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Extraction, Characterization, and Evaluation the Activity of Chia Seed (*Salvia hispanica* L.) as an Antibacterial for the Treatment of Gingivitis

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Abstract

This research aims to study the effect of chia seeds extract (*Salvia hispanica L.*) extracted by the method (alcohol extract with soxhelt and oil extract with a screw) in the treatment of periodontitis (gingiva), where the biological activity was tested against the most important bacteria and fungi that cause gingivitis for both extracts and compared with the control. The results of the current study showed that there is a superiority of the oil extract with a screw device over the alcoholic extract in inhibiting the most important bacteria that cause gingivitis, namely (*S.mutans*), in addition to other types of bacteria (*S. epidermidis, E. coli, S. aureus*) and the fungus (*C. albicans*). Some chemical components (active groups) of the oil extract of chia seeds were detected, and it has been found that they contain many active groups, including tannins, resins, flavonoids... etc. the Toxicity tests of the oily extract was carried out by both methods and it was observed that it was free from it after experimenting with several concentrations (125, 250, 500, and 1000 mg/kg) of body weight on laboratory animals (mice), where The results of the study showed at concentrations (12.5, 25, 50, and 75%) positive efficacy of both types of oil extract against oral microorganisms through the zone of inhibition and the minimum inhibitory concentration higher of %75 (16mm) for bacteria (*S. epidermidis, E. coli, S. aureus*)of the oil extract with alcohol and 13 mm for the oil extract with a screw device for bacteria (*S. epidermidis*) while the highest inhibition was for the second method of extraction against the fungus (*C. albicans*) where it was 25mm and bacteria (*S.mutans*) (20mm), that was conducted in the laboratories of Ibn Al-Bitar Center/Industrial Research and Development Authority.

1. Introduction

Most oral diseases are caused by bacteria and fungi that live in the oral cavity, which cause tooth decay and periodontitis (gum), and it is one of the most common pathological conditions. Accordingly, most preventive and curative interventions aim to reduce the bacterial load in a way that oral health can be maintained. The purpose and proven effectiveness, but nevertheless, its use led to significant side effects, as uncommon diseases appeared resulting from the resistance of microbes to these antibiotics. Instead, it was proven that plant extracts are better

and safer alternatives, as they were supported by scientific evidence where many common natural products were used such as peel Lemon for its antibacterial properties [1].

Therefore, in recent decades, interest in medicinal plants has increased, as they constitute one of the main sources in the preparation of medicines, as plants have been and still are an important source of many compounds necessary for human health for thousands of years. A huge number of modern medicines have been isolated from natural sources that depend on many of them in traditional medicine. Because it has no side effects and with the increase in public health awareness around the world, the demand for functional foods with multiple health benefits has increased.

Chia (*Salvia hispanica* L.) is a semi-annual flowering plant native to Mexico and northern Guatemala. It belongs to the family Labiatae and the genus Salvia. It is 1 meter tall and has simple leaves and purple flowers and white, black or gray oval seeds. With a diameter of one millimeter, it is a water-loving edible oil seed that absorbs from (1 to 12) times more than its weight and contains many minerals, fibers, vitamins, antioxidants and proteins [2].

Chia seeds are currently used for their nutritional and medicinal properties. They are used as an appetite suppressant for weight loss, blood glucose control, and intestinal regulation due to their high fiber content. Chia seed oil is unique in that it contains the highest percentage of (omega-3) more than any known natural source. Its high content of these fatty acids helps protect against inflammation, improves cognitive performance, lowers cholesterol levels, and prevents cardiovascular diseases. It also has a high diversity of secondary metabolic compounds such as terpenes, phenols, and flavonoids, and this enables it to be antimicrobial [3]. Where malnutrition is a key factor in gingivitis, in addition to poor dental cleaning, this study was conducted to find out the effect of the oil extract of chia seeds to get rid of gingivitis because its oil contains many unsaturated fatty acids such as (Omega-3,6), which have a prominent role in Inhibition of inflammation and many diseases in addition to secondary metabolism compounds and antioxidants [1] (Figure 1). Therefore, this study aims to evaluate the effect of the oil extract of chia seeds as an antibacterial for the treatment of gingivitis.

Figure (1) Seeds and flowers of the chia plant.

2. Materials and Methods

Materials

1. Chia Seeds Local Market (Pakistan)
2. Ethanol Alcohol 99% U.K\ HAYMAN\ETHANOL
3. Methanol alcohol 99% U.K \ HAYMAN \ METHANOL

4. Media culture INDIA
5. Hexane

Bacterial Types

1. S. mutans
2. E. coli
3. S. aureus
4. S. epidermidis

Fungi

C. albicans

Method of Work

Collection of Chia Seeds (Samples)

The commercial chia seeds used in this study were obtained from the local markets in Baghdad, where the source of these seeds is Pakistan. The seeds are clean and homogeneous and packed in sealed plastic bags and stored at a temperature of 15-20 ° C and then sent A sample of seeds to the herbarium of the College of Science / Department of Life Sciences / University of Baghdad for the purpose of classification and it turned out to be *Salvia hispanica* L.

