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Evaluation of Pies Containing Licorice Roots (*Glycyrrhiza glabra* L.) Extracts

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ABSTRACT

Licorice roots (*Glycyrrhiza glabra* L.) are an important medicinal plant, and have numerous uses. Two extractors (water and ethanol) were used to extract the phytochemicals from licorice roots. Antioxidant and antimicrobial activities of such extracts were evaluated. Three different concentrations (100, 250 and 500µg/ml) of water and ethanolic extracts were used to investigate inhibition zone and properties against some bacteria strains (*Staphylococcus aureus, Salmonella typhimurium*, *Bacillus subtilis and Escherichia coli)* and *fungi* **(***Asperigllus flavus*, *Asperigllus niger* and *Penicillum chrysogenu*). The present results indicated that ethanolic extract exhibited the highest antioxidant activity (AOA) at 600µg/ml. The AOA (IC_{50}) for water and ethanolic extracts was 110 and 212 µg/ml, respectively. Total phenols of water and ethanolic extracts was 21.01 and 45.24 mgGAE/gDW, respectively. The results of HPLC analysis fractionations showed that both water and ethanolic extracts contained a high level of pyrogallol, coumarin and catechin. The exist major flavonoids were hisperdin and quercetrin. It was revealed that the antimicrobial activity of the ethanolic extract was higher than of the water extract. Three levels of ethanolic licorice extract were used in pie preparing. The effect of addition licorice ethanolic extract (100, 200 and 300 mg/100g wheat flour) in preparing pies were evaluated and the results cleared that, specific volume was slightly increased at 200 and 300 mg/100g compared with control. Taste, volume and crumb color of pies were improved at 200 and 300 mg licorice extract. Addition of ethanolic licorice extract at 200 and 300 mg successfully extended the microbiological shelf life up to 12 days compared with 6 days for control.

***Keywords*:** licorice roots, aqueous extract, ethanolic extract, antioxidant, antimicrobial, bakery products, shelf life.

Introduction

The production of safe, high-quality and shelf-stable food become a challenge to the food industry. Some chemical additives when ingested in high amounts may provide undesirable reactions to the consumers. Therefore, consumers, food industries and the health authority are beginning to urge that these chemical preservatives should be replaced with natural components with properties that preserve the food throughout extended its shelf life (Campêlo *et al*., 2019). Bakery products are an important part of a balanced diet, it were commonly purchased by a wide range of consumers for their nutritional qualities, their palatability and their easy availability. Bakery products, like many processed foods, are subjected to physical, chemical and microbiological spoilage. Microbiological spoilage is the major factor limiting the shelf life of bakery products and is also a major cause of economic loss to the bakery industry (Smith *et al*., 2004).

Licorice roots (*Glycyrrhiza glabra* L.), as a popular traditional medicinal plant, belongs to the legume family Fabaceae (Alagawany *et al*., 2019). It is broadly used in the medicine, as a flavouring, food preservative agent and commercial purposes (Pastorino *et al*., 2018). It is used in foods as an antioxidant and antimicrobial agent to increase food quality (Jiang *et al*., 2013).

The Glycyrrhiza genus consists of about 30 species, it is a plant widely used in herbal medicines due to their several biological potentials. Licorice roots are an important commercial products that grows in tropical of the Mediterranean region, Asia, Minor and Middle East and also widely cultivated in southern Russia and Iran (Karahan *et al*., 2016 & Quintana *et al*., 2019).

Licorice has an active compounds, including alkaloids, flavonoids, tannins terpenoids, alkaloids, glycosides and phenolic compounds, which have been found *in vitro* to have antimicrobial

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properties (Rodino *et al*., 2015) and antioxidant activity which attracted great interest because of their beneficial effects on human health (Gülcin *et al*., 2005 and Butu *et al*., 2014). Licorice have been used as remedies for virus diseases which have been used to control COVID-19 infections (Murck, 2020), microbial diseases (Balunas and Kinghorn 2005), flavoring food, as preservatives and natural antimicrobial agents have gained attention as alternative therapeutic agents in food industry for improving the quality and nutritional value of food (Rodino *et al*., 2015; Ali *et al*., 2014 and Gyawali and Ibrahim, 2014).

Licorice is widely used worldwide in food, confectionery herbal supplements, chewing gums, drinks, and candy (Xu *et al*., 2002). It has been reported that the extracts of licorice have been widely used in the food industry as a sweetening agent, a flavor enhancer and a flavor modifier. It is, also, found a widespread usage as a foaming agent in alcoholic and nonalcoholic beverages (Tohma and Gulcin, 2010). Therefore, licorice extracts are commonly used in sweet foods such as sweet snacks, ice cream and sherbets to enhance their sweetness (Al-Turki *et al*., 2008).

Several studies have shown the ability of licorice aqueous, ethanol and methanol extracts, obtained by different extraction processes, to inhibit the growth of Gram-positive and Gram-negative bacteria, such as *Staphylococcus aureus*, *Bacillus subtilis* and *Echerichia* (Chandra and Gunasekaran, 2017).

Commercially, the root extract of licorice is supplied in concentrated liquid or powdered form for ease of transportation. For use in products, the concentrate or powder extract is diluted with water to the required concentration (Tohma and Gulcin, 2010).

The objective of this work was to study the effect of licorice extract addition as an antioxidant and an antimicrobial agent on pies quality characteristics.

