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Treatment of Some oral Injuries with Medical Extracts

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bstract:

Periodontitis is an infectious disease in periodontal tissue and is able to cause a plaque that contains bacteria on the teeth. These bacteria colonize the oral cavity, invade the periodontal tissue, and attack the body's host system. Iraq has many floras and one of them is called Water hyacinth. This flora is commonly known as waste because its ability to pollute the environment. However, water hyacinth has a useful material including alkaloids, flavonoids, and tannins that can be used as the antibacterial agents. The study aims to determine the effect water hyacinth leaf extract to limitation of dental diseases with aqueous plant extraction. in this study using two type of bacterial which caused periodontal diseases (Gram –ve *E.coil* and Gram +ve *S. auras* and prepared different concentrations from hyacinth plant extract The results has proven water hyacinth leaf extract evidenced better activity against the *E.coil* more than *S. auras* bacterial . The result adds to the value of the plant in pharmaceutical field as an antimicrobial agent. The global need for alternative prevention and treatment options and products for oral diseases that are safe, effective and economical comes from the rise in disease incidence (particularly in developing countries), increased resistance by pathogenic bacteria to currently used antibiotics and chemotherapeutics.

Keywords: oral injuries, medical extracts, dental caries, osteoporosis

ıtroduction

Oral diseases continue to be a major health problem worldwide. Dental caries and periodontal diseases are among the most important global oral health problems, although conditions such as oral and pharyngeal cancers and oral tissue lesions are also significant health concerns [1]. Despite general advances in the overall health status of the people living in industrialized countries, including oral and

dental health, the prevalence of dental caries in school aged children is up to 90% and the majority of adults are also affected [2]. Oral health is integral to general well-being and relates to the quality of life that extends beyond the functions of the craniofacial complex. There is considerable evidence linking poor oral health to chronic conditions, for example, there is a strong association between severe periodontal diseases and diabetes [3]. There is also evidence linking poor oral health and systemic diseases, such as cardiovascular diseases, rheumatoid arthritis and osteoporosis [4], while periodontal diseases and may also contribute to the risk of pregnancy complications, such as preterm low-birth weight [5]. Tooth loss, caused by poorperiodontal health (which affects up to 20% of the adult population worldwide) can lead to significant morbidity and premature death . The economic impact of oral diseases is an important consideration with up to 10% of public health expenditure in developed countries related to curative dental care . In most developing countries, expenditure in oral health care is low; access to dental healthcare is limited and is generally restricted to emergency dental care or pain relief . While there has been a marked improvement in oral health in most developed countries worldwide, populations of dentally disadvantaged individuals exist in these countries, often indigenous child populations and those people of low socio- economic status, where oral health is deteriorating.[6] The link between oral diseases and the activities of microbial species that form part of the microbiota of the oral cavity is well established [7]. Over 750 species of bacteria inhabit the oral cavity (~50% of which are yet to be identified) and a number of these are implicated in oral diseases . The development of dental caries involves acidogenic and aciduric Grampositive bacteria, primarily the mutans streptococci (Streptococcus mutans and S. sobrinus), lactobacilli and actinomycetes, which metabolize sucrose to organic acids (mainly lactic acid). that dissolve the calcium phosphate in teeth, causing decalcification and eventual decay. Dental caries is thus a supragingival condition [8]. In contrast, periodontal diseases are subgingival conditions that have been linked to anaerobic Gram-negative bacteria such as Porphyromonas gingivalis, Actinobacillus sp., Prevotella sp. and Fusobacterium sp [9]. In periodontal diseases, the areas at or below the gingival crevice become infected causing a cellular inflammatory response of the gingiva and surrounding connective tissue. These inflammatory responses can manifest as gingivitis (extremely common and seen as bleeding of the gingival or gum tissues) or periodontitis (the inflammatory response results in loss of collagen attachment of the tooth to the bone and in loss of bone). The global need for alternative prevention and treatment options and products for oral diseases that are safe, effective and economical comes from the rise in disease incidence (particularly in developing countries), increased resistance by pathogenic bacteria to currently used antibiotics and chemotherapeutics. Despite several agents being commercially available, these chemicals can alter oral microbiota and have undesirable side- effects such as vomiting, diarrhea and tooth staining [10, 11]. For example, bacterial resistance to most (if not all) of the antibiotics commonly used to treat oral infections (penicillins and cephalosporins, erythromycin, tetracycline and derivatives and metronidazole) has been documented [12]. Other antibacterial agents used in the prevention and treatment of oral diseases, including cetylpyridinium chloride, chlorhexidine, amine fluorides or products containing such agents, are reported to exhibit toxicity, cause staining of teeth or in the case of ethanol (commonly found in mouthwashes) have been linked to oral cancer [13,14]. Hence, the search for alternative products continues and natural phytochemicals isolated from plants used in traditional medicine are considered as good alternatives to synthetic chemicals [15]. Traditional Plant- Based Medicines Medicinal plants have been used as traditional treatments for numerous human diseases for thousands of years and in many parts of the world. In rural areas of the developing countries, they continue to be used as the primary source of medicine [16]. About 80% of the people in developing countries use traditional