Preparation of Plant Extracts

Alcoholic Extract

The oil was extracted from chia seeds using Soxhlet using hexane as a solvent. 25 gm of chia seeds were grinded using a coffee grinder for 60 seconds, then placed in a thumble and placed in the above device. The extraction continued for (4) hours, then it was disposed of. of the solvent in a Rotary evaporator vacuum) and then in the oven at a temperature of (60) ℃ until all the solvent is disposed of, after which it is kept in special bottles [6].

Extraction by Screw Press

The oil was extracted by placing a quantity of seeds in the upper hole of the device and collecting the extracted oil by means of a container from the lower hole [3].

Detection of Active Compounds

A set of qualitative examinations was conducted to identify the chemical components of each of the alcoholic chia seed extracts and the screw press, which are:

Tannins Test

Detection of tannins by adding (1) ml of aqueous lead acetate (1%) lead acetate to (1) ml of the extract, a white precipitate is formed, indicating the presence of tannins [7].

Carbohydrate Detection

Detection of carbohydrates using Molish's reagent, mixing (1) ml of the extract with (5) drops of alcoholic phenphthol in a tube and shake well and then added (2.5) ml of sulfuric acid to form a blue ring indicating the presence of carbohydrates [8].

Detection of Glycosides

Detection of glycosides by means of Fahlenk reagent, the appearance of a red precipitate indicating the presence of glycosides [7].

Detection of Phenols Test

It was detected by dissolving (0.1) g of the extract in (1) ml of distilled water, adding 1-2 drops of ferric chloride FeCl3 solution to it. The appearance of blue or green color indicates the presence of phenols [8].

Resins Test
It was detected by adding (1) ml of ethyl alcohol (95%) to (1) ml of the extract and adding to it (1) ml of Hcl, forming a white precipitate, evidence of the presence of resins [8].

Flavonoids Test
It was revealed by adding (1) ml of alcoholic potassium hydroxide reagent N (5) to (1) ml of the extract, the appearance of a yellow precipitate indicating the presence of flavonoids [9].

Sponin Test
Detection by adding (1) ml of aqueous mercury chloride reagent (5%) to (1) ml of the extract, forming a white precipitate indicating the presence of saponins [10].

Alkaloid Test
It was detected using Wagners reagent by adding several drops of the reagent to (1) ml of extract the appearance of positive turbidity indicating the presence of alkaloids [8].

Protein Test
Detection using Bayuret reagent, where (1) ml of the reagent is added to (1) ml of the substance, the violet color is an indication of the presence of proteins [10].

Coumarins Test
It was revealed by adding a quantity of the alcoholic extract of the plant in a test tube. The tube was covered with a filter paper moistened with dilute sodium hydroxide solution, and heated in a boiling water bath for a few minutes, then the filter paper was exposed to a source of ultraviolet rays. The paper was colored bright greenish yellow, indicating the presence of coumarin [9].

Detection of Terpens Test and Steroids Test
It was revealed by adding a little chloroform and a drop of anhydrous acetic acid and a drop of concentrated sulfuric acid. When the brown color appears, it indicates the presence of terpenes, but if after a period of time it turns dark blue, indicating the presence of steroids [7].

Examination of Biological Anti-Bacterial Activity
The method of diffusion with acar was used to test the effectiveness of the extracts, where the bacterial species were activated in a medium (Nutrient Broth), as 250 ml of the mentioned medium was prepared according to the manufacturer's instructions and sterilized with an autoclave at a temperature of 121 ℃ for 15 days. A minute and left to cool at 25℃ and inoculated with 1 ml of bacterial cell suspension, 250 ml of Muller Hinton agar was prepared according to the company's instructions, sterilized with the osmosis device, left to cool and inoculated with 1 ml of cell suspension. The activated bacteria, then pour 20 ml of the medium into each glass dish measuring 9 cm, the dishes were left to cool and a number of holes were made for each glass dish with a diameter of 8 mm for each hole using the perforator, and 50 microliters of the extract were added to each hole using the perforator. (Micropipette, Gentamycin antibiotic tablets) (as a control agent) were then incubated in an incubator 37 ℃ for (24) hours [11].