Material and Methods

1. Materials

The wheat variety (*Triticum aestivum* L.), named Sids 12, was obtained from the Wheat Research Department, Field Crops Research Institute (FCRI), Agricultural Research Center (ARC), Giza, Egypt. The plant material used was purchased from a well known traditional local supplier of medicinal herbs (Haraz). The licorice [*Glycyrrhiza glabra* Linn. (Family: Leguminasae)] plant was botanically identified and authenticated from the Department of Flora & Phyto-texonomy Researches. Horticultural research Inst., Agricultural Research Center, Dokki, Giza, Egypt. The specimens of the plants are deposited in the Department for further references. The licorice was grounded in a laboratory mixer grinder to obtain a fine powder, the powdered material was packed in poly ethylene and stored in an air tight container for use. Other materials included sugar, salt, yeast, shortening and dry milk powder were purchased from local market at Giza, Egypt.

1.1. Microorganisms

Staphylococcus aureus (ATCC-25923), *Salmonella typhimurium* (ATCC-9027), *Escherichia coli* (ATCC-2592**),** *Asperigllus flavus* (EMCC-101), and *Asperigllus niger* (EMCC-104) were obtained from Food Technology Research Institute-ARC. *Bacillus subtilis* (NRRL-4219) and *Penicillum chrysogenum* (ATCC-11710) were obtained from Cairo University Research Park (CURP), Faculty of Agriculture, Cairo University, Giza, Egypt. *Rhizopus stolonifer* (ATCC-14037) were obtained from Department of Biotechnology, National Research Center (NRC) Cairo.

2. Methods

2.1. Preparation of licorice extract

100 g of licorice root powder was mixed with 700ml water for water extract or 700 ml ethanol (80%) for ethanol extract, then left the extracts over night at refrigerator temperture (4ºC ± 2ºC). The extracts were filtered with cheesecloth and the supernatant was centrifuged at 33.540g speed for 20min after that evaporated under vacuum at 50 ºC to turns into a powder (Gupta *et al*., 2008).

2.2. Preparation of flour 72%

Wheat grains were separately cleaned, conditioned to14% moisture content at ambient temperature for 18h. Then milled by using fractionation Laboratory mill (Brabender Duisburg roller mill, Germany) to separate flours (72% extraction rates) from bran and shorts.

2.3. Preparation of pies

The ingredients used for making pies were described by Bedeir, (2014) as follows: per 100 g wheat flour (72% extract): 10g sugar, 1g salt, 1.5g yeast, 10g shortening, 3g dry skimmed milk powder and 30ml water. Various concentration of previously ethanolic licorice extract (100, 200 and 300 mg/100g flour) were added to the formula. Organoleptic characteristics (Symmetry, General appearance, Crust color, Crumb color, volume, texture, taste, oder and overall acceptability) of pies were evaluated by 10 panelists (n=10) of Food Technology Research Institute, Agriculture Research Center, Giza, Egypt to carry out the sensory evaluation test for the hedonic scale from 0 to 9, were a score of 9 represents excellent and a score of zero represents the lowest quality level (Arubayi and Ogbonyomi 2019).

3. Chemical analysis

3.1. Chemical composition of licorice root powder and wheat flour (72% extract).

The licorice and wheat flour were analyzed for chemical composition (protein, fat, moisture, fiber and ash) content according to the method of AOAC, (2012). Total carbohydrates were calculated by difference [100 – (protein + ash + fiber + oil)].

3.2. Determination of total phenolic content (TPC)

Total phenolic content in the water and ethanolic extracts were estimated by the Folin-Ciocalteu assay according to the method presented by Rodino *et al.* (2015). Gallic acid was used as a standard and results were expressed as mg of Gallic acid per g of dry sample. Quantification was done based on the calibration curve of gallic acid.

3.3. Determination total flavonoid content (TFC)

The total flavonoid content in the water and ethanolic extracts were determined by a modified aluminum chloride colorimetric method according to the method presented by Rodino *et al.* (2015). The standard curve was prepared using rutin by the same method. The results were expressed as mg of rutin per g of dry sample.

3.4. Identification of phenolic and flavonoids compounds by (HPLC)

HPLC has ability to separate and identify the compounds present in any specific sample that can be dissolved in a liquid in trace concentrations as low as parts per trillion. Therefore, in the present investigation, phytochemical analysis of licorice water and ethanolic extracts was investigated by HPLC (Gupta *et al*., 2013). High Performance Liquid Chromatography (HPLC), Agilent, Germany 1200 system equipped with a variable wave length detector autosampler, quaternary pump degasser and column compartment was used to determine. Analyses were performed with a C18 reverse phase packed stainless-steel column (Zorbax ODS 5 µm 4.5× 250mm).

3.5. DPPH radical scavenging activity

The ability of water and ethanolic extracts at different concentrations (50, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550 and 600 µg/ml) to scavenge 1, 1-diphenyl-2- picrylhydrazyl (DPPH) free radical. DPPH radicals was determined according to Brand-Williams *et al.* (1995). The extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph of inhibition percentage plotted against extract concentration (Chopra *et al*., 2013).

4. Mixolab properties

Mixolab properties (water absorption, dough stability, C1, C2, C3, C4 and C5) of wheat flour (72% extraction) and composite flours prepared by add ethanolic licorice extract 100, 200 and 300 mg/100g flour (72% extraction) were evaluated using the Mixolab Instrument (Chopin Technologies, Villeneuve La Garenne, France). The water absorption level for each sample was firstly determined by the consist graph. The mixing conditions of dough were as follows: initial temperature 30°C for 8 min, heating to 90°C (4°C/ min for 15 min), holding at 90°C for 7 min, cooling to 50°C (4°C/ min for 10 min) and holding at 50°C for 5 min. The mixing speed through the mixing process was at 80 rpm (Koksel *et al*., 2009).