medicines for their health care [17]. The natural products derived from medicinal plants have proven to be an abundant source of biologically active compounds, many of which have been the basis for the development of new lead chemicals for pharmaceuticals. With respect to diseases caused by microorganisms, the increasing resistance in many common Introduction

pathogens to currently used therapeutic agents, such as antibiotics and antiviral agents, has led to renewed interest in the discovery of novel anti-infective compounds. As there are approximately 500 000 plant species occurring worldwide, of which only 1% has been phytochemically investigated, there is great potential for discovering novel bioactive compounds. There have been numerous reports of the use of traditional plants and natural products for the treatment of oral diseases. Many plant-derived medicines used in traditional medicinal systems have been recorded in pharmacopeias as agentsused to treat infections and a number of these have been recently investigated for their efficacy against oral microbial pathogens. The general antimicrobial activities of medicinal plants and plant products, such as essential oils, have been reviewed previously [18, 19]. Therefore, the purpose of this review is to present some recent examples from the literature of studies that have served to validate the traditional use of medicinal plants with specific biological activity. In particular, traditional medicinal plant extracts or phytochemicals that have been shown to inhibit the growth of oral pathogens, reduce the development of dental plaque, influence the adhesion of bacteria to surfaces and reduce the symptoms of oral diseases. In addition, clinical studies that have investigated the safety and efficacy of such plant-derived medicines will be described. Plant Extracts and Phytochemicals with Activity against Oral Bacteria There have been numerous in vitro studies that have investigated the activity of natural plant substances against oral bacteria. These studies have focused on bacteria known to be involved in the etiology of oral and dental diseases. Early studies have clearly established that a number of substances had potential to be utilized in the dental industry, given their activity against cariogenic bacteria and those bacteria associated with periodontal disease . Substances that exhibited activity included spice and herb extracts, such as cinnamon bark oil, papua-mace extracts and clove bud oil and constituents of these extracts, such as cinnamic aldehyde and eugenol [22-28]. The solvent extracts of water hyacinth was tested against a bacteria Staphylococcus sp by disc diffusion method and a fungal stains Macor sp by streak plate method Acetone extract evidenced better activity against the microbex among other extracts, these result adds to the value of the plant in pharmaceutical field as an antimicrobial agent which prevention against different types of disease which prevention against different types of disease As such water hyacinth (Eickornia crassipes) is useful in bioenergy production (29-34) and wastewater treatment (35-39). In addition, it isused as vegetables in many countries due to its high carotene content and the presence of various secondary metabolites like terpenoids and alkaloids (Lalitha, Sripathi, & Jayanthi,2012 .(It also has the potential to absorb various harmful water pollutants like lead, mercury, and other carcinogenic chemicals. Their concentrations could be 10,000 times more than the surrounding water in E crassipest is a very fast-growing plant, which covers the pond very rapidly and affects the ecology of the pond. A study suggested that ponds with 10-15% E crassipes covering had a decreased number of phytoplanktons (due to nutritional competition) and zooplanktons (39-42) and hence a much lower fish production due to disturbed pond ecology Pathogens can enter the fish body. The aims of this study limitation of dental diseases with aqueous plant extraction. The study aims to determine the effect water hyacinth leaf extract to limitation of dental diseases with aqueous plant extraction. in this study using two type of bacterial which caused periodontal diseases **(Gram –ve *E.coil* and Gram +ve *S. auras*** and prepared different

concentrations from hyacinth plant extract The results has proven water hyacinth leaf extract evidenced better activity against the ***E.coil*** more than ***S. auras*** bacterial

2.Methodolgy

Culture media

Culture media used in this study are listed in table 2.

