



Exploring the potential of chewing gums containing plant extracts (clove, saffron, and clove-mint) as a natural solution for controlling obesity and associated health issues

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ABSTRACT

Background: Obesity is a global health concern resulting from an excessive accumulation of fat in the body, affecting individuals of all ages, including children. Modern lifestyles and the consumption of high-calorie foods have resulted in an increase in obesity prevalence. Cardiovascular disease, diabetes, blood pressure, and certain cancers are all serious health problems that obesity can lead to.

Objectives: Medicinal properties in functional foods are gaining popularity in the food industry for controlling or inhibiting diseases. Given the growing concerns about obesity and its associated health risks, the need for natural compounds with controlling or inhibiting properties is paramount. This study aimed to explore natural plant extracts for their functional properties, particularly their ability to control obesity by inhibiting lipase enzyme. To transport these biological compounds, chewing gums containing extracts of cloves (at concentrations of 0.1%, 1%, and 3%), saffron, and mint were developed. Chewing gums containing extracts can become a popular natural remedy for a variety of health

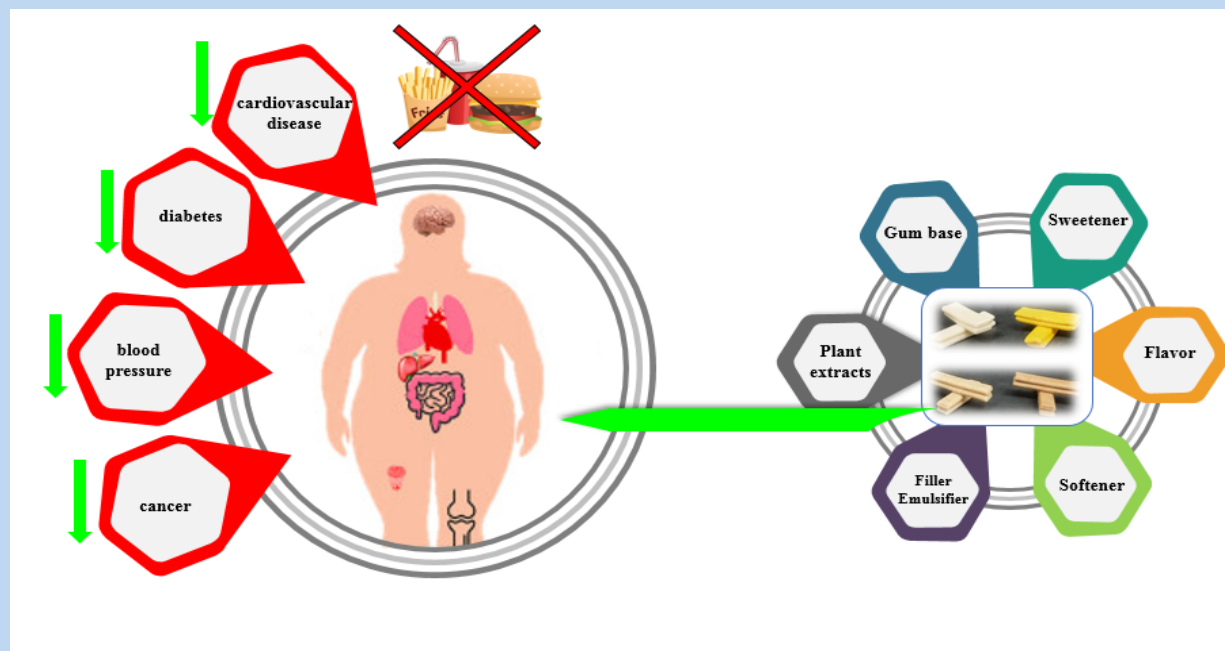
problems, including obesity. Using an effective delivery system, this solution is widely available to those looking for natural alternatives to address their health concerns.

Methods: The research centered on exploring the properties of natural plant extracts, which included their potential to inhibit enzymes linked to obesity and related diseases, their antioxidant properties to counteract the harmful effects of body fat oxidation, and their antibacterial properties. In addition, the research involved a sensory evaluation of chewing gum that contained these extracts.

Results: Clove extract had the highest amounts of total phenolic compounds. Also, clove extract exhibited the highest antioxidant properties and *Helicobacter pylori* inhibition (88.05% and 25.69% at ratio of 0.7% respectively), which is thought to be associated with its high content of eugenol and other phenolic compounds. Furthermore, the extracts were evaluated for other functional properties, including their ability to inhibit pancreatic lipase, angiotensin converting enzyme (ACE), and microbial activities against *Streptococcus faecalis* and *Candida albicans*. Among extracts, saffron extract showed the highest lipase inhibitory properties (40.07, and 36.06% at concentrations of 0.7% and 0.5% respectively). Also, clove and mint showed pancreatic lipase inhibition about 31.50% and 29.24 % at concentration of 0.7 and 0.5% respectively, revealing that all extracts can be a good candidate to be used as effective additives in preventing and controlling obesity. Also, clove, mint, and saffron extracts could inhibit blood pressure enzymes (25.54, 20.70, and 19.61 respectively), which can help maintain healthy blood pressure levels. Furthermore, release kinetics of extracts from chewing gums were obtained, and shown that after 20 min the release of extracts were 73.24, 64.34, and 63.2 for saffron, mint, and clove (1%) respectively.