5. Physical analysis

Pies weight (g) was recorded after cooling, pies volume (cm^3) was determined by rapeseed method as described by AACC (2002). Specific volume (cm^3/g) of pies was calculated by dividing volume by weight. Density (g/cm^3) was calculated by dividing weight by volume. The color of pies crumb was measured according to by McGurie (1992). Crumb color was measured on opposite sides of pie by using a hand-held tristimulus reflectance Colorimeter Minolta chromammeter (model CR-400, Konica Minolta, Japan). The apparatus provided *L* (lightness with *L* = 100 for lightness, and *L* = zero for darkness), *a* [(chromaticity on green (−) to red (+)], *b* [(chromaticity on a blue (−) to yellow (+)].

6. Microbiological analysis

6.1. Assay of antimicrobial activity

The antibacterial activity (ABA) and antifungal activity (AFA) of licorice extracts (water and ethanolic) at different concentrations (100, 250 and 500 µg/ml) was assessed by disc diffusion method as according to Thakur *et al*., (2016).

6.2. Microbiological quality of pies

The microbiological quality of stored pies for 12 days at a room temperature (35º ± 2ºC) was evaluated by determining aerobic plate count using total count media (Swanson *et al*., 1992). Total fungal count was determined by using malt yeast agar media to be as a good tool to estimate the shelf life according to Mislivec *et al*., (1992).

Statistical Analysis

For the analytical data, mean values and standard deviation are reported. The obtained data were subjected to one-way analysis of variance (ANOVA) at $P < 0.05$. It was performed and the results were separated using the Multiple Range Duncan's test using the SAS (1987) statistical software.

Results and Discussion

1. Chemical composition of wheat flour (72% extraction) and licorice (powder)

Chemical composition of wheat flour 72% and licorice are presented in Table 1. The chemical composition i.e. protein, ash, fiber, fat and total carbohydrates were varied between wheat flour 72% and licorice. Percentage of protein, ash, fat, fiber and carbohydrate are calculated based on dry weight. The results showed that protein content recorded 12.31% and 8.88 % in wheat flour and licorice, respectively. Fiber, fat, ash and total carbohydrates content of wheat flour recorded 0.54, 1.46, 0.72 and 85.08 %, respectively. The same results were found in (Mohsen *et al*., 2012), while the fiber, fat, ash and total carbohydrates content for licorice were recorded 25.10, 1.21, 8.38 and 56.55 %, respectively.

Table 1: Chemical composition (on dry basiss) of wheat flour (72% extraction) and licorice powder and total phenols, flavonoids of licorice extracts.

	Parameters %				
	Protein	**Fiber**	**Fat**	**Ash**	**Total carbohydrate**
Wheat flour	$12.31^{a} \pm 0.13$	$0.54^{b} \pm 0.03$	$1.46^{a} \pm 0.01$	$0.72^{b} \pm 0.00$	$85.08^{a} \pm 0.08$
Licorice	$8.88^{b} \pm 0.08$	$25.10^{a} \pm 0.05$	$1.21^{b} \pm 0.01$	$8.38^{a} \pm 0.08$	$56.55^{b} \pm 0.04$
Licorice extracts	**Total phenol (mg GAE/g DW)**		**Total flavonoid (mg RE/g DW)**		
Water	$21.01^{b} \pm 0.03$		$9.23^{b} \pm 0.01$		
Ethanolic	$45.24^{a} \pm 0.01$		$24.54^{a} \pm 0.00$		

Values are the average of 3 experiments ± SD. Mean values followed by different superscripts (within the same column) are significantly different at the 5%.

It could be noticed that licorice had a higher ash and fiber contents, while it had lower protein and total carbohydrate contents compared with wheat flour. Badr *et al*. (2013) found that, percentage

of protein, fat, moisture, ash, fiber and carbohydrates of licorice is 9.15, 0.53, 6.8, 7.7, fiber 24.42 and 47.11%, respectively.

2. Total phenols and flavonoids

Phenolic compounds are widely distributed in plants and are very important in human diet and health (Tohma and Gulcin 2010). Total phenolic and total flavonoid of water and ethanolic extracts amounts in licorice can be seen in Table (1). Total phenols of water and ethanolic extracts recorded 21.01 and 45.24 mg GAE/gDw. Tohma and Gulcin (2010) reported that, the phenolic compounds of one mg of water extract and ethanolic extract ranged from 75.7 to 185.7µg GAE, respectively.

Rodino *et al*. (2015) found that, total phenolic contents from *G. glabra* was 52.1 mgGAE/g DW compared with the ultrasonic extracts (63.39) mg GAE/g DW.

Concerning flavonoids content, the same Table (Table 1) showed significant differences between water and ethanolic extract where recorded 9.23 and 24.54 mgRE/g, respectively. The total flavonoid contents in the ethanolic extract by soaking, was 14.8 mg RE/g DW, compared with ethanolic extract by ultrasonic (17.28 RE/g DW). A higher flavonoid content was obtained for the extracts obtained by ultrasonication compared to the ones obtained by soaking method (Rodino *et al*. 2015).

