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Evaluation of The Properties of Frankincense Powder and Its Water Extracts and The Effect of Its Addition on Guava Nectar Characteristics

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FRANKINCENSE (*Boswellia carterii*) has many medicinal benefits. In Egypt, a few people like to use it in chewing and sometimes in incense. This study aimed to use the water extract of frankincense powder prepared by soaking in drinkable water for 24 hr (T1) and boiling in water for one hour (T2) for the fortification of guava nectar in concentrations ranging from 0.25 to 2% (volume/ volume (v/v)). The chemical composition, phenols, and flavonoids contents of the frankincense were determined. Anti-oxidant (DPPH method) and antimicrobials (pathogenic bacteria) and cytotoxicity (HepG-2 and MCF-7 human cancer cell lines) activities were measured. Sensory evaluation and the physical properties of the guava nectar mixed with water extracts of frankincense were evaluated. The phenols and total flavonoid contents of the T2 sample were 0.869 and 0.147 mg/g, respectively. However, it was 0.748 and 0.070 mg/g, respectively for T1. The results showed that frankincense powder has 93.74% antioxidant activity with 67.26 mg/ml IC_{50}. The results also showed that the T2 sample has antibacterial activity against *Salmonella typhi* and *Bacillus cereus*. The results also showed that the frankincense powder has cytotoxic activity and this activity is strong on HepG-2 human cancer cell lines compared to the positive control (doxorubicin), however, it is less activity on the human breast adenocarcinoma (MCF-7). The IC_{50} values of the frankincense powder were 7.8 and 33.9 µM, respectively, while doxorubicin values were 10.3 and 28.5 µM, respectively. The results of the sensory evaluation showed that water extracts of the frankincense can be added to guava nectar at concentrations up to1%. It can be concluded that it is possible to mix aqueous extracts of the frankincense with guava nectar to obtain a product with a pleasant taste, color, and aroma and having also the advantages of frankincense.

Keywords: Frankincense (*Boswellia carterii*), Anti-oxidant a nd anti-microbial activities, Cytotoxicity activity

Introduction

Frankincense is a resin obtained from the trees belong to the genus boswellia (family Burseraceae). It has orange-brown color and a direct relationship was found between levels of pigments and phenolic compounds (Pietta, 2000 and Elham et al., 2006). There are many species and varieties of frankincense trees, such as *Boswellia carterii*, *Boswellia serrata*, *Boswellia frereana*, and *Boswellia sacra* in East Africa and China, India, Somalia, and Arabia, respectively. The differences between frankincense resin species depend on soil and climate. The frankincense tree plays an important role in the economy of many Middle Eastern countries. It is also grown in many African countries such as Somalia, Sudan, and southern Egypt (Maloney, 1997 and Schmiech et al., 2019).

Frankincense has many medical benefits as it used in folk medicine and in the treatment of many

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diseases such as tumors, ulcers, leprosy, dysentery and chest diseases such as coughing, asthma, and distress breathing, also it is used as a tonic for the heart (Bonjar, 2014 and Aldory et al., 2018).

Boswellic resin improves memory and learning in treated Alzheimer's group, modulates the oxidative stress and be involved in the protective effect against amyloid deposition and neurodegeneration, and stimulates the immune system in mice's brain (Zerrouki et al., 2020).

Frankincense is used in most countries of the world. Frankincense is one of the most famous materials that are sold and found in aromatherapy shops and Arab medicinal herbs. Frankincense is used to treat many diseases, in addition to it has very slight side effects, and it is cheap natural sources, especially when compared to the side effects of some antibiotics that a human uses, in addition to the high prices of these antibiotics (Hamidpour et al., 2015).

Frankincense is commonly used in pharmaceutical products because it contains active compounds and is useful in treating many human diseases. The taste of frankincense is bitter, so few people like to chew like frankincense, so there is little research on frankincense in supporting foods. Among the nutritional applications is the use of frankincense in supporting broiler chicken diets with *Boswellia serrata* resin rich in biologically active ingredients as this application led to improve productivity, chemical composition and nutritional value of the meat produced (Al-Yasiry et al., 2017)

Guava is a tropical and subtropical fruit. Most guavas have a green peel and white pulp, whose shape is sphere and oval. Guava could benefit diabetes, wounds, diarrhea, inflammation, and hypertension. The extracts from guava showed antispasmodic, anti-inflammatory, anticancer, antioxidant, and hypoglycemic effect (Arakawa et al., 2004 and Guo et al., 2013)

Guava is a popular fruit for many people. Guava is present throughout the year in many forms, for example, guava nectar. Guava contains nutrients such as moisture, carbohydrates, protein, fats, and fiber with percentages 82, 15.7, 1.1, 0.4, and 5.3%, respectively (Hui, 2006). Also, guava contains calcium, magnesium, phosphor, iron, sodium, and zinc in percentages 18.5, 22.4, 36.5, 0.4, 2.2, and 0.2%, respectively (Mgaya et al., 2014).

This study aimed to measure the anti-oxidant and anti-microbial activities as well as cytotoxicity of frankincense (*Boswellia carterii*) powder and its water extracts and the effect of fortification of guava nectar with these extracts on some physical properties and sensory characteristics of the fortified guava nectar.