Conclusions: Extracts were found in our research to have a beneficial effect on controlling obesity. These compounds may be effective in preventing obesity, based on their observed inhibition of pancreatic lipase enzyme. However, further research is necessary to fully comprehend the efficacy of these substances for this purpose. The significant antioxidant properties of clove may have a promising impact on reducing free radicals, which can help prevent fat oxidation in the bodies of individuals with obesity. The inhibition of ACE enzyme by extracts could potentially have a positive impact on controlling blood pressure. The extracts showed potent antimicrobial properties that can potentially alleviate mouth and throat issues associated with *Streptococcus* bacteria. Moreover, extracts demonstrated efficacy in reducing the presence of *H. pylori*, which is the causative agent of stomach infections. Also, it was shown that chewing gum can be a convenient and easily accessible means of delivering natural compounds and may be a desirable option for individuals seeking natural alternatives for managing their health concerns.

Keywords: Chewing gum, Plant extracts, Obesity, Blood pressure, *H. pylori* infections



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INTRODUCTION

Obesity, a medical condition resulting from an excessive buildup of body fat, has been associated with various negative health outcomes. Regrettably, there has been a global increase in obesity rates, and if this trend continues, it is estimated that by 2025, the prevalence of obesity in men and women worldwide could reach 18% and 21%, respectively [1]. Obesity can result in various metabolic abnormalities including insulin resistance, dyslipidemia, and hypertension. Moreover, obesity is also linked to the occurrence of atherosclerosis, a condition where fatty deposits accumulate in the arteries. This build-up can obstruct blood flow, leading to a heightened risk of heart attack and stroke [2]. Due to the potential complications associated with drugs used to control obesity, such as increasing in heart rate and blood pressure leading to cardiovascular issues like heart attacks and strokes, as well as digestive side effects, there has been a growing interest in natural and herbal

compounds as alternative ways to manage and prevent obesity and related conditions [3-4]. Increasingly, consumers are seeking out healthier and safer products, and as a result, there has been a surge in demand for natural antioxidant ingredients, particularly those derived from plants. These ingredients are preferred over their artificial counterparts due to their perceived health benefits and safety [5].

The properties of clove extract are antioxidant, antifungal, and antibacterial, making it useful in protecting the body against oxidative stress, fighting fungal infections, and dealing with bacterial infections caused by listeriosis and salmonellosis [6]. Clove extract contains eugenol, which has a numbing effect and can help alleviate pain [7]. The potential of clove extract for regulating blood sugar levels and improving insulin sensitivity in individuals with diabetes has been suggested by research [8].

Saffron possesses bioactive compounds with special medicinal properties such as picrocrocin, crocin, and safranal, which are responsible for bitter taste, color, and aroma of saffron respectively [9]. Saffron extract contains many antioxidants, such as flavonoids and polyphenols, which can help protect the body against oxidative stress and damage caused by free radicals [10]. Saffron extract has been proven to improve mood and reduce symptoms of depression and anxiety. This may be due to its ability to increase levels of serotonin (a mood-regulating neurotransmitter) [11]. Saffron also has been found to have various effects, including anticonvulsant, anti-inflammatory, anti-tumor, and improvement of learning and memory [12].

Coughs, colds, and asthma symptoms can be alleviated by using mint extract that soothes the respiratory system. It also has the potential to clear congestion and reduce inflammation in the airways [13]. Mint has been shown to activate TRPM8 (Transient receptor potential cation channel subfamily M (melastatin) member 8), which can help reduce pain and inflammation. Additionally, mint extract has been found to alleviate symptoms of irritable bowel syndrome (IBS) [14].

The term "functional foods" is often used to describe foods that may provide health benefits beyond basic nutrition due to their physiologically active components. These foods are marketed as potentially contributing to overall well-being and health, often by promoting optimal body function or reducing the risk of disease [15].

Chewing gum's global popularity and its ability to release bioactive compounds over an extended time in the oral cavity make it a preferred option for drug delivery compared to tablets and syrups [16]. Chewing gum consumption stimulates saliva production, reduces gum inflammation, enhances alertness, and helps regulate stress levels. These benefits have contributed to a rise in the industry, with chewing gum ranking third in

terms of increased consumption [17]. Chewing gum is composed of a polymer mixture (elastomer softener), waxes, fats, emulsifiers, and antioxidants, which play a crucial role in determining its texture, mouthfeel, chemical stability, and release of bioactive compounds [18]. Chewing gum can increase saliva flow and volume, even in patients with reduced salivary gland function. It has also been shown to alleviate symptoms of head and neck cancer, such as dry mouth and chewing difficulties [19].

Fortunately, nowadays, the research area of food science has paid a lot of attention to finding alternative ways to develop methods to release herbal compounds that can effectively prevent obesity and related conditions. In this study, we thought that designing chewing gum as a delivery system for herbal extracts may have a beneficial effect on human health. The primary objective of this study was to determine the functional and nutritional properties of herbal extracts (saffron, clove, and mint). In the second step, the objective was to include extracts in the chewing gum formulation to investigate the release behavior of extracts in the body. As mentioned above, clove extract has a numbing effect, which may influence the final taste and acceptability of the product. Thus, in our study chewing gums enriched with mint-clove were also prepared to determine whether mint can improve the taste of clove or not.

