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

Sameer Hatem Abdulhaleem and Ali Hussein Makki Al Mamouri
University of Kerbala / College of Dentistry / IRAQ

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 aureus (ORF28 ~ similar to putative transposase. The study showed that the extract of the leaves of the plant 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 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 Hibiscus, plant Nerium oleander, inflammation and teeth

INTRODUCTION
Roselle (Hibiscus sabdariffa) is a species of Hibiscus probably native to West Africa,1,2 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 3–5 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.1,2,3

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 oualilît 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 fibre. Larvae of the common crow butterfly (Euptychia 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 frutescens 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 rhrub, 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 at temperature at 37 C after that culture in selected medium blood agar and MChbonekey 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 bacteria were washed twice with 40 ml of sterile saline (0.9% NaCl) and recovered by centrifugation at 4,300 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
PCR and sequencing:

1. 1.5% agarose gel in order to see DNA sequencing. (10)

The resulting PCR product was examined by electrophoresis on a DNA polymerase 5U μL, iNtRON Biotechnology, Seongnam). The proteinase K digestion was performed at 60°C 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 220°C until used(9).

The Streptococcus pneumoniae (pSpnP1_p2 replication protein) isolate was confirmed using the sense primer 5-TATTGAGTTGGCAAGTCA G-3 and antisense primer 5- TACGGTTCCCAATTCCATATT-3 with 876 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

**RESULTS:**

A study was conducted to investigate the microorganisms that cause inflammation of the gums and teeth; for this purpose, samples of inflamed teeth and gums were collected after 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 using 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 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.

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

<table>
<thead>
<tr>
<th>Extract</th>
<th>Guarat leaves 250 mg</th>
<th>Guarat leaves 500 mg</th>
<th>Buffalo leaves 250 mg</th>
<th>Buffalo leaves 500 mg</th>
<th>Buffalo leaves 1000 mg</th>
<th>Guarat leaves with Buffalo leaves 250 mg</th>
<th>Guarat leaves with Buffalo leaves 500 mg</th>
<th>Guarat leaves with Buffalo leaves 1000 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gujar (250 mg)</td>
<td>1.5</td>
<td>2</td>
<td>2.4</td>
<td>1.9</td>
<td>2.8</td>
<td>3</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Gujar (500 mg)</td>
<td>1.7</td>
<td>2.2</td>
<td>2.5</td>
<td>2.5</td>
<td>3</td>
<td>3.1</td>
<td>3.6</td>
<td>4.6</td>
</tr>
<tr>
<td>Gujar (1000 mg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Buffalo (250 mg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Buffalo (500 mg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Buffalo (1000 mg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table (2):** The calculated Chi-square values for the inhibition of bacteria

<table>
<thead>
<tr>
<th>Extract</th>
<th>Chi-square</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gujar (250 mg)</td>
<td>33.5**</td>
<td>0.0001</td>
</tr>
<tr>
<td>Gujar (500 mg)</td>
<td>27.1**</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

**significant**
**DISCUSSION:**

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

- Streptococcus pneumoniae (pSpnP1_p2 replication protein)
- Staphylococcus aureus (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 0 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 Polan(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 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 pSPnP1 replicer was unusual among other examined antibiotic-resistant pneumococcal clones. (12)

As Figure (2) between the bacteria Staphylococcus aureus (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 daphnillyline. Small amounts of myrtillin (delphinidin 3-monoglucoside), cyanidin (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)

**REFERENCE:**

1- Zhen, Jing, et al. (2016) "Phytochemistry, antioxidant capacity, total phenolic content and anti-inflammatory activity of Hibiscus sabdariffa leaves." Food chemistry 190, 673-680
5- Henary, H. A; Kurzrock, R; Falchuck, G; S; Naing, A; Moulder, S. L; Wheler, J. J; Tsimeridou, A; M; Durand; J; Yang, P; Johansen, M. J; Newman, R; Khan, R; Patel, U; Hong, D. S (1.1). "Final results of a first-in-human phase I trial of P01-05204, an inhibitor of AKT, FGFR-2, NF-Kb, and p70S6K in advanced cancer patients". Journal of Clinical Oncology. 29 (15 suppl.).
15- Zhen, Jing, et al.(2016) "Phytochemistry, antioxidant capacity, total phenolic content and anti-inflammatory activity of Hibiscus sabdariffa leaves." Food chemistry 190, 673-680