Materials and Methods

Materials

Frankincense (*Boswellia carterii*) resin was purchased from Harraz Herbs Company (http://www.harrazherbs.com), Cairo, Egypt and authenticated as the resin of *Boswellia carterii* Birdwood (Somalia) by Prof. Dr. Fathy M. Soliman by comparison with a genuine sample kept in the Drug Museum of Pharmacognosy Dept., Faculty of Pharmacy, Cairo University, Egypt. Guava was purchased from the local market. The cells of human liver carcinoma (HepG-2), and human breast adenocarcinoma (MCF-7) were purchased from the American Type Culture Collection (Rockville, MD). All cells were maintained in a DMEM medium, which was supplemented with 10% of heat-inactivated fetal bovine serum (FBS), 100U/mL of each of penicillin and streptomycin. The cells were grown at 37°C in a humidified atmosphere of 5% CO2. Microorganisms, which have been used in this study are bacteria (*Bacillus cereus, Bacillus subtilis, Staphylococcus aureus*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Salmonella typhi,* and *Enterococcus*) which were kindly provided by National Research Centre, Giza, Egypt. All chemicals used were of analytical reagent grade and were obtained from Sigma-Aldrich (St. Louis, MO, USA).

Methods

Preparation of frankincense extracts

Eighty grams of frankincense (*Boswellia carterii*) resin were ground and then 200 mL drinkable water was added. This mixture was divided into two equal parts. The first part was still in the water at room temperature for 24 h (T1). The second part was boiled at 100 °C for one h (T2). The two parts were purified using a piece of gauze filters and then dried at 55 °C in the electric oven overnight (Laboratory Drying Oven-Roshan Enterprises, 10-300 Degree Celsius, 200-240 V). The dried products were dissolved in drinkable water to prepare a 10% extract (weight/volume (w/v). The extracts were centrifuged (Lab Centrifuge 16,500 rpm - High-Speed Centrifuge (H-1650) to remove insoluble parts and then kept in dry and clean bottles at -18 °C until analysis.

The formula of guava nectar fortified by different concentrations of the frankincense water extract

Guava fruits were washed and cut, and the seed was removed. The prepared guava was put in an electric mixer (Moulinex, Super Electric Blender, 700 Watt, LM207125, France), and then the drinkable water, sugar, and different ratios of frankincense water extracts (0.25, 0.50, 0.75, 1, 1.5, and 2%) as shown in Table 1 were added and mixed well after adding 0.4 g of citric acid. The fortified nectar was pasteurized at 85 °C for one minute. Each nectar was stored in a dry and clean bottle at 4 °C until analysis.

Analytical methods

Chemical composition of frankincense (Boswellia carterii) powder

Proximate chemical components contents of frankincense (*Boswellia carterii*) powder including moisture, protein, ash, and fat were conducted in Food Technology Res. Institute according to the method described by the AOAC (2005). The minerals (iron (Fe), magnesium (Mg), copper (Cu), potassium (K), manganese (Mn), zinc (Zn), calcium (Ca), phosphorous (P) and sodium (Na)) were determined by using atomic absorption spectrophotometer (model 3300, Perkin-Elimer, Beaconsfield, UK) according to the methods outlined in AOAC (2005). Carbohydrate was calculated by difference.

Sensory evaluation of guava nectar fortified by different concentrations of frankincense (Boswellia carterii) water extracts

The fortified guava nectar by different concentrations of frankincense water extracts (T1 and T2) were evaluated for their sensory characteristics. The sensory attributes including color, odor, taste, consistency, appearance, and overall acceptability were evaluated by 15 trained members' panelist from Food Technology Research Institute, Agricultural Research Center, Giza, Egypt. Each panelist was provided with the guava nectar understudy in an unlabeled transparent cup under white lights and asked to cleanse the palate with the water between the samples tasting according to El-Gendy & El-Hadidy (2016).

Physical characteristics of guava nectar with and without different concentrations of frankincense (Boswellia carterii) water extracts

The pH value of the guava nectar with and without different concentrations of frankincense water extracts was determined according to AOAC (2005). Color values of the same previous samples were measured in terms of *L** (brightness), *a** (red to green color), and *b** (yellow to blue color) values, using Chroma meter (Konica Minolta, model CR 410, Japan).

Phenolic and flavonoids compounds contents of frankincense (Boswellia carterii) powder and its extracts

Phenolic compound contents of frankincense (*Boswellia carterii*) powder and its extracts (T1 and T2) were determined calorimetrically using Folin–Ciocalteu reagent (as gallic acid) according to Singleton et al. (1999). However, the total flavonoid compounds were determined (as quercetin) according to the method described by Marinova et al. (2005).

Antioxidant activity of frankincense (Boswellia carterii) powder and its extracts

The antioxidant activities of frankincense powder and its extracts were determined using the free radical, 2,2-diphenyl-1-picrylhydrazyl (DPPH) as a reagent, and the extract concentration providing 50% inhibition of radical scavenging activity (IC_{50}) was calculated and expressed as µl/mL according to the method described by Cuendet et al. (1997).

TABLE 1. Guava nectar ingredients with different concentrations of the frankincense water extract*

Blends	Frankincense (water extract 10%) (mL)	Water (mL)	Guava (g)	Sugar (g)
Guava nectar (Control)	-	100	80	20
Guava nectar + 0.25% frankincense	5	95	80	20
Guava nectar + 0.50% frankincense	10	90	80	20
Guava nectar + 0.75% frankincense	15	85	80	20
Guava nectar + 1% frankincense	20	80	80	20
Guava nectar + 1.5% frankincense	30	70	80	20
Guava nectar + 2% frankincense	40	60	80	20

* Frankincense water extract: prepared by soaking it in cold drinkable water for 24 hr (T1) - prepared by boiling it in water for 1 hr. (T2).