3. Identification of phenolic and flavonoid compounds

Identification of phenolic compounds in water and ethanolic extracts are presented in Table (2). The results showed that both water and ethanolic extracts contained a high level of pyrogallol (360.65 and 291.47 mg/100g) followed by coumarin (165.28 and 130.93 mg/100g) and 3, 4, 5-methoxy-cinnamic (147.05 and 84.13 mg/100g). Ethanolic extract had a higher benzoic amount (566.73mg/100g) compared with water extract (28.17 mg/100g). Caffeine and protocatchoic presented in a high level in water extract (78.61 and 106.88mg/100g) in relative to 27.45 and 11.56mg/100g in the ethanolic extract. On the other hand, salycillic and cinnamic were presented in ethanolic extract (154.90 and 31.90 mg/100g) respectively however they are undetected in water extract. Gupta *et al*. (2013) reported that, phytochemical analysis based on HPLC, both methanolic and acetonic extracts showed a noticeable variation in terms of absence or presence of certain compounds as well as in their quantification on percent dry weight basis of different compound such as chlorogenic and caffeic which recorded 0.01401 and 0.06223%, respectively. On contrary, for methanolic extract, chlorogenic didn't found in aceton extract but caffeic recorded 0.03432%.

Table 2: Identification of phenolic and flavonoid compounds in ethanolic and water licorice extracts

Phenolic compounds mg/100g	Ethanol extract	Water Extract	Flavonoids mg/100g	Ethanol extract	Water Extract
Pyrogallol	291.47	360.65	**Apiening 6-rhamnose 8-glucose**	5.68	ND
Gallic	9.37	1.17	**Apiening 6-rhamnose 8-glactose**	20.76	ND
Protocatchoic	11.56	106.88	**Naringin**	4.97	0.80
4-Aminobenzoic	0.26	2.90	**Rutin**	2.35	0.57
Catechein	129.97	32.40	**Quercetrin-3-O-glucose**	0.29	ND
Chlorogenic	33.89	31.37	**Apigenin-7-glucose**	15.54	ND
Catechol	96.49	24.16	**Apigenin 7-o-neohespiroside**	3.84	ND
P-OH- benzoic	75.39	11.47	**Kampferol 3-7-diramoside**	1.60	ND
Caffeic	8.27	3.43	**Quercetrin**	18.11	0.39
Vanillic	5.35	17.23	**Quercetin**	1.44	1.16
Caffeine	27.45	78.61	**Naringenin**	0.76	0.43
P-Coumaric	214.83	13.47	**Acacetin 7-neo rutinoside**	1.35	ND
Ferulic	121.46	39.31	**Hespirtin**	4.14	0.65
Iso-Ferulic	1.74	9.06	**Kampferol**	0.55	1.42
Salycillic	154.90	ND	**Apigenin**	0.44	2.49
Benzoic	566.73	28.17	**Hisperidin**	21.15	9.68
Coumarin	130.93	165.28	**Rhamnetin**	3.65	ND
3,4,5-methoxy-cinnamic	84.13	147.05	**Acacetin neo rutinoside**	1.65	ND
Cinnamic	31.90	ND	**Luteolin 7 glucose**	2.29	ND

ND: Not detected

The results Table (2) showed, also, that the type of extraction solvent affected the extracted flavonoids level. Flavonoids showed detectable differences between etnanolic and water extractors with respect to their types and quantities of different compounds. The ethanolic extract had higher total flavonoid compounds than water extract. The major flavonoid compound was hisperidin (21.15 and 9.68 mg/100g) in both of ethanolic and water extracts.

Ethanolic extracts had higher levels in apiening 6-rhamnose 8-glactose, quercetrin, and apigenin-7-glucose (20.76, 18.11 and 15.54 mg/100g, respectively) than water extract. Therewere, some flavonoids compounds presented in ethanolic extract and not found in water extract (i.e., Apiening 6-rhamnose 8-glucose, Apiening 6-rhamnose 8-glactose, Apigenin-7-glucose, Apigenin 7-o-neohespiroside and Rhamnetin, etc., ….).

Gupta *et al*., (2013) found that, rutin, quercetin and kaempferol were 0.0531, 0.00347 and 0.0071% for methanol extract and 0.0870, 0.0068 and 0.01388% for acetone extract, respectively.

Quercitin seems to exert antibacterial activity against almost all the strains of bacteria known to cause respiratory, gastrointestinal, skin and urinary disorders. Quercitin appeared active against different viruses including HIV, probably due to inhibition of reverse transcriptase (Rigano *et al*., 2007 and Gupta *et al*., 2013).

4. Antioxidant activity for licorice extracts

Free radical scavenging activity is a potent indicator for the bioactive compounds that acting as an effective phytotherapeutics (Chandra and Gunasekaran 2017).

1,1-diphenyl-2-picrylhydrazyl radical (DPPH) is used by most of the researchers to determine antioxidant activity. DPPH is a free radical that keeps its stability in aqueous or methanolic solutions. It accepts an electron or hydrogen ion to become a stable diamagnetic molecule (Ozusaglam and Karakoca, 2014).