MATERIALS AND METHODS

Saffron, mint, clove, and eugenol extracts were kindly provided by Masterfoodeh Company. 2,2-Diphenyl-1-picrylhydrazyl (DPPH), Folin-Ciocalteu, p-nitrophenyl butyrate, Orlistat, lipase (type II; from porcine pancreas), rabbit lung acetone extract, N-[3-(2-furyl) acryloyl]-1-phenylalanyl-glycyl-glycine (FAPGG) were procured from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals were purchased from Merck and Sigma-Aldrich or other producers with analytical grades.

Determination of total phenolic compound: The total phenolic content (TPC) was determined by preparing a mixture of 20 μL of the sample (0.001 mg/mL), 100 μL of Folin-Ciocalteu, and 1580 μL of distilled water. The reaction was terminated by the addition of 300 μL of sodium carbonate and incubation for 2 h. The resultant mixture's absorbance was measured at 765 nm using UV-Vis spectroscopy [20]. The TPC was expressed as gallic acid equivalent (mg GAE/g) ($Y = 1.1228 \times + 0.0328$, $R^2 = 0.9992$).

DPPH radical scavenging activity assay: To measure the antioxidant activity, 50 μL of the sample was mixed with 700 μL of 50% methanol and 750 μL of 0.4 mM DPPH, and incubated in the dark at 25 $^{\circ}\text{C}$ for 35 min. The absorbance of the mixture was recorded at 515 nm using a UV-Vis spectrophotometer [21]. Ascorbic acid and gallic acid were used as positive controls. DPPH radical inhibition was calculated by the following equation:

$$\text{DPPH radical scavenging activity\%} = \left(\frac{A_{\text{blank}} - A_{\text{sample}}}{A_{\text{blank}}} \right) \times 100$$

Helicobacter pylori inhibitory: Urease activity was determined by mixing 850 μL urea, 15 μL jack bean urease, and 120 μL phosphate buffer (0.1 M, pH 7.6) in each sample. The mixture was incubated for 30 min at 37 $^{\circ}\text{C}$. Next, a solution mixture of a (containing 4.47 g of salicylic acid, 2.5 g of NaOH, and 20 mg of sodium nitroprusside in 50 mL of distilled water) and b (containing 1.5 mL of chlorine water and 0.5 g of NaOH in 70 mL of distilled water) was added. Then the mixture was incubated for another 30 min at 37 $^{\circ}\text{C}$. *H. pylori* inhibitory was calculated according to the following equation:

$$H. \text{pylori\%} = \left(\frac{A_{\text{blank}} - A_{\text{sample}}}{A_{\text{blank}}} \right) \times 100$$

Pancreatic lipase inhibitory assay: To assess the activity of lipase, the sample was combined with the substrate, which was composed of 10 mM 4-nitrophenyl butyrate dissolved in Dimethyl sulfoxide (DMSO), in a ratio of 40

μL to 20 μL . The resulting mixture was then treated with 40 μL of the enzyme, which had been dissolved in 0.1 M phosphate buffer at a concentration of 2.5 mg/mL. After incubating the mixture at 37 $^{\circ}\text{C}$ for 20 min, the ELISA reader Expert 96 (Biotech, USA) was utilized to measure the absorbance at 405 nm [22]. Orlistat was used as a positive control. Pancreatic lipase inhibition was calculated according to the following equation:

$$\text{Pancreatic lipase inhibitory\%} = \left(\frac{\Delta A_{\text{blank}} - \Delta A_{\text{sample}}}{\Delta A_{\text{blank}}} \right) \times 100$$

ACE inhibitory assay: To perform this measurement, 10 mL of 0.05 M Tris-HCl buffer (pH 8.3) containing 5% (v/v) glycerol was used to dissolve 1 g of rabbit lung acetone powder. After storing it overnight at 4 $^{\circ}\text{C}$, the mixture was centrifuged at 30,000 $\times g$ for 40 min. The resulting translucent liquid was carefully gathered. To determine the inhibitory activity of ACE, a solution of FAPGG (0.001 M) in a Tris-HCl buffer (pH 8.3) containing NaCl (0.4 M) was prepared. Subsequently, 50 μL of each sample was combined with this solution and allowed to incubate at a temperature of 37 $^{\circ}\text{C}$ for a duration of 2 min. Following this, a precise quantity of ACE extract (10 μL) was introduced, and the absorbance of the mixture was measured using a ELISA reader Expert 96 (Biotech, USA) at a wavelength of 340 nm over a time period of 25 min, all while maintaining a constant temperature of 37 $^{\circ}\text{C}$ [23]. ACE inhibitory was calculated by the following equation:

$$\text{ACE inhibitory\%} = \left(1 - \frac{\Delta A_{\text{sample}}}{\Delta A_{\text{blank}}} \right) \times 100$$

Determination of minimal bactericidal and fungicidal concentrations: To assess the antimicrobial properties of the extracts, the agar well diffusion technique was employed. The microbial cultures were introduced into Lysogenia Broth (LB broth) media and left to incubate at 37 $^{\circ}\text{C}$ for 3 h until they reached a turbidity level of 0.5 McFarland's index in phosphate-buffered saline. LB agar wells with a diameter of 6 mm were created using a

sterilized syringe cap, and a diluted culture was spread over the agar surface using a sterile cotton swab to establish a lawn culture. Following this, 20 µL of various extracts were placed in the wells, and the plates were incubated at 37 °C for 24 h [24].