Antibacterial activity of frankincense (Boswellia carterii) water extracts

Antibacterial activity of frankincense water extracts was analyzed by the agar well diffusion method according to (Srinivasan et al., 2001). The MIC (the minimum inhibitory concentration of the frankincense water extracts) was determined according to the method described by NCCLS (2003), Also MBC (the minimum bactericidal concentration of the frankincense water extracts) was determined according to Zhao et al. (2010).

Cytotoxicity and antitumor activity of frankincense (Boswellia carterii) powder on human cell lines

The cytotoxicity activities on the HepG-2 and MCF-7 human cancer cell lines were estimated, employing the 3-[4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay, which was grounded on the reduction of the tetrazolium salt by the mitochondrial dehydrogenases in viable cells (Alminderej et al., 2019).

Statistical analysis

Means ±SD of the results are statistically analyzed using one-way analysis of variance (ANOVA), $p<0.05$ was used to indicate significance. Statistical software (Assistat Version 7.7, Brazil) was used for all statistical analyses according to Silva & Azevedo (2009).

Results and Discussion

Chemical composition and minerals content of frankincense (Boswellia carterii) powder

The results presented in Table 2 showed the chemical composition and minerals contents of frankincense powder on a dry weight basis. It could be noticed that frankincense powder contained a high amount of oil (70.72%) and carbohydrate (25.20%). It also indicated that the Ca showed the highest content (9833.74 ppm), followed by K (3482.99 ppm) then Na (1595.50 ppm), and finally Mg (1268.58 ppm). However, the lowest contents were observed for Cu, Mn, Fe, Zn, and P. Al-Yasiry et al. (2017) reported that the frankincense resin (*Boswellia serrata*) comprised 95.34% dry matter. They found that the contents of ash, protein, fat, and gum resin acetyl-11-keto-ß-boswellic acid on the dry matter were 1.59%, 2.65%, 63.88%, and 2.38%, respectively.

Sensory evaluation of guava nectar fortified by different concentrations of frankincense water extracts.

Frankincense was soaked in cold water for 24 h (T1) and in another treatment was boiled with water for 1hr (T2) and the obtained extracts were added to guava nectar at different concentrations as indicated in Table 1. All treatments were sensory evaluated as well as the control sample and the obtained results are shown in Table 3. From these results, it could be observed that the addition of 0.25% and 0.50% of T1 to guava nectar showed no effect on color, and no significant differences were found compared to the control sample ($p > 0.05$). A similar result was found when 0.25% of T2 was added to guava nectar and such treatment was found also to be not significantly different from other treatments which contained 0.50, 0.75, 1.5, and 2% of T2 ($p > 0.05$). The same results also indicated that the addition of T1 up to 0.75% and T2 up to 0.50% led to obtaining guava nectar not differed significantly from control for odor ($p > 0.05$). The result indicated that the taste of guava nectar which contained 0.25% of T1 was found to be not significantly different from control and also from the samples contained 0.50% and 0.75% of T1 ($p > 0.05$). The addition of 0.25% and 0.50% of T2 to the nectar showed a significant difference from the control sample for taste ($p < 0.05$) but it was not significantly different in between ($p > 0.05$).

TABLE 2. Chemical composition and minerals content of frankincense powder (on dry weight basis).

Components (%)	Values	Minerals (ppm)	Values
Protein	2.90±0.54	**Fe**	13.55±0.72
Oil	70.72±0.62	**Mg**	1268.58±0.20
Ash	1.10±0.53	**Cu**	8.33±0.47
Fiber	0.08±0.04	**K**	3482.99±0.68
Carbohydrate*	25.20±0.59	**Mn**	11.47±0.63
Moisture	7.25±0.50	**Zn**	31.28±0.35
		Ca	9833.74±0.45
		P	161.11±0.67
		Na	1595.50±0.80

* Carbohydrate was calculated by the difference. Values are mean of three replicates ± SD.

The highest scores for taste were obtained with the addition of T1 up to 1% and T2 up to 0.75%. The results indicated that the addition of frankincense (T1 or T2) was found to not affect the consistency of guava nectar ($p > 0.05$). However, the addition of 1.5% and 2% of T2 showed no significant difference from the sample that contained 1% of T2 for consistency ($p > 0.05$). The highest scores for appearance were obtained with the addition of T1 up to 1% and T2 up to 1.5% with no significant differences compared to control($p > 0.05$). However, the samples contained 1.5% and 2% of T1 were found to be not significantly different from those contained 0.50%, 0.75, and 1% ($p > 0.05$) but it was significantly different from control ($p < 0.05$). Also, the samples contained 2% of T2 were not significantly different from other samples but it was significantly different from the control sample for appearance. The results also indicated that the addition of T1 to guava nectar up to 0.50% showed no significant differences compared to control for overall acceptability. However, the addition of T1 up to 1% showed the highest scores for overall acceptability, and the samples of the treatments (0.25, 0.50, and 0.75%) were found to be not significantly different in between. Also, the addition of T2 to guava nectar up to 0.75% showed the same trend.

From these results, it could be revealed that frankincense water extracts (T1 and T2) could be added to guava nectar at levels up to 1% to obtain products having the best scores for all the evaluated characteristics.