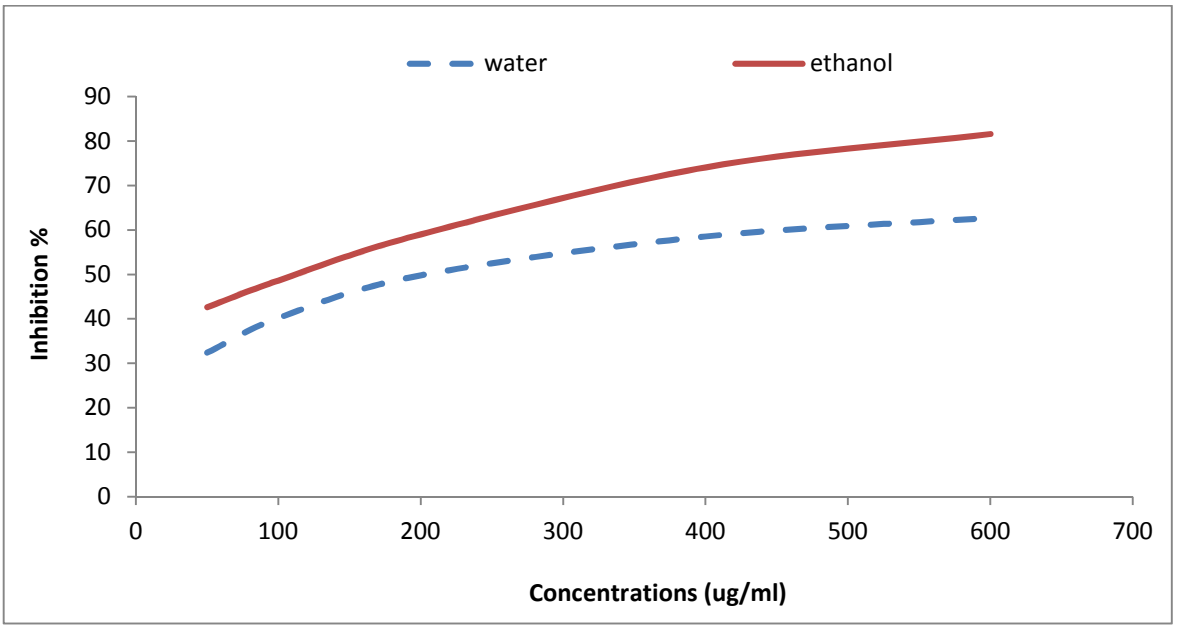

Fig. 1: The antioxidative effect of various concentrations (50 - 600 µg/ml) of water and ethanolic licorice extracts.

Results indicated that the activity increase with increasing the licorice extractor concentration. The ethanolic licorice extract was superior compared with water extract at all various concentrations. It was found that in maximum antioxidant activity was achieved with ethanolic extract at the concentration of 600µg/ml. The calculated IC_{50} for the water and ethanolic extracts of licorice is 110 and 212µg/mL, respectively. The antioxidant activity of licorice extract with 100 µg and 500 µg exhibiting minimal 70.33 and maximal 87.70% scavenging activity (Chandra and Gunasekaran, 2017).

Thakur *et al*., (2016) found that, the antioxidant activity (IC_{50}) of extracted licorice using different solvents water, methanol and ethanol by DPPH method recorded 189.93, 238.06 and 287.14, respectively.

5. Antimicrobial activity (AMA) for licorice extracts

The antibacterial activity (ABA) and antifungal activity (AFA) of various concentration water and ethanolic extracts of licorice are displayed in Tables (3 and 4), respectively. In general, results indicated that in both cases (water and ethanolic extracts), there were increasing in the ABA and AFA by increasing its concentrations. High level in inhibition zone was observed at 500µg of ethanolic extract compared with water extract. Data in Table (3) indicated that, significant differences in inhibition zones at tested concentrations of licorice extracts for the following bacteria *Staphylococcus aureus, Sallmonella typhimurium, Bacillus subtilis, and E. coli*. Data also revealed that the largest inhibition zones (14.33 mm) was found with *Staphylococcus aureus* at 500 µg of ethanolic extract. This indicates that *Staphylococcus aureus* strain was the most sensitive among the tested bacteria species. On the other hand, *Sallmonella typhimurium* showed the smallest zones compared with other bacteria. This results agree with Irani *et al* (2010), who found that the relative antibacterial activity of ethanolic extract was higher than water extract.

Staphylococcus aureus is a major clinical pathogen. During the past decade, this bacterium has developed resistance to many commonly used antibiotics. The extracts of licorice showed activity against *Staphylococcus aureus* and can be used as raw materials for phytotherapy (Irani *et al*., 2010). The presence of polyphenol compounds in this plant extracts could account for their inhibitory effect on bacterial growth (Rodino *et al*., 2015).

Table 3: The antibacterial activity of various concentrations of the water and ethanolic extracts of licorice.

Bacteria	Inhibition zone (mm)					
	100µg/ml		250µg/ml		500µg/ml	
	Water	Ethanolic	Water	Ethanolic	Water	Ethanolic
Staphylococcus aureus	6.67^{a}_{f} ±0.58	10.00^{a}_{d} ±0.00	8.33^{a}_{e} ±0.57	11.83^{a}_{b} ±0.29	10.83^{a}_{c} ±0.29	14.33^{a}_{a} ±0.58
Salmonella typhimurium	4.00^{c}_{c} ±0.00	4.33^{c}_{c} ±0.58	4.00^{c}_{c} ±0.00	6.00^{d}_{a} ±0.00	5.17^{c}_{b} ±0.29	6.33^{c}_{a} ±0.58
Bacillus subtilis	4.33^{c}_{d} ±0.58	6.33^{b}_{b} ±0.57	5.00^{b}_{cd} ±0.00	9.67^{b}_{a} ±0.58	5.33^{c}_{c} ±0.29	10.00^{b}_{a} ±0.00
E coli	5.33^{b}_{c} ±0.57	6.00^{b}_{c} ±0.00	8.00^{a}_{b} ±0.00	8.33^{c}_{b} ±0.58	8.67^{b}_{b} ±0.58	9.67^{b}_{a} ±0.58

Values are the average of 3 experiments ± SD. Mean values followed by different superscripts (within columns) and different subscripts (within rows) are significantly different at the 5%.

