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Manufacturing of Medicate Chewing Gum Against Oral Pathogens by the Extract of Seeds of *Lepidium sativum L.*

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Abstract

The results of the qualitative chemical detection of the active ingredients revealed that the *Lepidium sativum L.* (SLS) extract contained on the tannins, flavonoids, and saponines, as well as resins and alkaloids but lacked the glycosides. As well as determination of total phenols in SLS extract 44.72 mg/g also flavonoids 19.96 mg/g. Medical gum was manufactured from the following ingredients gum base, glycerine, xylitol, sun flower oil, flavor and color with the addition of the extract tested with different concentration (1.5 and 2.5 mg/g), the release of phenols compounds of chewing gum samples were estimated after each period of crushing the sample of gum in phosphate buffer. It was found that the ratio of the release of tis group of active compounds (phenols) increased with the increase of the duration of crushing gum samples until reaching 100% for all samples after 20 min. The results showed that sample of gum added to the extract (1.5 mg/g) was acceptable and no significant differences was found in comparison to the control treatment except for the sample added to the SLS extract at a concentration of 2.5 mg/g which was not accepted by the consumer due to the unpalatable taste.

Keywords: *Lepidium sativum L.*,Phenols, Flavonoids, Chewing gum, phenol release

INTRODUCTION

Many plants have been used because of their antimicrobial traits, which are due to compounds synthesized in the secondary metabolism of the plant. These products are known by their active substances, for example, phenolic compounds, which are part of essential oils, tannins, terpenoids, alkaloids and flavonoids, these metabolites have been tested in vitro to have antimicrobial properties [1]. Various compounds in plants that are produced for self-protection could support each other in inhibiting bacterial growth, while also reducing the bacterial strains [2]. Chemotherapeutic agents from natural products have proved to be a promising source for the development of new drugs throughout human history [3]. Recently, several studies have shown the feasibility of using medicinal plants as a source of chemotherapeutic agents for the prevention of oral diseases [4].
Lepidium sativum L. (SLS) is a fast-growing edible herb which is due to family Brassicaceae (5). It has been reported that phytochemicals, which are considered as secondary products components, are directly responsible for an activity such as antioxidant, antimicrobial, antifungal, anticancer, and anti-inflammatory, among others [6].
Oral diseases are major health problems with dental caries and periodontal diseases among the most important preventable global infectious diseases. *Struptococcus mutans*, a member of endogenous oral microflora, has long been implicated to play a vital role in the pathogenesis of these diseases [7]. Dental caries prevention is preferable to treatment. Convention preventive methods, such as the use of alcohol or antibiotics, like chlorhexidine, erythromycin, ampicillin, and penicillin, have proven effective in preventing dental caries [8].
However, excessively used of these chemicals has been reported to change the oral and intestinal flora, and can cause other problems such as vomiting, tooth staining or oral cancer, importantly antibacterial agents can also promote the development of

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resistant bacteria strains [9,10]. For these reasons, alternative methods such as the use of medicinal plants are of increasing interest.

In recent years, research is developing new methods of dental disease prevention, releasing natural bioactive compounds included in traditional or innovative medical devices in the mouth to improve tooth protection. Chewing gums is a particularly useful means for delivering and maintaining bioactive compounds, included in the gum formulation able to have an anti-cariogenic effect [11] Gum advantages over the other delivery methods include no needing to water or liquids to eat, increasing the systemic effects, low dose administration, faster onset of action and good stability relieving of dry mouth strengthening the mastication muscles and prevention of dental caries [12]. In the present study apply different concentrations from the SLS extracts in the manufacturing of medicating gum to protect teeth from the effect of the bacteria and thus prevent teeth decay.

MATERIALS AND METHODS

Plants collection

The seeds of *Lepidium sativum L.* (SLS) were purchased from the local market for herbs in Baghdad. After that these seeds were grounded into powder, and kept till use.

Extraction

The applied method according to the method described by [13] to 10 g of the extract powder of LSL added 300 ml of distilled water at boiling point and the mixture was left for 30 min on a magnetic stirrer. After that, the mixture was filtered through filter paper (What man No.1), then concentrated by rotary evaporator under reduced pressure at 50˚C, the concentrated extracts were dried in the oven at 40˚C, scraped and stored the powder in labeled sterile screw-capped bottles at 5˚C in the refrigerator, until when required for use.

Phytochemical screening

The aqueous extract were subjected to various chemical tests in order to estimate the active compounds by employing multiple methods described by [14] which are reported in [15] for detection of glycosides, tannins, flavonoids and saponins, while employing the method mentioned in [16] for alkaloids and [17] for steroids and terpenes.

Determination of total phenolic compounds

Folin-ciocalteu's reagent colorimetric method was used as described by [18] add 0.5 ml of the extract (1mg/ml) to 2.5 ml of Folin-ciocalteu's reagent, and 2ml of sodium carbonate 7.5% then left the mixture for 30 min at room temperature, the absorbance was recorded at 760 nm, the total phenolic compounds were determined according to gallic acid standard curve fig (1).