Preparation of chewing gums: For preparation of chewing gums, gum base was mixed with appropriate amounts of plasticizers including lecithin and glycerin. Then, a half amount of sorbitol, as well as sweeteners (sucralose and acesulfame), were added to the mixture. Flavoring agents, in the form of herbal extracts such as saffron, clove, and clove-mint (at concentrations of 0.1, 1, and 3), were incorporated and thoroughly blended with the other ingredients. Then, the rest of the sorbitol was added followed by kneading at 55 °C using a z-blade mixer for 30 min. After obtaining a homogenous mixture for both types of chewing gums (flavorless and flavored chewing gums), the mixed solution was transferred into the Forming machine, rolled, and cooled at 28 °C. The rolled mixture was then cut into 1.5×1.5×1 cm dimensions and stored in airtight containers for 48 h at 20 °C until they were analyzed. The entire preparation process took place in the Research and Development section of MasterFoodeh Company, and the resulting flavored chewing gums were designated as the Nature Dent series.

Sensory evaluation of chewing gum samples: Ten panelists between the ages of 20 to 35 were subjected to sensory analysis of chewing gums containing 3%, 1%, and 0.1% clove extract, saffron extract, and clove-mint extract-flavored chewing gums. A control group was also included in the experiment, consisting of chewing gum with no flavor. To eliminate any potential bias, environmental conditions were kept standardized throughout the test. The participants brushed their teeth beforehand and then chewed the samples for 20 min.

The chewing gum samples were rated on a 10-point scale for appearance, hardness, chewiness, taste, and total acceptability, with 1 indicating extreme dislike and 10 indicating extreme liking.

Release kinetics of extracts from chewing gum samples:

For this measurement, three volunteers were chosen. They brushed their teeth before the start of the experiment. Participants were assigned to chew 2 pieces of each chewing gum containing 1% clove, saffron, and clove-mint extracts at different times (0, 5, 10, 15, and 20 min). The chewing duration of 20 min was chosen to simulate typical gum chewing time by individuals [25].

For each run of chewing time, the mastication process was started with an un-chewed sample. After each time the chewed samples were collected from volunteers, stretched out up to the maximum, and stored at -18 °C in separate bags. The frozen pieces were then cut into small pieces and ground. 2.5 g of each sample was transferred to separate falcon tubes and mixed with 25 mL of 80% methanol. Afterward, the samples were vortexed and centrifuged at 2700 × g for 30 min, and then the supernatants were collected, transferred to separate tubes, and stored at 4 °C for the next analysis. The remaining amount of extract in supernatants was determined using standard curves, which were prepared for pure extracts of clove, saffron, and mint before analysis. To prepare each calibration curve, an appropriate amount of each extract was diluted, and the absorbance of each concentration was determined by a UV spectrophotometer at 420, 260, and 350 nm for saffron, clove, and mint extracts respectively. Then, the relationship between these two variables was recorded. The amount of extract released during mastication was calculated by subtracting the amount of the residual active ingredient present in the gum after chewing from the total content of each extract.

Statistical analysis: Three replicates of each experiment were conducted, and statistical analysis was carried out using Minitab software (version 18) via one-way ANOVA. The means were then compared using SPSS software (version 16) through Duncan's test.

RESULTS AND DISCUSSION

Total Phenolic compound (TPC): According to Figure 1, the total phenolic content (TPC) values were measured for different extracts at two concentrations of 0.5% and 0.7%. Eugenol was also examined as the most important index in clove extracts. Among the extracts tested, clove extract had the highest TPC values, (0.48 and 0.78 mg of gallic acid/g of the sample in concentration of 0.5, and 0.7%), which were significantly different ($p < 0.05$) from

those of saffron, and mint extract. Conversely, mint extract had the lowest TPC values, with 0.05 and 0.06 mg of gallic acid /g of sample. The type of extract had a significant effect on the amount of TPC ($p < 0.05$). The abundance of TPC in cloves can be attributed to the presence of eugenol, which is the main component of the extract [26]. This relationship is proved by the high concentration of total phenol in eugenol during our research. The low TPC values observed in mint and saffron extract may be attributed to various factors, such as the plant species, the extraction method employed, or the growing conditions of the plants. Environmental factors, such as temperature, light, and soil conditions, may also play a role in determining the TPC levels in plants [27, 28]