Table 4: The antifungal activity of various concentrations of the ethanolic and water extracts of licorice.

Mold	Inhibition zone mm					
	100µg/ml		250µg/ml		500µg/ml	
	Water	Ethanol	Water	Ethanol	Water	Ethanol
Asperigllus flavus	2.33^{b}_{d} ±0.58	5.00^{b}_{c} ±0.00	5.00^{b}_{c} ±o.00	8.00^{a}_{b} ±0.00	8.33^{a}_{b} ±0.58	10.33^{b}_{a} ±0.58
Asperigllus niger	4.00^{a}_{d} ±0.00	5.00^{b}_{c} ±0.00	5.33^{b}_{c} ±0.58	8.67^{a}_{b} ±0.58	8.67^{a}_{b} ±0.58	11.67^{a}_{a} ±0.58
Rhizopus stolonifer	2.67^{b}_{f} ± 0.58	6.00^{a}_{e} ±0.06	6.83^{a}_{d} ±0.29	8.67^{a}_{b} ±0.58	8.00^{a}_{c} ±0.00	10.00^{b}_{a} ±0.00
Penicillum chrysogenum	0.00^{c}_{a} ±0.00	0.00^{c}_{a} ±0.00	0.00^{c}_{a} ±0.00	0.00^{b}_{a} ±0.00	0.00^{b}_{a} ±0.00	0.00^{c}_{a} ±0.00

Values are the average of 3 experiments ± SD. Mean values followed by different superscripts (within columns) and different subscripts (within rows) are significantly different at the 5%.

Results for AFA (Table 4) indicated that, *Aspergillus niger* strain showed a maximal inhibition zone of 11.67mm at concentration of 500µg/ml, but the lowest inhibition zone observed for

Aspergillus flavus (10.33mm) was detected at the same concentration (500µg/ml). Statistic analysis showed significant differences in inhibition zone between fungi strains at ethanolic and water extracts for all concentrations. It was also noted that *Penicillum chrysogenum* not affected at all concentrations of ethanolic and water extracts even at 500µg/ml. Licorice extracts exhibited antimicrobial activities against food pathogenic microorganisms which used in this study with the exception of *Penicillum chrysogenum*.

The methanolic extract of *G. glabra* was most potent against *Staphylococcus aureus* at 500µg/mL (inhibition zone 13mm) among bacteria and showed maximum potency against *Rhizopus spp.* at 500µg/ml (inhibition zone 11 mm) among fungi (Chopra *et al*., 2013).

All the licorice plant methanolic extracts inhibited the growth of *B. cereus*, *E. faecalis* and *Staphylococcus aureus***.** However, there was no activity against *E. coli* (Karahan *et al*., 2016). The ethanolic extract of licorice root revealed considerable antimicrobial activity against some pathogenic bacteria. It had also, antibacterial activity toward *Streptococcus mutans* (Karami *et al*., 2013 and Phaiboon *et al*., 2019)**.** Fukai *et al*. (2002) reported that, flavonoids for licorice showed antibacterial activity against resistant strain of *Staphylococcus aureus*. Gupta *et al*., (2013) found that, production of *Aspergillus flavus* aflatoxin can be decrease by caffeic acid.

6. Rheological evaluation

6.1. Mixolab properties of dough

The results indicated that ethanolic extract exhibited the highest antioxidant and antimicrobial activities compared with water extract, so ethanolic extract is used for preparing the dough.

Mixolab properties of dough prepared by addition ethanolic licorice extract with different levels (100, 200 and 300 mg/100g flour 72%) were presented in Table (5). Mixolab shows the mixing and heating of dough to determine protein quality, α-amylase activity and starch properties (Koksel *et al*., 2009). Water absorption of dough with ethanolic licorice extract were so close to wheat flour dough value (59.80 %) except licorice addition level (100mg) which showed a slight increase in water absorption. The stability value is an indication of the flour strength. High stability values suggest strong dough. The stability values were slightly increased at addition levels of 100 and 200 mg in the ethanolic licorice extract (9.60 min) compared with control and addition level 300mg samples (8.88 min) for both. The C1 and C2 values showed the protein quality whereas, C3, C4 and C5 values show the starch properties (Koksel *et al*., 2009). The C1 (1.06 - 1.10 Nm) values not affected by ethanolic licorice extract addition.

However C2 (0.49 - 0.61Nm) values were slight affected by addition of ethanol licorice extract levels. 200mg addition level showed slight increase in C2 value (0.61 Nm) compared with control (0.53Nm) and the two addition levels 100 and 300 mg (0.49 and 0.53Nm), respectively. Similar results were reported by Aly-Aldin (2016) for wheat flour dough replaced with different replacement levels of germinated flaxseed flour.

The C3 is the maximum larger obtained during the heating stage. The C3 values (1.67-1.94Nm) of wheat flour dough licorice extract added with different levels showed a slight increase at 200mg addition level (1.94 Nm) compared with control (1.86 Nm). Addition level 300 mg was the same control (1.86 Nm).