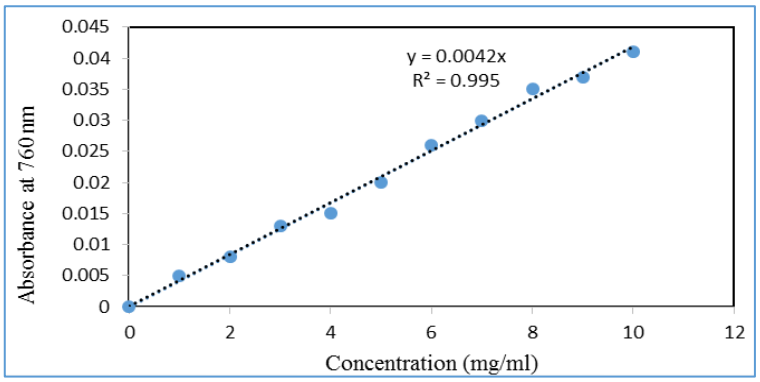

Figure 1: Standard curve of gallic acid

Determination of total flavonoids compounds

The total flavonoids in the aqueous extract were determined according to [19] mixing 1ml of the extracts (1mg/ml) in a 10ml volumetric flask with 5ml of distilled water and 0.3ml of $NaNo_2$ 5%. After 5 min added 0.6 ml of AlCl3 10%. After another 5 min added 2ml of 1M NaOH and the volume was made up to 10ml with distilled water. The mixture was mixed thoroughly, and the absorbance was measured at 510 nm. The total flavonoids compounds were determined according to catechin standard curve fig (2).

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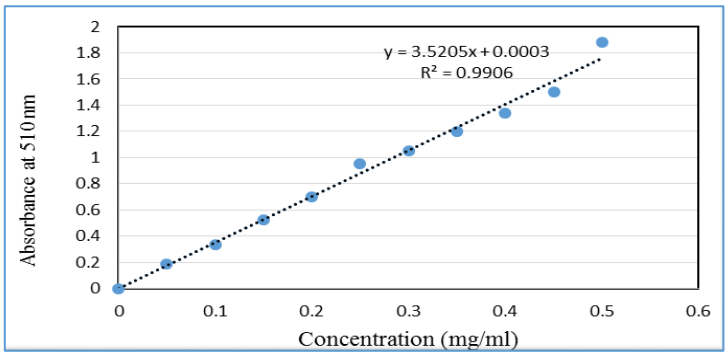

Figure 2: Standard curve of catechin

Chewing gum preparation

Chewing gum was formulated of the gum base, oil, glycerin, xylitol, color, and flavor. The combination of the base was softened in a water bath at 60°C. Oil, glycerin, and xylitol, finally color and flavor and plant extract (1.5 and 2.5 mg/g) were added at 40 °C table (1). The homogeneous mixture was extended on a glass plate. Then it was cooled and cut in small pieces and kept for 48 hours at room temperature.

Table 1: Ingredient of medicated chewing gum

No.	Ingredient	g
1	Gum base	75
2	Glycerine	15
3	Xylitol	1.5
4	Sun flower oil	2.5
5	Flavor and color	0.7

In vitro: phenol release test

One gram of the formulation was taken in a mortar, added to it 50 ml of pH 6.8, 0.2M phosphate buffer and for 20 min crushed. The temperature was maintained at 37˚C by a water bath. 0.5 ml aliquots of the mixture were removed at the times of 0, 5, 10, 15 and 20 min since the start of crushed. The aliquots were replaced by 0.5 ml fresh phosphate buffer subsequently. After that, added the solutions of total phenols determination (2.5 ml folin-cicalteu's reagent and 2ml sodium carbonate 7.5%) and absorbance was recorded at 760nm by using a spectrophotometer.

Evaluating the organoleptic characteristics

To evaluate organoleptic features of this product, ten healthy volunteers were asked to chew the gum and give comments on the hardness/softness, adhesion to teeth, the volume of the gum mass and taste according to the Likert scale on the evaluation forms (20) Table (2).

No	Organoleptic properties	Samples						
		1	2	3	4	5	6	7
1	**Chewing gum volume**							
2	**Softness**							
3	**No adherence**							
4	**Taste**							
5	**Persistence of taste**							

1. The bulk volume of gum was evaluated as Huge=5, much=4, right=3, little=2, very little=1.
2. The softness/Hardness was evaluated as very hard=5, hard=4, suitable=3, soft=2, very soft=1.
3. The adherence to the teeth was evaluated as never adheres=5, rarely adheres=4, sometimes adheres=3, often adheres=2, always sticks=1.
4. The taste was evaluated as excellent=5, good=4, fair=3, poor=2, very poor=1.
5. The persistence of the taste was evaluated as strong persistence=5, good persistence=4, intermediate persistence=3, weak persistence=2, very weak persistence=1

Statistical analysis

The Statistical Analysis System- SAS [21] program was used to show the effect of the difference of treatments in study parameters. The least significant difference –LSD ($p > 0.05$) was used to significant compare between means in this study.