Effect of addition of frankincense water extracts on physical properties of guava nectar

The effect of the addition of frankincense either that soaked in cold water for 24 hr (T1) or that boiled in water for 1 hr (T2) to guava nectar on physical properties was studied and the results presented in Table 4. From these results, it could be noticed that the Brix values were 12°Bx for control and all treatments. pH values were also the same for control and all treatment samples since it varied between 3.9 and 4.1 concerning color parameters, the same results indicated that little changes could be observed for L^* values since it varied between 54.33 for the addition of 1% of T1 and 57.12 for the addition of 0.50% of T2 compared to 54.43 for the control sample. The same trend was observed for a^* values since it varied between 11.27 for the addition of 0.75% of T1 to 11.92 for the addition of 1% of T2 comparing with 11.70 for the control sample. Concerning the b^* parameter, the same results indicated that

TABLE 3. Sensory evaluation of guava nectar fortified with different concentrations of the frankincense water extract*

Treatments			Color	Odor	Taste	Consistency	Appearance	Overall acceptability
guava nectar			$9.6^{a}\pm0.51$	$9.5^{a}\pm0.52$	$9.5^{a}\pm0.70$	$9.5^{a}\pm0.70$	$9.2^{a}\pm0.78$	$9.4^{a}\pm0.49$
T1: guava nectar +	frankincense water extract (%)	0.25	$8.9^{ab}\pm0.87$	$9.1^{ab}\pm0.73$	$8.7^{ab}\pm0.82$	$9.5^{a}\pm0.52$	$9.1^{a}\pm0.73$	$9.0^{ab}\pm0.58$
		0.50	$8.9^{ab}\pm0.73$	$8.8^{abc}\pm0.91$	$8.5^{bc}\pm0.82$	$9.4^{ab}\pm0.69$	$9^{abc}\pm0.95$	$8.9^{ab}\pm0.62$
		0.75	$8.6^{bc}\pm0.96$	$8.5^{abc}\pm0.97$	$8.0^{bcd}\pm0.76$	$9.5^{a}\pm0.52$	$8.9^{abc}\pm0.88$	$8.6^{bc}\pm0.59$
		1	$7.7^{cde}\pm0.82$	$8.0^{bcd}\pm0.94$	$7.5^{cde}\pm0.89$	$9.2^{ab}\pm0.78$	$8.8^{abc}\pm0.91$	$8.2^{bc}\pm0.69$
		1.5	$7.0^{e}\pm1.15$	$6.4^{g}\pm1.17$	$6.1^{f}\pm0.99$	$8.4^{ab}\pm1.57$	$7.9^{c}\pm1.28$	$7.1^{d}\pm0.96$
		2	$7.4^{de}\pm0.96$	$6.8^{efg}\pm1.31$	$5.7^{f}\pm1.33$	$8.4^{ab}\pm1.26$	$8.0^{bc}\pm1.24$	$7.2^{d}\pm0.90$
T2 guava nectar +	frankincense water extract (%)	0.25	$8.9^{ab}\pm0.73$	$8.7^{abc}\pm1.16$	$8.4^{bc}\pm1.07$	$9.2^{ab}\pm0.78$	$9.0^{ab}\pm0.66$	$8.8^{ab}\pm0.80$
		0.50	$8.6^{bc}\pm1.17$	$8.3^{abc}\pm1.05$	$7.5^{cde}\pm0.90$	$8.9^{ab}\pm1.37$	$8.5^{abc}\pm1.08$	$8.3^{bc}\pm0.97$
		0.75	$8.6^{bc}\pm0.96$	$7.9^{bcde}\pm1.44$	$7.2^{de}\pm1.03$	$8.9^{ab}\pm1.37$	$8.5^{abc}\pm0.85$	$8.2^{bc}\pm0.88$
		1	$8.6^{bc}\pm0.84$	$7.7^{cdef}\pm1.41$	$6.5^{ef}\pm0.97$	$8.7^{ab}\pm1.25$	$8.2^{abc}\pm1.03$	$7.9^{cd}\pm0.88$
		1.5	$8.0^{bcd}\pm1.24$	$6.7^{fg}\pm1.82$	$5.9^{f}\pm1.15$	$8.3^{b}\pm1.33$	$8.0^{bc}\pm1.24$	$7.3^{d}\pm1.0$
		2	$8.0^{bcd}\pm1.05$	$6.0^{defg}\pm1.52$	$5.6^{f}\pm1.64$	$8.3^{b}\pm1.49$	$7.9^{c}\pm1.44$	$7.3^{d}\pm1.1$

* Frankincense water extract: prepared by soaking it in cold drinkable water for 24 hr. (T1) - prepared by boiling it in water for 1 hr. (T2).Values are mean of three replicates ± SD, number in the same column followed by the same letter is not significantly different at 0.05 level

it varied between 31.47 for the addition of 0.75% of T1 to 36.29% for the addition of 0.50% of T2 compared to 36.21 for the control sample. These results indicated that in general addition of frankincense water extracts to guava nectar has no effects on the determined physical properties. The results of the control guava nectar are in agreement with Bogha et al. (2020) who reported that the Brix and the pH of guava were 11.4 and 3.9, respectively.

Phenolic and flavonoid contents of frankincense powder and its extracts

Many health-promoting compounds such as high concentrations of phenolic compounds, vitamins, fiber, and minerals may protect people from some chronic diseases, although they are not necessary to stay life (Mullen et al., 2007). The total phenolic compounds (as gallic acid) and flavonoids compounds (as quercetin) of frankincense powder and the two treatments T1 and T2 were determined (Table 5). The results presented in Table 5 indicated that frankincense powder contained 2.9 mg/g and 0.262 mg/g of total phenolic and flavonoid compounds, respectively. The frankincense which heated with water for 1 h (T2) contained the highest amount of total phenolic compounds and of total flavonoid compounds being 0.869 mg/g and 0.147 mg/g, respectively comparing to 0.748 mg/g and 0.070 mg/g, respectively for frankincense that soaked in water for 24 hr (T1). Sadek et al., (2013) determined the total phenol and flavonoids contents in the water extract of frankincense (*Boswellia carterii*) and they found that every 100 mL of the water extract contained 180 mg as gallic acid equivalent and 0.58 mg as catechin equivalent, respectively. Kim et al. (2008) found that heat treatment increases the biological activities of different foods, due to the chemical changes that occur during heat treatment. They also reported that heat treatment in addition to soaking in a solvent, such as ethanol led to increasing phenolic compounds.