Table (2): Culture media used in the current study No.

lture media	mpany
trient agar	media (Indian)
ıeller-Hinton agar	
trient broth	
ıitol salt agar	
ɔod agar	

Preservation of bacterial isolates

The following methods were used for microbial preservation

Short term preservation :

Short time storage up to triple weeks was carrying out by culture on Nutrient agar slant using screw capped tubes. The tubes were incubated for 24h; then stored at 4°C for month (43-45).

Long term preservation

Preserved for long time (at least three month) was carried out by culturing on Nutrient broth containing 20% glycerol. Inoculated tubes by one pure colony then incubated at 37°C for 24 hrs and stored in deep freezing at -20C (43-45).

Preparation of Plants for Extraction

After collected the plant, we wash it well with distilled water to remove dust and dirt and the possibility of any other plant residues that may affect the result shown in figure (2) . Then we cut the parts of the plant and separate the leaves from the stem and then leave it to dry in the sun shown in figure (3), then we put it inside the oven at a temperature of 100-120 C for two hours to dry it shown in figure (4), then we grind the plant to get the powder of the leaves and powder of the stem so that we can prepare the extract using the powder

Figure 1 : hyacinth plant after wash

Extract preparation

We dilute the ethanol by adding 75 ml of distilled water to 175 ml of ethanol to obtain ethanol with a concentration of 70%. We weighted 5 g of dried leaves plant powder by using electric balance figure (5) ,

Then put it in a beaker, and then immerse it in 125 ml of diluted ethanol, has been covered the flask and place it on the stirrer Leave the powder to soak for 24 hours with continuous movement in the stirrer figure (1)

After obtaining the mixture, we filter it through filter paper to remove the remainingimpurities from the extract

Antimicrobial Susceptibility Test

Disc Diffusion Method

Kirby-Bauer diffusion method was followed as described by WHO (2003), the media prepare to carry out the antibiotics susceptibility test for two antibiotic; suspensions prepared by picked 2-3 colonies from microb and introduced into 4 ml of normal saline within test tube to produce a microbial suspension, turbidity compared with the standard turbidity solution (1.5x108 CFU/ml) by comparing the suspension with McFarland tube (0.5). By using a sterile cotton swab, suspension was transferred carefully and finally spread on Mueller- Hinton agar medium; then it was left for 5-10 min, after that antimicrobial disc was placed on the agar with a sterile forceps pressed firmly, incubated at 37ºC for 18-24h.,

developed Inhibition zones diameter around the discs were measured by the metric ruler (mm) according to Clinical and Laboratory Standards Institute (46).

Phytochemical screening

Benedect Reagent

It was used to detect the glycosides and was prepared by dissolving (137) sodium citrate and (100) ml of distilled water, and after filtering the mixture, a solution of copper sulfate (17.3 g) was added in 100 ml of distilled water, then completed the

volume to (1000) by distilled. The examination was conducted by taking (2) ml of the test reagent and adding (1) ml of the plant extract, then placing it in a boiling water bath for (5) minutes and after cooling it, as the appearance of a red scab indicated the positive test.

Ferric chloride

It was used to detect phenolic substances. Dissolve ferric chloride salt at a percentage of 1% in distilled water, and infer the result. A green or blue color is formed when sprayed on stains or mixed with solutions containing phenolic compounds.

Mayer′s Reagent

It was used to detect alkaloids, mercury chloride (1.36 g) and potassium iodide (5.00 g) in water (100.0 ml). Most alkaloids are precipitated from a neutral or slightly acidic solution by Mayer's reagent (potassium mercuric iodide solution) to give a cream-colored precipitation.

Terpene Steroid

The reagent was prepared by mixing (1) ml of plant extract with (2) ml of chloroform, and a drop of anhydrous acetic acid and a drop of concentrated sulfuric acid were added to it. For a while it is an indication of the presence of a steroid.

Saponin

An aqueous mixture of vegetable powder was prepared by taking (1) ml in a test tube and adding (5) ml of distilled water, and after shaking vigorously, it was observed the appearance of a thick foam that remained for a long time as evidence of the positive test.