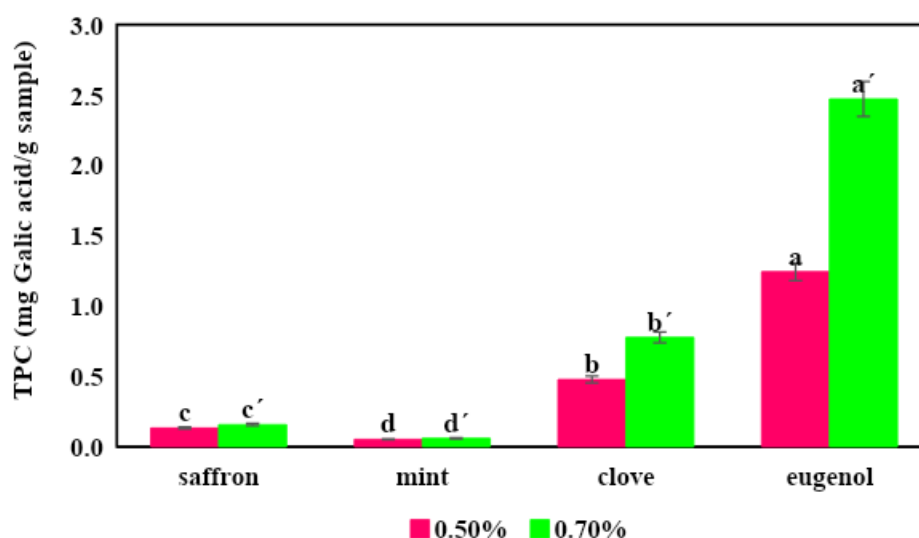


Fig 1. The total phenolic content (TPC) was determined and presented as gallic acid equivalent. The results were expressed as mean \pm SD ($n=3$). The ANOVA and Duncan methods were used to determine significant differences between the samples, with $p < 0.05$ being the threshold for significance. Different lowercase letters were used to indicate significant differences at the 95% level. "a" is better than other examples.

DPPH radical scavenging activity assay: An antioxidant's capacity to neutralize the DPPH free radicals can be determined using the DPPH scavenging method. Plant extracts are frequently evaluated for their antioxidant capacity using the DPPH assay [29]. Figure 2 shows the DPPH radical scavenging activity at two concentrations of extracts (0.5% and 0.7%). The DPPH radical inhibition by

clove and eugenol extracts were significantly different from other extracts ($p < 0.05$). It is reported that clove extract shows the highest antioxidant activity among variety of other spices [30]. The presence of antioxidant compounds like eugenol, eugenyl acetate, α -humulene, 2-heptanone, and β -caryophyllene in clove oil enables it to safeguard cells from free radical damage, resulting in

one of the most potent antioxidant activities observed among herbal medicines [31]. In our study, it seems that clove extract has significant DPPH inhibitory activity, which is attributed to the presence of its bioactive compounds, especially eugenol in accordance with other articles [32]. It is reported that the hydroxyl group, which is available on the aromatic ring of eugenol is responsible for the antioxidant activity of clove oil [33]. Karimi et al. [34] highlighted that saffron's bio actives, such as

phenolic compounds, safranal, crocetin, crocin, and carotenoids contribute to synergistic antihyperlipidemic and antioxidant potential of saffron. In the case of mint extract, it is a good source for a variety range of phenolic compounds, tannins, terpenes, terpenoids and sesquiterpene, flavonoids, quinones, coumarins, alkaloids, and sterols, which are responsible for antioxidant activity of it [35].

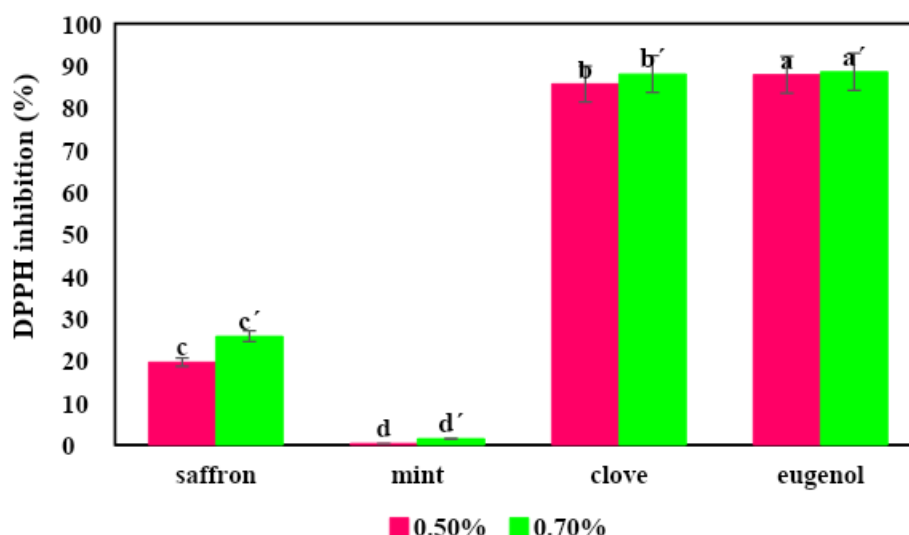


Fig 2. DPPH radical scavenging activity assay. The results were expressed as mean \pm SD (n=3). The difference at $p < 0.05$ between samples can be indicated by different small letters using ANOVA and Duncan method at the 95% level. "a" is better than other examples.

***Helicobacter pylori* inhibitory:** Around half of the world's population is affected by the common infection known as *H. pylori*. This bacterial infection is directly linked to stomach cancer, making it crucial to control its spread. Fortunately, managing *H. pylori* can significantly reduce the risk of developing stomach cancer. However, long-term use of conventional medications to control this bacterium is not advisable. Instead, herbal remedies are highly valued as an effective alternative [36]. Several scientific articles have reported that clove, saffron, and mint extracts are effective on various diseases including *H. pylori* infections [37]. In the current study, the effectiveness of each extract was tested against *H. pylori*

and the results were shown in Table 1. The concluded results showed that all the extracts were able to effectively inhibit the growth of *H. pylori*. Among samples, the highest and lowest activity in both concentrations were related to clove, and mint extracts respectively. Clove extract and eugenol had more inhibition than other samples ($p < 0.05$). In numerous articles effectiveness of β -caryophyllene from clove extracts has been proven to inhibit *H. pylori* growth [38, 39, 40].