Antioxidant activity of frankincense powder and frankincense water extract

The antioxidant activities of the frankincense powder and frankincense water extract can be attributed to the presence of total phenolic and flavonoid compounds that are found in the resin. The results presented in Table 6 showed the DPPH radical scavenging activity of frankincense powder, T1, and T2 (frankincense water extracts). Noteworthy, there is a direct correlation between the concentration of the frankincense powder as well as the water extracts (T1 and T2) and the antioxidant activity. Results showed that the antioxidant activity of frankincense powder was higher than that of

TABLE 4. Physical properties of guava nectar fortified with different concentrations of the frankincense water extract* .

Treatment			Brix (°Bx)	pH	Color		
					*L**	*a**	*b**
guava nectar			12	4.1^{a}±0.058	54.43^{c}±0.57	11.70^{a}±0.59	36.21^{a}±0.61
T1 guava nectar +	frankincense water extract (%)	0.25	12	4.0^{ab}±0.057	55.80^{bc}±0.55	11.66^{a}±0.62	34.23^{abc}±0.76
		0.50	12	4.0^{ab}±0.057	55.41^{bc}±0.59	11.63^{a}±0.64	33.2^{4cd}±0.55
		0.75	12	4.0^{b}±0.057	54.38^{bc}±0.48	11.27^{a}±0.70	31.47^{d}±0.70
		1	12	3.9^{b}±0.057	54.33^{bc}±0.50	11.74^{a}±0.60	33.20^{bc}±0.64
T2 guava nectar +	frankincense water extract (%)	0.25	12	4.0^{ab}±0.057	55.42^{abc}±0.51	11.60^{a}±0.72	35.60^{a}±0.56
		0.50	12	4.1^{ab}±0.057	57.12^{a}±0.64	11.80^{a}±0.53	36.29^{a}±0.74
		0.75	12	4.1^{ab}±0.057	55.46^{abc}±0.43	11.66^{a}±0.61	34.51a^{bc}±0.36
		1	12	4.0^{ab}±0.057	56.06^{ab}±0.55	11.92^{a}±0.43	35.57^{ab}±0.67

Frankincense water extract: prepared by soaking it in cold drinkable water for 24 hr.(T1) - prepared by boiling it in water for 1 hr. (T2). L (lightness with L* = 100 for lightness, and L* = zero for darkness), a* [(chromaticity on a* green (–) to red (+)], b* [(chromaticity on a blue (–) to yell low (+)].Values are mean of three replicates ± SD, number in the same column followed by the same letter is not significantly different at 0.05 level

TABLE 5. Total phenolic and flavonoid contents of frankincense powder and its extracts (T1 and T2)*.

Treatments	Total phenolic (mg/g GAE?)**	Flavonoid (mg/g quercetin)***
Frankincense powder	2.9±0.56	0.262±0.05
T1	0.748±0.06	0.070±0.007
T2	0.869±0.05	0.147±0.05

*Frankincense water extract: prepared by soaking it in cold drinkable water for 24 hr.(T1) - prepared by boiling it in water for 1 hr. (T2). **Total phenolic (mg/g): calculated as gallic acid - ***Flavonoid (mg/g): calculated as quercetin. Values are mean of three replicates ± SD

other samples, followed by the water extracts T1 then T2. The maximum antioxidant activity was found using a concentration of 1000 µl/ml of frankincense powder (97.64%) and it was 74.13% for T1, while it was 62.07% for T2. However, the antioxidant activity of the frankincense powder at 500 µl/mL was 93.74%. Also, it could be observed that IC_{50} for frankincense powder was 67.26 µl/mL, while for T1 and T2 it was 407.76 µl/mL and 603.84 µl/mL, respectively. The concentration of all frankincense water extracts which added to guava nectar covered these IC_{50} values so, the resulted nectar will be having an antioxidant activity that led to an increase the shelf life. These results are in coincided with the results of Sadek et al. (2013). They found that the frankincense water extract (*Boswellia carterii*) prepared by dissolving 433 mg of powdered gum resin in 100 mL of boiling water and soaking for 10 hr had 94.1% free radical scavenging activity. Also, Sharma et al. (2011) found that the aqueous extract of *Boswellia serrata* had 94.6% free radical scavenging activity at the concentration of 500 mg/mL. Pandey et al., (2005) found that the water-soluble fraction of the Boswellia frankincense possesses antioxidant activity as it can inhibit induced nitric oxide. Celep et al., (2012) reported that the phenolic compounds have been proved to be an antioxidant better than vitamins E and C because they can chelate metal ions and be electron donors. Briones-Labarca et al., (2015) and Singh et al. (2020) reported that there is a positive relationship between antioxidant activity and total phenols content.