Toxicity Test
In conducting the acute toxicity examination, healthy and unmeasured laboratory animals (male albino mice) were used that were approved according to the specifications of the Animal House / Al-Razi Diagnostic Research Center / Industrial Research and Development Authority, where mice with a weight range of 30-25 were used. g. Its ages range from 15-18 weeks. It was left for a week for the purpose of adapting to providing appropriate food and exposing it to appropriate environmental conditions designated inside the animal house in terms of exposure to light 12 hours and temperature of 5+25 degrees Celsius, where The mice were dosed with a specific dose that did not exceed 2000 mg/kg of body weight, and after all groups of mice were dosed, they were

returned to the cages and given the food designated for them, where the apparent signs were recorded after the individual administration.

The method used in studying the acute toxicity of the oily extract in both ways of the plant above adopted the same method used in previous studies [12,13] with a slight modification of the doses used orally and using a group of white adult mice and before feeding these mice were starved of food for (2) An hour, but it was allowed to be given drinking water, as it was dosed once orally (125,250,500,1000 mg/kg of body weight), and it was divided into two large groups, each of which was divided into five small groups of mice to determine the (LD50) and the control group (the control group) Only distilled water was given. The animals were kept under observation for (72) hours post treatment in order to check for any behavioral changes or clinical signs, such as agitation, sleep, feeding change, vomiting, diarrhea, ataxia, etc.

1. The first group: A control group, mice were dosed orally with distilled water 5 mice.
2. The second group: The oily extract with alcohol group, where the group was divided into five groups, orally dosed with the above extract.
3. The third group: The oil extract group with a screw pressure device, where the group was divided into five groups and dosed in the same way as the previous one.

3. Results and Discussion

Table (1) shows some of the secondary metabolites that were detected in the oil extracts of Chia seeds (*Salvia hispanica* L.), which are considered to have an inhibitory effect on bacteria. The quality of the active compounds present in the oil extract varies according to the extraction method and the environment of the plant in which it is grown. These active compounds have an important role in inhibiting the growth of most microorganisms, and therefore they are important because most of the therapeutic effects are attributed to them.

Flavonoids are water-soluble pigments that have most of the therapeutic effects of medicinal plants, as they act as anti-inflammatory, histamine, antiviral, and anti-oxidant [12]. The glycosides are a substance that helps kill germs, while the resins have an antiseptic and tonic properties for the gums, and the tannins have an effective role in treating burns and wounds and act as antiseptics as they cleanse the gums and teeth and have uses against bleeding and knew healing of the mucous membranes [13]. as it is an antioxidant compound and prevents the growth of microorganisms, and has a medical benefit in the formation of new tissues and healing of the mucous membranes [14].

Table (1). Chemical qualitative tests of oil extracts of chia seeds.

detection	tannins	carbohydrates	clacosides	phenols	resins	flavonoids	saponins	alkaloids	protein	coumarins	terpenes	steroids
Oily extract with alcohol	+	+	-	+	+	+	+	+	-	-	-	-
Oil extractor with screw press	-	+	-	-	+	+	+	+	-	-	-	-

(+) an indication of the presence of the active compounds, (-) an indication of the absence of the active compounds)

The toxicity test that was conducted on laboratory animals (rats) (paragraph E, the toxicity examination test) proved that no abnormal symptoms were recorded on the animals after being dosed with the extract prepared from chia seeds at the four concentrations during the first four hours of the test, and no fatalities were recorded

after (24 hours) of the test. This test proves that the oil extract in both ways of the chia plant seeds is non-lethal and safe for the concentrations used in the experiment (Table 2).

Table (2). Results of the toxicity examination for both alcoholic oils and the screw press for chia seeds.

Dosed substance	Concentration (mg/kg)	Number of mice/cage	Dosage Duration
(Control)	١٢٥	٥	1day
	٢٥٠	٥	
	٥٠٠	٥	
	١٠٠٠	٥	
Oily extract with alcohol	125	5	
	250	5	
	500	5	
	1000	5	
Oil extractor with screw press	125	5	
	250	5	
	500	5	
	1000	5	

Experiments were conducted to examine the effect of the effectiveness of oil extracts of the seeds of the chia plant, where the concentrations (12.5%,25%,50%,75%) were prepared on bacterial species and fungi (*C.albicans*) using the method.

Diffusion and determine the reading period of the results after (24 hours) by measuring the diameters of the inhibition zones, as the results showed a difference in the rates of the diameters of the inhibition zones that have a direct relationship in the sensitivity of each type of bacteria and the types of solvent used, and the method of extraction, which is important in The quality and quantity of the effective primary metabolites present in the dry plant, where each of (*E.coli*, *S.aureus*, *S.epidermidis*, *C.albicans*, *S.mutans*) was used as the first type and for teeth, as it is the basis in the events of caries through dynamic complex processes It affects the dental plaque and gums, which leads to the gradual destruction of tooth enamel and its loss, in addition to gingivitis. Without it, the rest of the bacteria and fungi in the mouth would not be able to cause caries and gingivitis because they are built on them [17]. Where research has shown the main role of (*S. mutans*) in causing caries. In addition to their ability to convert sugar into acid, they can manufacture a substance called (Peptidoglycans), a starchy substance that deposits and sticks to the tooth, which contributes to the events of caries after bacteria transforms Sugary and starchy materials to acids such as lactic and formic that dissolve the calcareous mineral salts found in the enamel of the tooth [18].