In our study, however, it seems that eugenol is responsible for high inhibition of *H. pylori*. Also, *H. pylori* inhibitory values of saffron at 0.5 and 0.7% were

16.75±0.17, and 23.36±0.10 respectively. According to observation of Nakhaei et al., anti-*H. pylori* activity of saffron extract is mainly attributed to both safranal and crocin [41]. In the case of mint extract, inhibition percentage of 13.42 was observed at concentration of 0.7%, which can be associated with phytochemicals like flavanoids and tannins [42]. These findings suggest that incorporation of chewing gums with clove, saffron, and mint extract can have potential effect for inhibition of *H. pylori* infection as a natural remedy in human beings.

Pancreatic lipase inhibitory assay: Pancreatic lipase is an important enzyme involved in the digestion and absorption of dietary fats within the small intestine. This enzyme is synthesized and secreted by the pancreas and works to break down triglycerides present in ingested fats into smaller fatty acids and other substances that can be absorbed and utilized by the body, which causes obesity [43]. Chemical medications have demonstrated effectiveness in inhibiting the activity of lipase enzyme and have been utilized in treating obesity. However, prolonged use of these drugs can lead to significant digestive complications. Consequently, the utilization of natural compounds to inhibit lipase activity seems essential. Such natural compounds may provide a safer and more sustainable approach to managing obesity and related conditions. All the extracts tested in our study demonstrated an inhibitory effect on lipase enzyme activity. However, saffron extract exhibited the most significant reduction in lipase enzyme activity ($p < 0.05$). A study conducted on rats revealed that crocetin (an active compound found in saffron) prevented the accumulation of visceral fat and insulin resistance caused by a hyper-caloric diet, without affecting food intake [44], indicating that crocetin may have therapeutic potential as a natural

agent for the prevention and management of obesity and related metabolic disorders. In another study, saffron showed superior effectiveness over crocin suggesting that other active constituents in saffron, may be involved to prevent obesity [45]. Saffron is known to contain several active compounds, including crocin, crocetin, safranal, and picrocrocin, all of which have been reported to have inhibitory effects on lipase activity. These compounds may work in synergy to further enhance the overall inhibitory effect of saffron on lipase activity. In the case of mint, mint extract contains active compounds such as menthol and menthone that may interact with lipase enzyme molecules and inhibit their activity [46]. The inhibitory activity of lipase enzyme is influenced by the amount of phenolic content in plant extracts [47]. Our study showed that cloves, which contain high levels of phenolic compounds, particularly eugenol, demonstrated a significant inhibitory effect on lipase activity. However, saffron and mint extracts, which contain lower levels of total phenols, also exhibited significant inhibitory effects on lipase activity. It is possible that the lipase enzyme may be inhibited by the presence of other active compounds in these extracts.

ACE inhibitory: Blood pressure is regulated by the ACE enzyme, which converts angiotensin I into angiotensin II, a powerful vasoconstrictor. The result of this feature is the narrowing of blood vessels, which leads to an increase in blood pressure. One of the available methods for controlling blood pressure is to inhibit the ACE enzyme [48]. . Table 1 shows that eugenol, clove, and saffron extracts had the highest and lowest inhibitory effects on ACE (30.02, 25.54, and 19.61% respectively). Clove and eugenol extracts had a significantly different effect on ACE inhibition activity than other extracts

($p < 0.05$). Consuming foods that are rich in phenolic compounds, particularly flavanols, regularly may help lower blood pressure [49]. Based on the current study, it was found that clove and eugenol had the highest

phenolic content and exhibited the most significant level of ACE inhibition. This indicates that they may have high potential as natural remedies for hypertension by regulating blood pressure through ACE inhibition

Table 1. Enzymatic inhibitory properties of extracts

Concentration	plant extract	<i>Helicobacter pylori</i> inhibitory (%)	Pancreatic lipase inhibitory (%)	Concentration	ACE inhibitory (%)
0.5%	saffron	16.75±0.17 ^c	36.06±0.27 ^a	0.1%	19.61±0.51 ^c
	mint	11.15±0.14 ^d	25.61±0.36 ^c		
	clove	21.25±0.21 ^b	26.03±0.21 ^c		20.70±0.68 ^c
	eugenol	21.83±0.18 ^a	29.90±0.10 ^b		
0.7%	saffron	23.36±0.10 ^c	40.07±0.15 ^a		25.54±0.85 ^b
	mint	13.42±0.23 ^d	29.24±0.21 ^c		
	clove	25.69±0.16 ^b	31.50±0.17 ^b		30.02±0.34 ^a
	eugenol	29.65±0.29 ^a	31.64±0.12 ^b		

The results were expressed as mean ±SD (n=3). The ANOVA and Duncan method at the 95% level indicate samples differ significantly at $p < 0.05$ when using different small letters. "a" is better than other examples.