Antibacterial activity of frankincense water extracts

Antimicrobial activity of frankincense water extracts was studied against Gram-positive (*Bacillus cereus*, *Bacillus subtilis*, *Staphylococcus aureus,* and Enterococcus) and Gram-negative (*Klebsiella pneumonia, Pseudomonas aeruginosa* and *Salmonella typhi*) bacteria. The antibacterial activity was assessed by evaluation of the inhibition zone (IZ) and the determination of MIC and MBC values. The results are shown in Table 7. It could be observed that the T1 and T2 samples showed varying degrees of antibacterial activity against all strains tested. The inhibition zones were in the range of 6 – 13 mm. The results indicated that the obtained inhibition zones with T2 treatment were higher than those obtained with T1 treatment for most of the tested microorganisms. However, the highest inhibition zone obtained with T2 treatment (13 mm) was against *Salmonella typhi* followed by 11 mm against *Bacillus cereus* then 9 mm against *Bacillus subtilis* and *Klebsiella pneumonia* and the lowest inhibition zone value (7 mm) was found with *Staphylococcus aureus*, Enterococcus, and *Pseudomonas aeruginosa.* The same trend was also observed with T1 treatment. Concerning MIC values the same results indicated that T1 treatment showed values varied between 1.25 for Enterococcus and *Klebsiella pneumonia* and 25 mg/ml with *Staphylococcus aureus*. Concerning T2 treatment the MIC values varied between 1.25 mg/mL with Enterococcus, *Klebsiella pneumonia*, and *Salmonella typhi,* and 50 mg/mL with *Pseudomonas earuginosa.* Moreover, MBC values for T1 treatment also varied between 50 mg/mL with *Staphylococcus aureus* and 3.1 Enterococcus and for T2 treatment the MBC values varied between 50 mg/mL with *Staphylococcus aureus* and *Pseudomonas earuginosa* and 3.1 mg/mL for all other microorganisms under study. The obtained results reviled that T2 has a slightly greater effect than the effect of T1 and this may be due to the fact that the hot extract contains total phenolic and flavonoid compounds (which had antimicrobial activity Prakash et al., 2020) more than cold extract as shown in Table 5. Concerning MBC was found the same trend but it was equal

TABLE 6. Radical scavenging activity (%) of frankincense powder and its extracts (T1 and T2)*

Concentration (µL/mL.)	Radical scavenging activity (%)		
	frankincense powder	T1	T2
1000	97.64 ± 0.02	74.13 ± 0.16	62.07 ± 0.23
500	93.74 ± 0.32	54.67 ± 0.0.23	44.75 ± 0.35
250	87.68 ± 0.12	42.93 ± 0.45	41.39 ± 0.33
125	62.59 ± 0.23	34.138 ± 0.23	25.63 ± 0.54
62.5	48.60 ± 0.12	20.183 ± 0.1	15.44 ± 0.21
IC_{50}**	67.26±0.42	407.76±0.22	603.84±0.47

*Frankincense water extract: prepared by soaking it in cold drinkable water for 24 hr.(T1) - prepared by boiling it in water for 1 hr. (T2). **$IC_{50:}$ IC_{50} was calculated as the frankincense water extracts concentration providing 50% of inhibition. Values are mean of three replicates ± SD.

for T1 and T2 in case *Staphylococcus aureus* and Enterococcus. These results are in agreement with the results of El Kichaoui et al. (2017). They found that the distilled water extract of *Boswellia carterii* had antibacterial activity against *Bacillus subtilis*, with an 8 mm zone of inhibition. Also, they found that the MIC value of the distilled water extract of *Boswellia carterii* against *Klebsiella pneumonia* was 12.5 mg/mL. Ismail et al., (2014) found that the highest effect of antimicrobial activity of the frankincense of *Boswellia serrata* was found against *E. coli* with a zone of inhibition of 21.87 mm followed by *Staphylococcus aureus* with a zone of inhibition of 19 mm, then *Bacillus subtilis* with a zone of inhibition of 15 mm. Mustafa et al. (2020) found that the essential oils of the oleo-gum resins of *Boswellia papyrifera* showed high antibacterial activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhi* with minimum inhibition concentration (MIC) of 5 - 10 µg/ mL. Furthermore, all tested bacteria (excluding *Bacillus subtilis*) showed resistance to the water extracts (the inhibition zone was about 11 mm). Finally, the differences between these results and our results may be due to the difference in the type of frankincense and the type and proportions of total phenolic and flavonoid.

Cytotoxicity and antitumor activity of frankincense powder on human cell lines

The frankincense was designated in vitro for its cytotoxicity activity on the HepG-2 and MCF-7 human cancer cell lines through the employment of the MTT assay. The percentage of the viable cells and the IC_{50} value were measured and were, subsequently, assessed with those of the control, doxorubicin (Figure 1-2 and Table **8**). The attained results revealed that the frankincense powder presented dose-dependent cytotoxicity activities against both cell varieties (Fig. 1&2). The constructed deduction from these outcomes is that an assessment with the positive control doxorubicin, the frankincense displayed slightly fewer activities relative to the positive control, regarding human liver cancer (HepG-2) (Fig. 1 and Table 8). Regarding breast cancer cells (MCF-7); the frankincense was more potent relative to the positive control (Fig. 2 and Table 8). Paradkar et al. (2004) found that there is a relationship between the intake of dietary flavonoids and cancer prevention and that may be due to their anti-inflammation and antioxidant activity. Sun et al. (2011) declared that if one gram of phenolic compounds is taken daily from fruits and vegetables which are rich in antioxidants, this discourages many diseases such as mutagenesis and carcinogenesis. Baghel et al. (2012) found that kaempferol can control cancer, and quercetin showed antioxidant and anti-carcinogenic activities. Sun et al. (2020) isolated a new eight active compounds from the gum resin of *Boswellia carterii* as mainly as cembrane-type diterpenoids and they found that these compounds have many activities like anti-inflammatory with (IC_{50} of 14.8 µM), cytotoxic with (IC_{50} > 100 µM), and hepatoprotective with (IC_{50} > 30 µM). Alyahya & Asad (2020) found that *Boswellia sacra* extract does not possess any toxic effect on the testis, revealed its antioxidant potential that may protect testes against the effect of toxicants.