Through the results of the biological activity of the types of extracts studied, it was noted that there is a difference in the inhibitory role of the tested bacteria compared to the control (Gentamycin). The oil extract of chia seeds with a screw pressing device at a concentration of 75% gave high efficacy with diameter 20 mm and diameters (25, 15, 13, 15 mm), respectively, for the rest of the above bacteria and fungi compared to the rest of the concentrations and higher than the antibiotic (Gentamycin) as well as higher than the extract The alcoholic oil has an effect on the occurrence of gingivitis and teeth, as shown in Table (3).

Table (3) shows the antimicrobial activity of the oil extract (alcoholic and with a screw press) of chia plant seeds on bacterial species (*S.mutans* ,*S.epidermidis* ,*S.aureus* ,*E.coli*) In addition to the mushroom (*C.albicans*). Whereas, the highest inhibition rate was for the concentration 75% (25 mm) of the oil extract using a screw press

on *C. albicans* and 20 mm on *S. mutans* compared to the rate of inhibition of the oil extract with alcohol was 17 mm. And 18 mm respectively, for the above concentration and the least inhibition for the concentration 12.5% (11 mm) of the oil-alcohol extract on (*S.aureus*) and (*C.albicans*) bacteria, while gentamicin gave the highest inhibition rate (17 mm) for (*E.coli*) bacteria. And (*S.aureus*) in comparison with the two alcoholic extracts and the screw press of chia seed oil, which had a lower inhibitory effect, and the lowest percentage of inhibition (15 mm) on the fungus (*C. albicans*) compared to the above two extracts whose inhibitory effect was higher. The obvious for chia oil because of its composition to contain effective groups such as alkaloids, phenols and flavonoids. It was reported [20, 19] that alkaloid extracts have anti-pathogenic activity through their effect on enzymatic attachments. And its inhibitory effect may be due to the fact that it contains many nutrients such as calcium, iron, potassium and magnesium, in addition to that it contains about 17-26% linoleic acid and 50-57% linolenic acid [21]. The study conducted by (Divyapria, G.K.; Veeresh, D.J.and Yavagal, P.C.) [1] showed that the presence of antioxidants in the chia plant, such as omega-3 fatty acids, shows an anti-microbial effect.

Table (3). shows the results of the biological activity of types of oil extracts from the seeds of the chia plant (alcoholic and with a screw pressure device) against bacteria and fungi (*C.albicans*) and their comparison.

Microorganisms	Type of extract	Inhibition zone diameter (mm)				Positive control (Gentamycin)mg\ml
		75%	50%	25%	12.5 %	
E.coli	Chia seeds extract (screw)	15	14	13	R	17
	Chia seeds extract (alcoholic)	16	16	15	R	
S.epidermidis	Chia seeds extract (screw)	13	12	R	R	16
	Chia seeds extract (alcoholic)	16	15	14	12	
S.aureus	Chia seeds extract (screw)	15	14	13	12	17
	Chia seeds extract (alcoholic)	16	13	12	11	
S.mutans	Chia seeds extract (screw)	**20**	16	12	R	16
	Chia seeds extract (alcoholic)	**18**	15	14	R	
C. albicans	Chia seeds extract (screw)	**25**	16	14	13	15
	Chia seeds extract (alcoholic)	**17**	14	13	11	

(1g/ml=100% , R=resistance)

4. Conclusions

We conclude through the qualitative lists of the active ingredients of the oil extract in both ways (alcoholic and by screw pressure device) that they are affected by the extraction method, as each group has specific specifications and a specific extraction method. The results of examining the biological activity of bacteria that cause gingivitis and dental caries showed that the oil extract with the screw pressure device gave the highest inhibition result at the concentration (75%), and therefore it is considered the best because it is economically feasible. The results of examining the biological activity of C. albicans fungus, which is also one of the causes of

gingivitis, showed that the oil extract in the screw pressure device gave the highest inhibition result at the concentration of 75%, and therefore it is considered the best because it is economically feasible.

5. Recommendations

- ❖ Conducting several applied studies of chia seed oil inside the body of the living organism to know its effect.
- ❖ Take advantage of the inhibitory role of chia seed oil for gingivitis in an applied way by making a combination in toothpaste or gargle for the mouth.

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