Antibacterial effect: Figure 3 shows the significant inhibitory effect of extracts on pathogenic microorganisms such as *Enterococcus faecalis* and *Candida albicans*. Plant extracts are recognized for their robust antibacterial properties, which make them a highly favored and effective natural treatment for a range of bacterial infections. Compared to synthetic antimicrobial compounds, plant extracts offer a safe and natural alternative for treating these bacterial infections [50]. Several bioactive compounds, including alkaloids, flavonoids, phenolics, and terpenoids, can be found in plant extracts that have antibacterial properties. Inhibiting the growth and reproduction of bacteria is made possible by these compounds in plant extracts,

which makes them effective in treating bacterial infections [51]. The antibacterial properties of clove, saffron, and mint extracts against *Enterococcus faecalis* and *Candida albicans* can be attributed to the presence of various bioactive compounds in these extracts. Clove extract contains eugenol, which has been shown to have strong antibacterial properties [52]. Crocin and safranal, which are present in saffron extract, possess significant antimicrobial properties against multiple bacteria and fungi [53]. Mint extract contains various bioactive compounds such as menthol, menthone, neomenthol, and iso menthone, which are responsible for its antibacterial activities [5].



Fig 3. Antimicrobial activity of *Streptococcus faecalis* and *Candida albicans*

Sensory evaluation of chewing gums incorporated

extracts: Sensory evaluation analysis aids in identifying essential factors that should be taken into consideration during the process stages, future commercialization of the product, and consumer acceptance. The results of several organoleptic properties of chewing gum are given in Table 2. The results revealed that the control group had all chewing gum formulations that were like each other in appearance and hardness, except for chewing gum that was flavored with saffron. The more score for appearance may be due to the shiny-yellow color of saffron, and the lower score (Softer texture) can be associated with the presence of saffron's polyphenols within the gum matrix. In terms of taste, chewing gum flavored with saffron and mint-clove obtained more scores than other samples. Moreover, although the taste of chewing gums enriched with clove at concentration of

0.1 and 1% were like that of control, clove at concentration of 3% gained the lowest score due to creating the astringency sensation in some panelists, which results from interactions of flavonoids with salivary proteins. Thus, it can be concluded that mint could effectively improve off-flavor caused by clove. In addition, it appears that altering the concentration of clove in the formulation could result in notable modifications to the sensory characteristics of the chewing gum. However, incorporating even 1% of clove does not adversely impact the sensory evaluation, thanks to the gum base's capability to conceal the aftertaste of clove extract in chewing gums. Finally, from the results, it can be concluded that chewing gums enriched with saffron and mint-clove can be a suitable carrier for the delivery of bioactive compounds with the final acceptance by the customers.

Table 2. Sensorial properties of chewing gums enriched with clove, saffron, and mint-clove extracts

	Control	Clove 3%	Clove 1%	Clove 0.1%	Saffron	Mint-Clove
Appearance	8.10±0.20 ^b	7.60±0.35 ^b	7.80±0.30 ^b	7.90±0.25 ^b	9.30±0.10 ^a	8.06±0.32 ^b
Hardness	6.16±0.51 ^a	6.06±0.15 ^a	6.38±0.48 ^a	6.23±0.51 ^a	5.33±0.35 ^b	6.43±0.30 ^a
Chewiness	5.80±0.02 ^a	5.53±0.35 ^a	5.85±0.41 ^a	6.03±0.68 ^a	5.33±0.35 ^a	5.57±0.20 ^a
Taste	7.60±0.26 ^b	3.26±0.80 ^c	7.50±0.30 ^b	7.43±0.30 ^b	8.40±0.45 ^a	8.43±0.20 ^a
Total acceptability	7.43±0.28 ^b	3.17±1.05 ^c	7.16±0.25 ^b	7.20±0.36 ^b	8.16±0.37 ^a	8.46±0.41 ^a

The outcomes were presented as mean ±SD (n=3). The usage of distinct lowercase letters in the same line indicates a significant disparity among samples at $p < 0.05$. "a" is better than other examples.

Releasing behavior: Since chewing gum is not entirely consumed, the initial digestion of its components primarily occurs in the oral cavity. As a result, the release behavior of the functional components can be evaluated during mastication and recorded. In our investigation, we utilized a swift approach employing spectrophotometer apparatus to analyze the dispersion of integrated extracts from chewing gums, opting for commonly available laboratory equipment rather than intricate devices. In the case of chewing gums enriched with clove extract, chewing gum containing clove at a concentration of 1% was preferred to investigate releasing characteristics, because this formulation was closer to the control group than chewing gums composed of clove at concentration of 3%. For this objective, a pair of volunteers were selected, and each participant was given the opportunity to chew two samples of chewing gum for specific durations, namely 5, 10, 15, and 20 min. The results of the release behavior of functional compounds from extracts at different time intervals are shown in

Figure 4. As expected, results showed that as the chewing time increased, the releasing amounts of extracts increased, and the highest release was observed at the end of the chewing period (73.24, 64.34, and 63.2% in saffron, mint, and clove respectively). Also, it was observed that the release amounts of the extracts varied according to the type of extract, and the difference between extracts remaining in the gum after 5, 10, 15, and 20 min was significant ($p < 0.05$) in chewing gum incorporated with clove extract. Considering the releasing amounts, it was observed that saffron was released faster than clove or mint during mastication. This behavior might be associated with the fact that the gum matrix is composed of hydrophobic components, therefore, mint and clove, which are more fat-soluble than saffron, are more likely to interact with the gum base than partition into the saliva. No negative alterations associated with extract oxidation, such as alterations in taste or color, were detected throughout the 6-month storage duration of the chewing gums.