Coumarin

By placing (5) ml of the alcoholic extract of the plant in a test tube and covered with a filter paper moistened with dilute sodium hydroxide solution, the tube was placed in a boiling water bath for (5) minutes, after which the filter paper was exposed to a source of ultraviolet light (Trans illuminated), indicating the appearance of Greenish- yellow color on the filter paper on the presence of coumarin

4.Results and Discussion

Phytochemical screening

The phytochemical screening tests carried out for the extract of water hyacinth pla shows that the plant possesses many phytochemicals which have therapeutic applicationThe results of the phytochemical screening tests for the extracts of water hyacinth a given in table)4-1). The ethanol extract tested positive for Glycosides, phenolics, protei the ethanol extract indicated the presence of metabolites such as flavonoids, ,protei alkaloids, phenol, steroid, tannin and saponins ,sterols, terpenoids and Coumarin [30].

Figure (11): The hyacinth plant extract after dried

Table (4-1): Shows the results of the phytochemical screening tests for the extracts ofwater hyacinth plant

Test	Procedure	Ethanol70%
Glycosides	**enedict test enedict reagent + waterbath 'e blue +ve green to red**	++
Alkaloids	**Mayer′s test yellowish precipitate**	±
Phenols	**erric chloride + bluish black**	++
Coumarin	**xtract + concentrated aOH +ve bright yellowishgreen**	+
Saponins	**eavy shake ve long lasting foam -ve clear solution**	++

Steroids+ Terpenoids	**Golden yellow Bright green color**	++
Flavonoid		++
Test	**Procedure**	**Ethanol70%**
Glycosides	**enedict test** **enedict reagent + waterbath** **e blue** **+ve green to red**	++

4-1-2 Antibacterial activity

The antibacterial activity of hyacinth plant extract and Antibiotic in vitro using 32 µg/ml for hyacinth plant extract and Antibiotic concentrations revealed the evolution inhibitory effect of hyacinth plant extract and Antibiotic against *S. auras and E.coli* bacteria by using the well diffusion method. As illustrated in figures ( )and(), Gram- positive bacteria are more susceptible to S. auras bacteria than Gram-negative bacteria. While the antibiotic/hyacinth plant extract activity when combined with **gentamicin and Imipenem** hyacinth plant extract were studied via the well diffusionmethod at a concentration of 32 µg/ml (antibiotic). The inhibitory zone was studied using 32 µg/ml for hyacinth plant extract andAntibiotic. The results showed the inhibition zone increased with the increasing of mixed hyacinth plant extract and Antibiotic. The maximum antibacterial effect of Antibiotic alone against *S.* auras was 9.15 mm, while the maximum antibacterial effect of hyacinth plant extract alone against *S. auras* was 17.34 mm. The synergistic effects of the inhibitory activities of hyacinth plant extract and Antibiotic against S.auras were 21.23 mm, in comparison to the control.(47)

Results antibacterial effect of Antibiotic alone against *E.coli* was 7.56 mm while the antibacterial effect of Results antibacterial effect of Antibiotic alone against *E.coli* was 7.56 mm while the antibacterial effect of Results antibacterial effect of Antibiotic alone against *E.coli* was 7.56 mm while the antibacterial effect of Results antibacterial effect of Antibiotic alone against *E.coli* was 7.56 mm while the antibacterial effect of hyacinth plant extract alone against *E.coli* was 9.10 mm. While the synergistic effect of of the inhibitory activities of hyacinth plant extract and Antibiotic and against *E.coli* were 11.23 mm, in comparison to the control. Show in figure (2).

Gram-positive bacteria's cell walls have a thin layer of peptidoglycan on top of teichoic acid and numerous pores that allow foreign particles to pass through, causing cell membrane damage and death. Gram-negative bacteria, on the other hand, have lipoproteins, LPS, and phospholipids, which form a penetration barrier thatonly permits big molecules to pass through, explaining why *S.aureas* is more sensitivethan *Ecoli*. Furthermore, Gram-positive bacteria have a larger negative charge on their cell walls than Gram-negative bacteria,. For bacteria resistance to the outside world,

cell walls and membranes are crucial defense barriers. The natural shape of the bacterium depends on the bacterial cell wall in particular. Adsorption pathways for sample, Gram-positive bacteria, and Gram negative bacteria are generated by cell

membrane components. The combination of nanoparticles with antibiotics is thoughtto be responsible for the observed synergistic activity against microorganisms(48,49)