TABLE 7. Antimicrobial activity, minimal Inhibitory concentration and minimal bactericidal concentration of frankincense water extracts (T1 and T2)*

Microorganisms	Antimicrobial activity Inhibition zone IZ (mm)		Minimal inhibitory concentration MIC (mg/mL)		Minimal bactericidal concentration MBC (mg/mL)	
	T1	T2	T1	T2	T1	T2
Bacillus cereus	9	11	3.1	3.1	12.5	3.1
Bacillus subtilis	8	9	3.1	3.1	25	3.1
Staphylococcus aureus	7	7	25	12.5	50	50
Enterococcus	7	7	1.25	1.25	3.1	3.1
Klebsiella pneumonia	8	9	12.5	1.25	12.5	3.1
Pseudomonas aeruginosa	6	7	0	50	0	50
Salmonella typhi	12	13	6.25	1.25	6.25	3.1

* Frankincense water extract: prepared by soaking it in cold drinkable water for 24 hr (T1) - prepared by boiling it in water for 1 hr (T2).

TABLE 8. The cytotoxic IC50 values of the frankincense according to the MTT assay on the two human cell types

Compound	IC_{50} (µM) ± SD	
	HepG-2*	MCF-7**
Frankincense	33.9±3.5	7.8 + 1.3
Doxorubicin	28.5± 1.9	10.3± 0.8

*HepG2 human liver carcinoma cell - **MCF-7 human breast adenocarcinoma cell. Values are mean of three replicates ± SD.

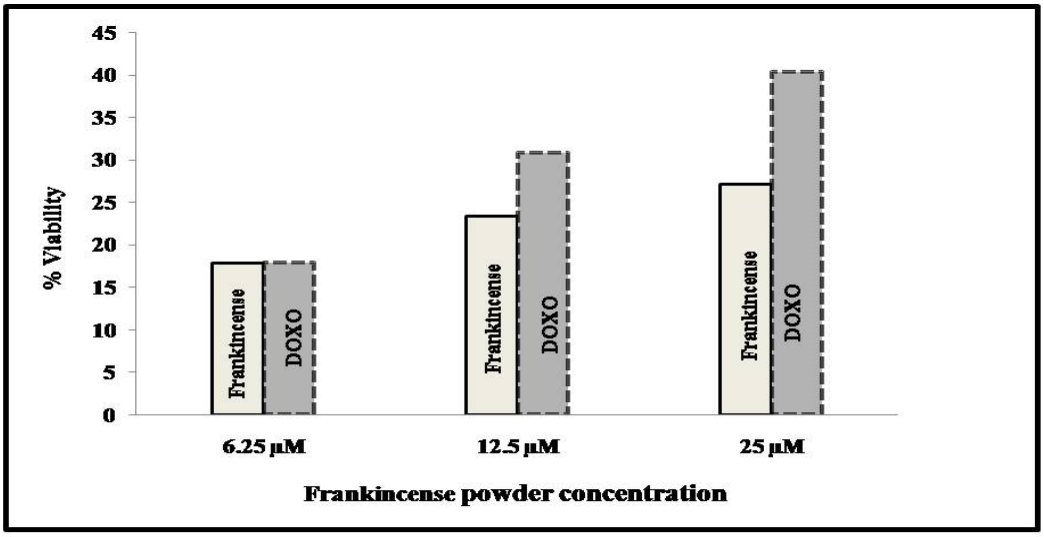

Fig. 1: Dose-dependent cytotoxicity data of the frankincense powder on the HepG-2 human cancer type, according to the MTT assay after 48 h of exposure.

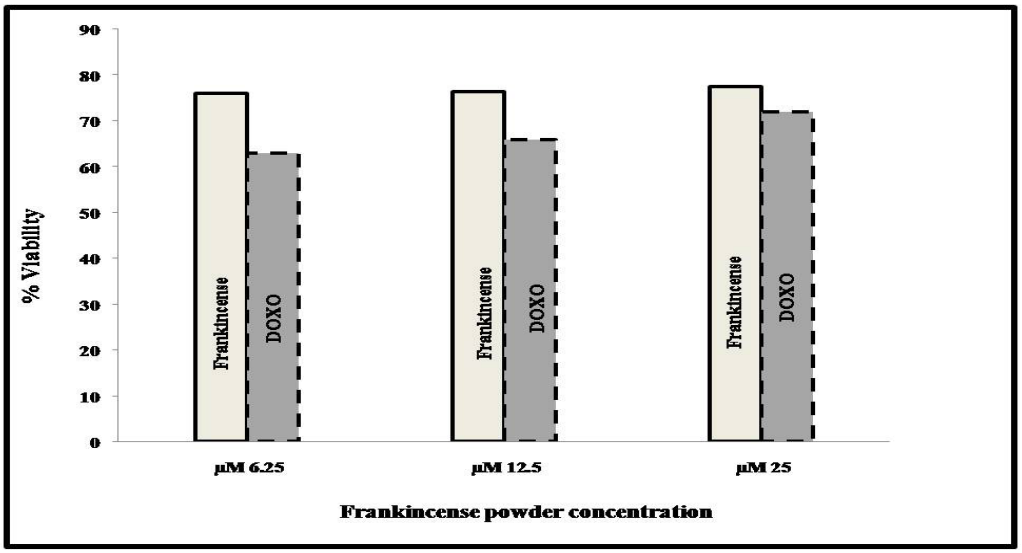

Fig. 2: Dose dependent cytotoxicity data of the frankincense powder on the MCF-7 human cancer type according to the MTT assay after 48 h of exposure.

