate the bacteria causing inflammation of the gums and teeth by the The study revealed the presence of bacteria isolated from the gum and teeth, as follows:-

Streptococcus pneumoniae (pSpnP1_p2 replication protein) ,Staphylococcus aureas (ORF28 ~ similar to putative transposase

The study showed that the extract of the leaves of Gujarat tea and leaves leaves affected the bacteria causing inflammation of the gums and teeth and showed a concentration of 1000 mg of the mixture the highest effect in the isolated bacteria (4.6.4.8) followed by the water extract concentration of 500 mg and 250 mg, either extract the leaves of the amount of damping The concentration of the tea leaves was less effective in the isolated bacteria, which caused inflammation of the gums and teeth. The concentration of 1000 mg, 500 mg and 250 mg had an inhibition of 2.2.1.7.1 for Streptococcus pneumoniae and 2.4 , 2,1.5) for Staphylococcus aureas bacteria

The results showed that there were significant differences (below 0.01 and 0.05) in the diameter of the inhibitory region of the isolated bacteria that caused gum and tooth inflammation with different concentrations of plant water extracts

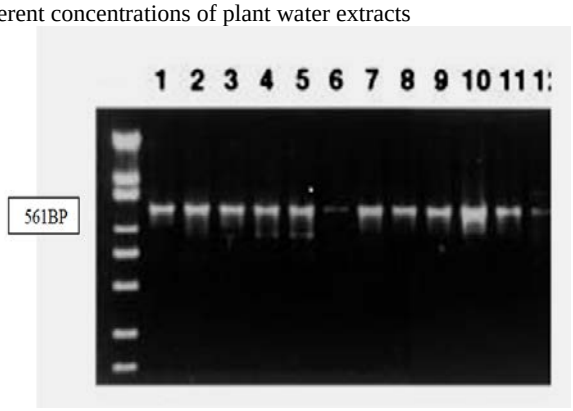

Figure (2) shows the package bacteria Staphylococcus aureas (ORF28 ~ similar to putative transposase)

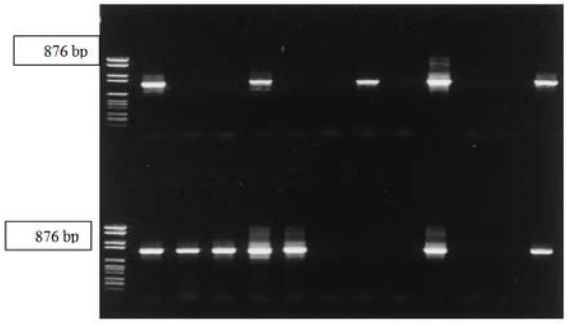

Figure (3) shows the package bacteria Streptococcus pneumoniae (pSpnP1_p2 replication

Table (1): - The effect of plant extracts on the diameter index indicates the inhibitory region in mm

Staphylococcus aureas (ORF28 ~ similar to putative transposase) isolate was confirmed using the sense primer 5- ATGGTACAGGAATATGCTCC-3 and antisense primer 5-TAACTAGCTAGCATGCATGC-3 561BP	Streptococcus pneumoniae (pSpnP1_p2 replication protein) isolate was confirmed using the sense primer 5-TATTGAGTTGGCAAGTCAGT -3 and antisense primer 5- TACGTTCCCAATTCCATATT-3 with876 Bp	Bacteria / Extract
1.5	1	Guarat leaves 250 mg
2	1.7	Guarat leaves 500 mg
2.4	2.2	Guarat leaves 1000 mg
1.4	2	Buffalo leaves 250 mg
1.9	2.5	Buffalo leaves 500 mg
2.8	3	Buffalo leaves 1000 mg
3	3.1	Guarat leaves with Buffalo leaves250 mg
3.6	3.8	Guarat leaves with Buffalo leaves500 mg
4.8	4.6	Guarat leaves with Buffalo leaves1000 mg
33.5**	27.71**	Calculated X^2
18.7	18.7	X^2 Scalability 0.01
16.32	16.32	X^2 Scalable 0.05

**significant

DISCUSSION:-

The study revealed the presence of bacteria isolated from the gum and teeth include:-