Table 5: Mixolab parameters of tested dough treated with different concentrations of ethanolic licorice extract

Treatments	Water absorption (%)	Dough stability (min.)	C1 (Nm)	C2 (Nm)	C3 (Nm)	C4 (Nm)	C5 (Nm)
Control	59.80	8.88	1.10	0.53	1.86	1.78	3.12
100mg/100g flour	60.06	9.60	1.06	0.49	1.67	1.60	2.61
200mg/100g flour	59.80	9.60	1.10	0.61	1.94	1.75	3.33
300mg/100g flour	59.80	8.88	1.10	0.53	1.86	1.78	3.12

The C4 and C5 values of licorice added dough were so close with control (1.60-1.78 Nm) and (2.61-3.33 Nm) for C4 and C5, respectively. In general, addition level (200mg) showed a high dough stability, C2 and C3 values compared with control wheat flour dough and the other two ethanolic licorice addition levels.

7. Sensory evaluation of pies

Sensory characteristics, such as symmetry, general appearance, crust color, crumb color, volum, texture, taste, odor and overall acceptability, of pies treated with ethanolic licorice extracted at concentration 100, 200 and 300mg/100g flour compared with control, are shown in Table 6. The results showed that, no significant differences in symmetry, general appearance, texture, odor and overall acceptability. Significant differences are shown between control and samples in crust color, volume, crumb color and taste. Crust color value showed a slightly decrease in sample treated with 300mg of ethanolic licorice extract. The pies treated with 200mg had a higher mean scores for volume.

Simurina *et al*. (2008) found that, the herbal blend affected bread flavour providing a pleasant aroma associated with the used herbal drugs. The bread crumb was well developed and spongy, the elasticity, compressibility and melting-in-mouth of bread were excellent.

Table 6: The effect of various concentration of ethanolic licorice extract on sensory in pies.

Treatments	Symmetry	General appearance	Crust color	Crumb color
Control	8.56 ^{a} ± 0.53	8.78^{a} ± 0.44	8.83^{a} ± 0.35	8.11^{b} ± 0.93
100mg/100g flour	8.44 ^{a} ± 0.53	8.78^{a} ± 0.44	8.56^{ab} ± 0.53	8.56^{ab} ± 0.73
200mg/100g flour	8.61 ^{a} ± 0.49	8.44^{a} ± 0.53	8.61^{ab} ± 0.60	8.67^{a} ± 0.71
300mg/100g flour	8.67 ^{a} ± 0.50	8.56^{a} ± 0.53	8.22^{b} ± 0.79	8.78^{a} ± 0.67

Table 6: Continued

Treatments	Volume	texture	Taste	Odor	Overall acceptability
Control	8.17^{b} ± 0.68	8.28^{a} ± 0.67	8.11^{b} ± 0.74	8.78^{a} ± 0.44	8.44^{a} ± 0.53
100mg/100g flour	8.39^{ab} ± 0.60	8.22^{a} ± 0.51	8.22^{ab} ± 0.62	8.67^{a} ± 0.71	8.50^{a} ± 0.50
200mg/100g flour	8.67^{a} ± 0.50	8.44^{a} ± 0.53	8.56^{a} ± 0.46	8.78^{a} ± 0.44	8.5^{a} ± 0.50
300mg/100g flour	8.56^{ab} ± 0.53	8.33^{a} ± 0.55	8.61^{a} ± 0.42	8.89^{a} ± 0.33	8.50^{a} ± 0.50

Values are the average of 10 experiments ± SD. Mean values followed by different superscripts (within the same column) are significantly different at the 5%.

8. Physical properties of pies

Physical properties such as volume, weight, specific volume, density and color parameters (*L*, a** and *b**) were evaluated for pies prepared with ethanolic licorice extract 100, 200 and 300mg (Table 7). It cleared that pies contained 200 and 300mg ethanolic licorice extract had the highest volume and weight (193.00 and 194.33 cm^3 and 87.22 and 87.66g, respectively) compared with (100mg) addition level and control whom recorded 183.33 and 179.67 cm^3, respectively, for volume and 86.26 and 85.65g for weight, respectively. Pies volume slightly increased with increase the addition level. Specific volume was slightly increased at 200 and 300mg ethanolic licorice extract, compared with control. But the density was decreased by increasing the addition level. Low density was observed at 200 and 300mg (0.45 and 0.45 g/ cm^3), respectively, with non significant differences between them.

Bread made with the herbal extract did not significantly differ from the control sample in the specific volume (Simurina *et al*., 2008).

The color of pies were slightly affected with the addition of licorice extract. The *L** value indicates for lightness and it close to 100. Significant differences were found between control and two licorice extract 200 and 300 mg/100g flour where the addition decreased the lightness, this result agreed with Simurina *et al*. (2008). The *a** value indicates that, no significant differences in values for control and three levels of ethanolic licorice extract. *b** values indicates that, significant differences between control and licorice extracts (100, 200 and 300 mg./100g flour). No significant differences

were found between 200 and 300 mg of licorice extract for *L* and *b* values. Simurina *et al*. (2008) found that, the supplementation increased the yellow tone of the crumb as compared to the control.

Ma *et al*. (2020) found that, bread crumb samples containing vine tea extract were darker compared with the control sample, this is due to the presence of phenols in the extract.