Conclusion

This study revealed that frankincense water extracts (T1 and T2) could be added to guava nectar at levels up to 1% to obtain products having the best scores for all the evaluated sensory characteristics. The addition of frankincense water extracts to guava nectar has no effects on the Brix, pH, and color properties ($p > 0.05$). The frankincense which heated with water for 1 hr (T2) contained the highest amount of total phenolic and flavonoid compounds comparing to frankincense that soaked in water for 24 hr (T1). The antioxidant activity of the frankincense powder at 500µl/mL was 93.74%, with the IC_{50} of 67.26 µl/mL. This study showed also that frankincense and its water extracts have antimicrobial activity against many pathogenic positive and negative bacteria. Also, it provides that the frankincense and its water extracts have more potent cytotoxicity activity on the breast cancer cells compared to the positive control (doxorubicin), while it has slightly fewer activities compare to the positive control on the human liver cancer.

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تقييم خصائص مسحوق اللبان الدكر (*Boswellia carterii*) ومستخلصاته المائية وتأثير إضافته على خصائص نكتار الجوافة

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يتميز راتنج اللبان (*Boswellia carterii*) بالفوائد الطبية العديدة وفي مصر يستخدمه بعض الناس في المضغ كما يستخدم في البخور. أجريت هذه الدراسة بهدف استخدام مميزات المستخلص المائي للبان المحضر بطريقتين { النقع في الماء لمدة ٢٤ ساعة (T1) والغليان في الماء لمدة ساعة واحدة (T2) } في تدعيم نكتار الجوافة بتركيزات تتراوح بين ٠,٢٥ الي ٢ ٪ (حجم/حجم). تم تقدير التركيب الكيميائ للبان كما تم تقدير محتواه من العناصر المعدنية. تم ايضا تقدير محتوي اللبان من الفينولات والفلافونيدات وتم قياس الانشطة المضادة للاكسدة (بطريقة DPPH) والمضادة للميكروبات المرضية (٧ انواع من البكتيريا المرضة) والسمية الخلوية { خطوط خلايا السرطان البشرية HepG-2 (الكبد) و MCF-7 (سرطان الغدة الثديية)} . كما تم التقييم الحسي وتقدير الخصائص الطبيعية لنكتار الجوافة المضاف اليه المستخلصات المائية للبان. دلت النتائج علي ان اللبان يحتوي علي ٧٠,٧٢ ٪ زيت ، ٢٥,٢ ٪ كربوهيدرات كما انه يحتوي علي الكالسيوم والبوتاسيوم والصوديوم والماغنسيوم بتركيزات ٩٨٣٣,٧٤ ، ٣٤٨٢,٩٩ ، ١٥٩٥,٥ و ١٢٦٨,٥٨ جزء في المليون علي التوالي. اكدت النتائج علي ان المستخلص المائي T2 يحتوي علي مركبات الفينولات والفلافونيدات بنسب ٠,٨٦٩ و ٠,١٤٧ ملجم/جم علي التوالي وكانت اكبر من قيمها في T1 حيث كانت ٠,٧٤٨ و ٠,٧٠ ملجم/جم علي التوالي. تبين من النتائج ان اللبان له ٩٣,٧٤ ٪ نشاط مضاد للاكسدة عند تركيز ٥٠٠ ميكروليتر/مل وكانت الـ IC_{50} له ٦٧,٢٦ ملجم/مل. اكدت النتائج ايضا ان للبان نشاط مضاد لبكتيريا *Salmonella typhi* . وكذلك بكتيريا *Bacillus cereus* حيث كان متوسط قطر منطقة التثبيط ١٣ و ١١ ملم علي التوالي في حالة T2 . وكانت قيم الـ MIC ١,٢٥ و ١,٣ ملجم/مل علي التوالي . كما وجد ان MBC كانت ١,٣ لكل منهما. كما تبين من النتائج ان اللبان له نشاط مضاد للسمية الخلوية وان هذا النشاط طانية خطوط خلايا السرطان البشرية (HepG-2) بالمقارنة بعينة الكنترول . ونشاط اقل بقليل من عينة الكونترول علي خلايا الصدر السرطانية {سرطان الغدة الثديية البشرية (MCF-7)} حيث كانت قيم IC_{50} للبان ٧.٨ و ٣٣.٩ علي التوالي بينما كانت لعينة الكنترول ١٠.٣ و ٢٨.٥ علي التوالي. كما اكدت النتائج ان اضافة المستخلصات المائية للبان لم تؤثر علي الخصائص الطبيعية لنكتار الجوافة من حيث درجة البركس و pH واللون. واخيرا دلت نتائج التقييم الحسي لنكتار الجوافة المضاف له المستخلصات المائية للبان لم يكن بينها وبين عينة الكنترول فروق معنويه حتي تركيزات تصل الي ١ ٪.

من ذلك يمكن استنتاج انه يمكن خلط المستخلصات المائية للبان مع نكتار الجوافة للحصول علي منتج له الطعم واللون والرائحة المحببة وله ايضا مميزات اللبان.