Streptococcus pneumoniae (pSpnP1_p2 replication protein) ,Staphylococcus aureas (ORF28 ~ similar to putative transposase

The main reason why Streptococcus pneumoniae can cause disease is because it has a capsule that surrounds it and protects it from your immune system. In addition to the capsule, it has an enzyme called IgA1 protease. You have antibodies called IgA that defend your respiratory system against foreign substances; IgA is in the mucous membrane that lines your respiratory system. Streptococcus pneumoniae uses the IgA1 protease to destroy your IgA antibodies, so it can live in the mucous membrane of your upper respiratory system. It also has a substance called pneumolysin O that damages your respiratory lining. It will then multiply.(11)

Streptococcus pneumoniae (pSpnP1_p2 replication protein) Gene was part of a package 876 base pair because A novel Streptococcus pneumoniae plasmid (pSpnP1; 5413bp) has been isolated from the multidrug-resistant clone Poland(23F)-16, and its complete nucleotide sequence has been determined. Sequence analysis predicted seven co-directional open reading frames and comparative analyses revealed that plasmid pSpnP1 is different to pDP1, the only previously described pneumococcal plasmid as well as Detection of single-stranded DNA by Southern blot analysis indicated that pSpnP1 replicates via a rolling circle mechanism. Interestingly, the product of orf1 has a putative Zonular occludens toxin conserved domain present in toxigenic strains of Vibrio cholerae. Real-time PCR assays revealed that this ORF was expressed. Hybridization experiments showed that the pSpnP1 replicon was unusual among other examined antibiotic-resistant pneumococcal clones. (12)

As Figure (2) between the bacteria Staphylococcus aureas (ORF28 ~ similar to putative transposase) Was in the package genetic base pair 561 resulte They include two virulence-related genes, the etb gene and a gene encoding a novel ADP-ribosyltransferase closely related to EDIN, which belongs to the C3 family of ADP-ribosyltransferases modifying Rho GTPases. They also include genes for a cell wall-anchoring surface protein and a phage resistance protein. Based on the determined sequence of pETB, the genome structures of etb-bearing plasmids (ETB plasmids) from various clinical isolates were analyzed by the PCR scanning method. The data indicate that, although the ETB plasmids are highly heterogeneous in genome size, the fundamental genome organization is well conserved. The size variation of the plasmid is mainly attributed to defined regions which may be hot spots for gene shuffling(13), This is attributed to the pathogenic role of these bacteria and their secretion of toxins, which affects the necrosis of the teeth Necrosis as well as the secretion of dental materials affecting the teeth and yellow color (7)

It was found in the study that the extract of the mixture (leaves of Gujarat tea with leaves of the powder) affected the bacteria isolated and caused by inflammation of the gums and teeth, and this is because these plants contain effective substances have a very harmful effect to revive the microscopic material (flavons)

The research showed that the tea leaves and the leaves of the plant are effective to the antibiotic, affecting the positive bacteria for the dye of chromium and the dyes of the chromium dye either the leaves of tea Gujarat has an effect because it contains oils and amino acids and tanning materials as well as the presence of Terpinene substance, which has an effective effect against bacteria The tea leaves of Gujarat contain:-neochlorogenic acid, chlorogenic acid, cryptochlorogenic acid, caffeoylshikimic acid and flavonoid compounds such as quercetin, kaempferol and their derivatives. The flowers are rich in anthocyanins, as well as protocatechuic acid. The dried calyces contain the flavonoids gossypetin, hibiscetine and sabdaretine. The major pigment, formerly reported as hibiscin, has been identified as daphniphylline. Small amounts of myrtillin (delphinidin 3-monoglucoside), chrysanthenin (cyanidin 3-monoglucoside), and delphinidin are present. Roselle seeds are a good source of lipid-soluble antioxidants, particularly gamma-tocopherol.[15]

Therefore, we note that the mixture (leaves of tea plant as leaflets and leaf leaves) play a role in the elimination of bacteria isolated in the study due to "the contents of the mixture of substances which have a wide range in the elimination of bacterial growth and also does not contain those extracts on the severe side effects that may come to Effects in the tissues of gums and teeth (16)

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