Table 7: The effect of various concentration of ethanolic licorice extract on the physical properties of pies

Treatments	Volume (cm^3)	Weight (g)	Sepicific Volume (cm^3/g)	Density (g/cm^3)	Color L^*	a^*	b^*
Control	179.67^{c} ±0.58	85.65^{b} ±0.51	2.10^{b} ±0.01	0.48^{b} ±0.00	78.20^{a} ±0.47	$- 2.20^{a}$ ±0.09	22.38^{c} ±0.38
100mg/100g flour	183.33^{b} ±0.58	86.26^{b} ±0.51	2.11^{b} ±0.01	0.47^{b} ±0.00	78.12^{a} ±0.40	$- 2.21^{a}$ ±0.05	23.70^{b} ±0.17
200mg/100g flour	193.00^{a} ±0.58	87.22^{a} ±0.43	2.22a ±0.01	0.45^{a} ±0.00	77.74^{ab} ±0.45	$- 2.10^{a}$ ±0.05	26.09^{a} ±0.19
300mg/100g flour	194.33^{a} ±0.58	87.66^{a} ±0.35	2.21^{a} ±0.00	0.45^{a} ±0.00	77.00^{b} ±0.02	$- 2.11^{a}$ ± 0.03	26.41^{a} ±0.16

Values are the average of 3 experiments ± SD. Mean values followed by different superscripts (within the same column) are significantly different at the 5%.

9. The microbial quality of pies

Pies shelf life or microbial quality were affected by ethanolic licorice extract addition. After baking and during un-properly stored for prolonged time, contamination can result in the spoilage of baked goods. Fresh baked goods often contain the acceptable ranges of molds < $10\text{-}10^3$ CFU.g^{-1}; yeast, < $10\text{-}10^3$ CFU.g^{-1} and aerobic bacteria < $10^2\text{-}10^3$ CFU.g^{-1} (Mayou and Moberg, 1992). Foods are spoiled when they contained more than 10^5 or 10^7 CFU.ml^{-1} or g^{-1} of mold or bacteria, respectively (Mossel *et al.,* 1995). Tables 8 and 9 display the effect of different concentrations of ethanolic licorice extract on total bacteria and total fungi count in pies during storage at room temperature for 12 days. Data showed significant differences were found between control and different concentrations. A gradual increase in bacteria count at 100mg of ethanolic licorice extract samples where reaching its maximum shelf life at the 9^{th} day of storage.

The addition of 200 and 300 mg of licorice extract positively delayed of spoilage bacteria up to 3 days for 12 days. Fungi count was relatively lowered by the addition of 100mg extract where increased the shelf life compared with control (6.87 and 5.36 CFU.g^{-1}) at the 6^{th} day for control and 100mg, respectively. On the other hand, 200 and 300 mg significantly affected fungi growth where, shelf life was extended to 9 and12 days (6.95 and 6.31 CFU.g^{-1}), respectively.

Table 8: The effect of various concentrations of ethanolic licorice extract on total bacterial count (log CFU.g^{-1}) in pies during storage at room temperature (35ºC ± 2) for 12 days.

	Storage (Days)				
Treatments	**0**	**3**	**6**	**9**	**12**
Control	0.92^{a}_{e} ±0.06	3.95^{a}_{d} ±0.01	6.11^{a}_{c} ±0.01	8.55^{a}_{b} ±0.04	9.71^{a}_{a} ±0.01
100mg/100g flour	0.89^{a}_{e} ±0.01	3.91^{b}_{d} ±0.01	6.04^{a}_{c} ±0.07	7.08^{b}_{b} ±0.03	9.00^{b}_{a} ±0.03
200 mg/100g flour	0.86^{a}_{e} ±0.03	2.91^{c}_{d} ±0.01	4.32^{b}_{c} ±0.06	5.79^{c}_{b} ±0.04	7.18^{c}_{a} ±0.06
300 mg/100g flour	0.88^{a}_{e} ±0.04	2.13^{d}_{d} ±0.03	3.81^{c}_{c} ±0.04	5.18^{d}_{b} ±0.00	6.95^{d}_{a} ±0.05

Values are the average of 3 experiments ± SD. Mean values followed by different superscripts (within columns) and different subscripts (within rows) are significantly different at the 5%.

Table 9: The effect of various concentrations of ethanolic licorice extract on total fungi count (log CFU.g^{-1}) in pies during storage at room temperature (35ºC ± 2) for 12 days.

Treatments	Storage (Days)				
	0	3	6	9	12
Control	0.46^{a}_{e} ±0.15	2.99^{a}_{d} ±0.09	6.87^{a}_{c} ±0.03	8.02^{a}_{b} ±0.07	8.94^{a}_{a} ±0.01
100mg/100g flour	0.40^{a}_{e} ±0.17	2.69^{b}_{d} ±0.01	5.36^{b}_{c} ±0.10	6.42^{b}_{b} ±0.04	8.38^{b}_{a} ±0.04
200mg/100g flour	0.42^{a}_{e} ±0.10	2.24^{c}_{d} ±0.07	3.88^{c}_{c} ±0.09	5.04^{c}_{b} ±0.04	6.95^{c}_{a} ±0.05
300mg/100g flour	0.46^{a}_{e} ±0.10	1.95^{d}_{d} ±0.05	2.93^{d}_{c} ±0.02	4.18^{d}_{b} ±0.02	6.31^{d}_{a} ±0.03

Values are the average of 3 experiments ± SD. Mean values followed by different superscripts (within columns) and different subscripts (within rows) are significantly different at the 5%.

Conclusions

The ethanolic extract of licorice roots have a great potential as antioxidant compounds, and antibacterial and antifugal activities against *Staphylococcus aureus, E coli, Asperigllus flavus* and *Asperigllus niger.* This plant extract can prevent the spoilage in foods. Where the addition of ethanolic licorice extract to bakery products, successfully reduced bacteria and fungi count and extended microbilogical shelf life.

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