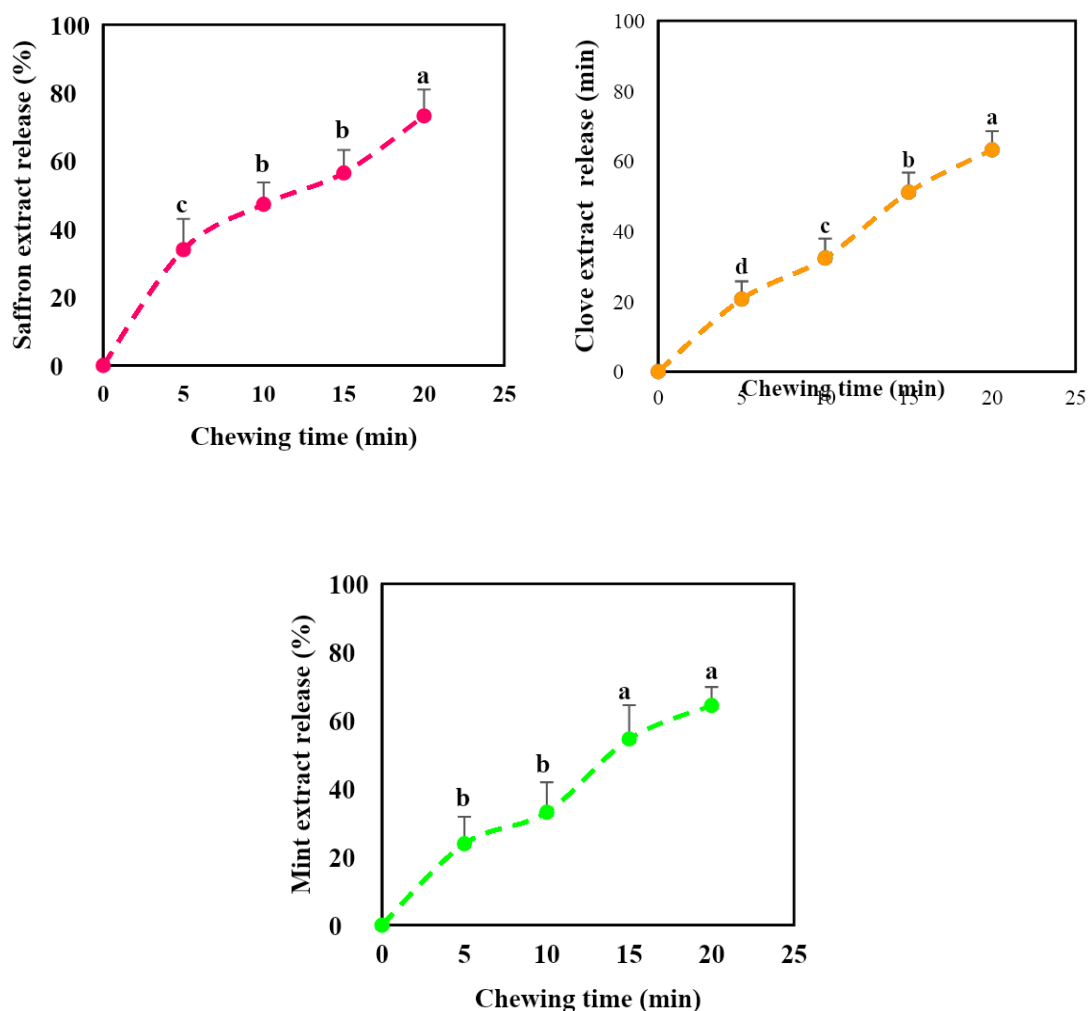


Fig 4. Releasing amounts of extracts from chewing gums chewed by individuals at 5, 10, 15, and 20 min. "a" is better than other examples.

CONCLUSION

In recent years, there has been a notable increase in obesity rates, leading to the development of related diseases. Therefore, natural and herbal compounds such as plant extracts are suggested as potential alternatives to obesity drugs with adverse effects, to manage obesity and improve overall health. Based on our study findings, it was observed that extracts exhibited a favorable influence in managing obesity. The observed suppression of pancreatic lipase enzyme implies that these compounds hold significant potential in effectively combating obesity. However, further research is necessary to fully comprehend the efficacy of these substances for this purpose. The significant antioxidant

properties of clove may have a promising impact on reducing free radicals, which can help prevent fat oxidation in the bodies of individuals with obesity. The inhibition of ACE enzyme by extracts could potentially have a positive impact on controlling blood pressure. The extracts showed potent antimicrobial properties that can potentially alleviate mouth and throat issues associated with *Streptococcus* bacteria. Moreover, extracts demonstrated efficacy in reducing the presence of *H. pylori*, which is the causative agent of stomach infections. Also, it was shown that chewing gum can be a convenient and easily accessible means of delivering natural compounds and may be a desirable option for individuals

seeking natural alternatives for managing their health concerns.

Abbreviations: TPC: Total phenolic content, DPPH: 2,2-Diphenyl-1-picrylhydrazyl, DMSO: Dimethyl sulfoxide, ACE: Angiotensin converting enzyme.

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