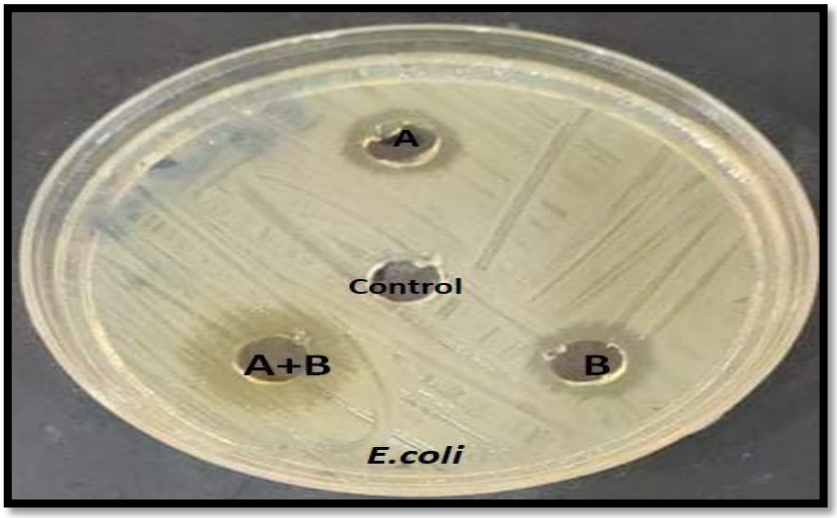

Figure (2). Synergistic effect of hyacinth plant extract and Antibiotic against *E.coli* .A (Antibiotic alone), B (hyacinth plant extract alone), A+B (hyacinth plant extract alone and Antibiotic) and Control.

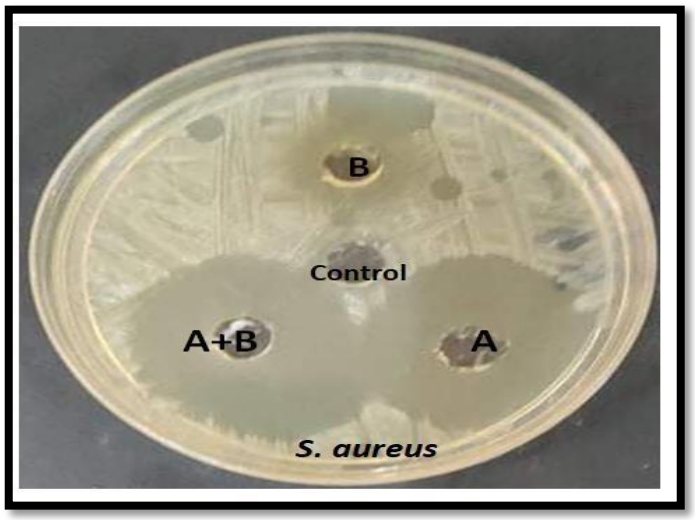

Figure (3). Synergistic effect of hyacinth plant extract and Antibiotic against*S. aureus*. A: (Antibiotic alone), B : (hyacinth plant extract alone), A+B :hyacinth plant extract and Antibioticand Control.

The result proved was water hyacinth leaf extract contains alkaloids, flavonoids, and tannins which are known to have antibacterial power that can inhibit bacterial growth[]. The alkaloids contained in the hyacinth leaf extract has antibacterial biological activity that can interfere with the constituent

components of the bacterial cell that make the cell wall not formed and easily get lysed [] Antibacterial flavonoids has a working mechanism that can interfere bacterial membrane synthesis, and inhibits bacterial metabolism – which can damage bacterial breeding pathways and damage bacterial membrane walls that will be followed by the bacterial death[]. This compound will also interfere with the energy metabolism in a similar manner to the respiratory system because the energy demand for active absorption of various metabolites and the biosynthesis of macromolecules insufficient so that lead to bacterial dead[]. Tannin known to has antibacterial activity related to their ability to form hydrogen bonds which results in protein denaturation in the membrane so that cells experience damage. Membrane damage causes the fulfillment of nutrients needed by bacteria cannot be achieved. This can be disrupted the bacterial metabolism and reduce the bacterial energy.

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