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RESULTS AND DISCUSSION

The phytochemical tests were done on aqueous extract, which was prepared from seeds of *Lapidium Sativum L.* (SLS). The results are summarized in table (3). Most of the active compounds were isolated and identified in the crude extracts of the plant [22]. Phytochemical screening of SLS extract presence of various medically active constituents, the phytochemical compounds present in the SLS extract is rich in tannins, flavonoids, saponins, and terpenes. On the other hand, they lack glycosides. Other researcher who has demonstrated the presence of flavonoids, alkaloids, and saponins, and absence of tannins, sterols, and polyterpenes in SLS extract [23]. The results of the phytochemical tests indicated that the plant possesses various biologically active compounds which could serve as a potential source of drugs in herbal medicine [24]. Phytochemicals such as saponins, terpenoids, flavonoids, tannins, steroids, and alkaloids have anti-inflammatory effects [25], glycosides, flavonoids, tannins, and alkaloids have hypoglycemic activities [26,27], steroids and triterpenoids showed the analgesic properties [28]. Total phenols and flavonoids content of SLS aqueous extract 44.72, 19.96 mg/g, respectively.

Table 3: Phytochemical screening of aqueous extract of SLS

Compounds	Detection	Detected indicator	Result of detection
Tannins	Lead acetate 1%	White gel precipitate	+
	Ferric chloride 1%	Bluish green	+
Glycosides	Fehling indicator A, B	Red precipitate	-
Flavonoids	Ethyl alcohol 95%+ KOH	Yellow ring	++
Saponins	Cold distilled water	Persistent foam	+-
Resins	Ethyl alcohol 95%+ boiling	Clear crumb	+
Alkaloids	Mayer's reagent	White precipitate	+ +
	Picric acid	Yellow precipitate	++
	Dragendrroff's reagent	Orange precipitate	++
Stereol and terpenes	Chloroform+ anhydrous acid	Blue-black color	+

+/presence - /none detected

Fig (3) show the rate of release of phenols compounds found in the chewing gum to the phosphate buffer solution, which represents saliva in the manner of estimating phenols compounds. The results of the current study showed that the phenols release to the solution increased by crushing time of the chewing gum samples. The phenols release rate was 36.44 and 38.22% after 5 min at 1.5 and 2.5 mg/g respectively — all the tested formulations released approximately 100% of their active agents after 20 min. In general, the results showed that the samples of chewing gum with a higher concentration of extracts had a higher phenols release rate than the samples with a lower concentration. This may be due to the increased concentration of phenols polymerized in the high concentration of the extract. Phenols are either small molecules such as phenolic acids or high polymerization compounds such as tannins [29]. The drug-polymer ratio was found to affect the drug release characteristics of the prepared chewing gums, at higher drug-polymer ratio, the drug release from the chewing gums was faster as compared to lower drug polymer ratio, this was because the high drug-polymer ratio promotes the increase saliva uptake and lead to the greater solubilization of the drug present in the polymer matrix causing faster diffusion of drug through the gum base (30).

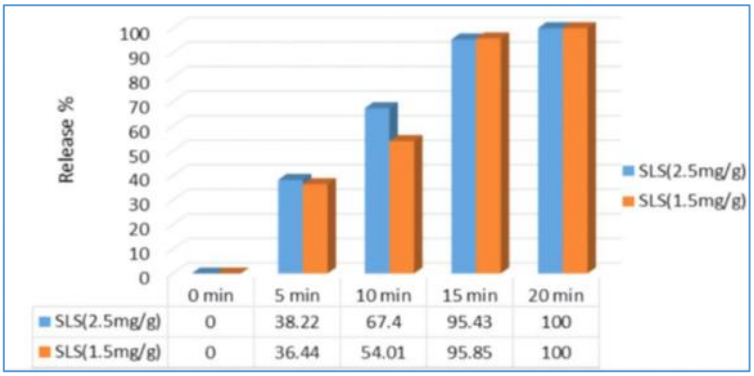

Figure 3: In vitro release of phenols from chewing gum formulations in pH 6.8 phosphate buffer at 37 ˚C with various concentrations of SLS extract.

Shows the results of the statistical analysis of the organoleptic properties of the chewing gum, which are treated with different concentrations of the SLS extract compared with the control (without adding the extract). The results showed no significant differences ($p< 0.05$) for the chewing gum samples added to the extract SLS (1.5 and 2.5 mg/g) compared to the treatment of the control in the qualities of chewing gum volume, softness, hardness, no adherence and persistence of taste. As for the taste characteristic, there was no significant difference for the chewing gum samples added to the extract SLS (1.5 mg/g), while SLS (3.0 mg/g) had a significant difference ($p< 0.05$) in taste compared to the control treatment. Fig (4) shows the resulting chewing gum forms for two treatment with control.

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Figure (4): Experimental chewing gums produced

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