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Production and Evaluation of healthy drinks prepared from dried willow leaves *(Salix safsaf L.)*.

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ABSTRACT

In recent years, the willow (*Salix safsaf*), is widely distributed along the River Nile in Egypt, leaves used as a traditional medicine. Thus, the objective of this study was to evaluate the healthy drinks from dried willow leaves, as well as, its water extracts, as natural antioxidant and antimicrobial agents as well as protection from thrombosis. The leaves of willow were dried with three methods; oven, sun and vacuum drying. The methanolic extract of vacuum drying leaves gave the highest concentrations of phenolic and flavonoid compounds. The resultant of different dried Willow leaves *(Salix safsaf)* was evaluated for their proximate composition (on dry weight basis), Besides, DPPH, total phenolic compounds and total flavonoid. Results of the proximate analysis revealed that the moisture content recorded 5.95 ,5.48 ,7.26% in oven, sun and vacuum drying, respectively, whoever ash and fibers were 0.42& 1.22, 0.34& 1.5, 2.04 and 2.23% in oven, sun and vacuum drying, respectively. Chlorophyll a and b contents were 3.12&12.64, 1.52 &5.88 and 3.42& 14.19 in samples dried by oven, sun and vacuum drying, respectively.

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Carotenoids were 8.27, 8.64 and 6.98% oven, sun and vacuum drying, respectively. The total phenolic compounds content was 114.53, 88.17 and 93.61mg Gallic acid equivalent/g for oven, sun and vacuum drying, respectively. While, total flavonoids were 26.66, 14.85 and 24.43 (mg querctin equivalent /g) in samples dried by oven, sun and vacuum drying, respectively. The fractionation of phenolic compounds exhibited Ellagic, Pyrogallol and Caffeine in oven, sun and vacuum dried samples, respectively. Results ascertained that the highest Ellagic and Pyrogallol contents were detected in vacuum drying followed by sun and oven drying meanwhile, Caffeine was the highest component in vacuum drying, followed by oven drying however, it was not detected in sun drying sample. Water extracts of dried willow leaves were tested for their antimicrobial activity against different pathogenic microorganisms by using Disc Diffusion assay (zones of inhibition in mm). Our findings indicated that water extracts of willow leaves exhibited antibacterial activity against all tested pathogenic bacteria except fungal strains, which indicating the effective use of willow leaves *(Salix safsaf)* extracts as antimicrobial agents. Regarding to overall acceptability, it could be observed that control and (G2) Willow powder was added to lemon with ginger by 0.1% received nears scores to control, while he lower acceptability score values of (G4) Willow powder was added to lemon with ginger 0.3%that willow leaves oven powder.

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the sensory acceptance of the samples, it was found that GC2 (green coffee fortified with 2.5% willow leaves) is closest to the control, followed by GC3 (green coffee fortified with 5% willow leaves) and the least of them, GC4 (green coffee fortified with 7.5% willow leaves) due to the increase in the concentration of willow leaves.

Key words: Willow leaves, antioxidant, water extract, sensory evaluation, antimicrobial activity, proximate analysis.

INTRODUCTION

The genus Salix, commonly known as willow, includes almost 350 species. Salix species are cultivated in countries with temperate and semi-tropical climates as in the Middle Eastern country Egypt. In the Ebers papyrus, ancient Egyptians reported that willow could be used as an analgesic (Mahdi *et al.,* 2006). The high phenolic content of these plants and the consumer drive towards natural products demonstrated that these herbal extracts would be ideal ingredients for incorporation into healthy drinks with potential anti-inflammatory properties. Like other willow trees, extracts from safsaf also, have been used in traditional medicine. For example, treatment of stomach pain, fever and headaches with roots, brewed tea from leaves to treat rheumatism and powdered bark for soothing and curing burns. Previous phytochemical investigations on the genus Salix have led to reports on phenolic compounds, flavonoids, terpenes and lignans (Kim *et al.,* 2014).

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Coffee and willow are known to be valuable sources of biologically active phytochemicals such as chlorogenic acid, caffeine and salicin. These active substances demonstrated anti-inflammatory and antioxidant activities *in vitro* and thus, may be a potential complement to the treatment of civilization diseases associated with excessive generation of reactive oxygen species (Durak *et al.,* 2015). For a high quality extract for incorporation into drink the level of phenolic should be maximized, in particular the non-tannin fractions which will be included the active ingredients with anti-inflammatory properties. One supplement that has gained considerable popularity in the recent year is green coffee extract (GCE). GCE is a supplement made from green unroasted coffee beans. The supplement is available in capsule form, or it can be added to beverage products or chewing gum. The supplement contained naturally occurring caffeine and chlorogenic acid, a polyphenol antioxidant. The theory behind this product is that the chlorogenic acid is thought to be responsible for several of its pharmacological effects in GCE. It has been shown to inhibit fat accumulation and reduce weight in animal models and humans. In addition, GCE is thought to reduce postprandial glucose concentrations. It is also thought to reduce glucose absorption in the intestine Also, the tannin fractions should be minimized as they cause astringency, an undesirable gustative attribute.

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The phenolic compounds isolated, salicylic glycosides were the most abundant with reported analgesic, antipyretic, anti-inflammatory and anti-rheumatic properties (Chrubasik *et al.*, 2000; Chrubasik *et al.*, 2001 and Biegert *et al.*, 2004). Coffee and willow were known to be valuable sources of biologically active phytochemicals such as chlorogenic acid, caffeine and salicin. Therefore, the current study was undertaken to investigate healthy drinks from dried willow leaves, as well as its water extract, as natural antimicrobial and antioxidant agents.

MATERIALS AND METHODS

1. MATERIALS

a) Plant materials

The willow leaves *(Salix safsaf L.)* were collected from the Salix Farm of the Faculty of Agriculture, Cairo University, Giza, Egypt.

Green Coffee beans: it was purchased Obtained from the market under the trade name Abu Auf, Giza.

Ginger and lemon: Lemon and dried ginger were purchased from the local market, Giza.

a) Chemicals

All chemicals, used in this study were of analytical grade and were purchased from El-Gomhoria Co., Egypt.

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b) Microbial

Four bacterial strains of significant importance were used to assess the antibacterial properties of the willow leaves strains. The Gram positive were ***Bacillus subtilis*ATCC6538** and ***Staphylococcus aureus*ATCC25923** and two Gram Negative as ***E. coli* ATCC25922** and ***Salmonella typhimurium*ATCC9027**. Two fungal strains were ***Penicillium spp.*** and ***Aspergillus niger.*** The cultures were obtained from Microbiological Resources Centre (MIRCEN), Faculty of Agriculture, Ain Shams University, Cairo, Egypt.

2. Methods

Drying methods

1- Oven drying

The leaves were dried at (48°C) for six hours in oven drier (Fisher scientific U.S.A)

2- Vacuum oven drying

The leaves were dried at 45°C for 4 hours in (vacuum Drier SPT. 200 U.K)

3- Sun dried

Willow leaves were dried in the sun, where they were exposed to the sun at 39- 42°C for a period of 81 hours. The dried leaves were ground milled using a moulinex mill machine to obtain powder and it was used in the preparation of functional drinks (green coffee with willow powder added).

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Preparation of functional drinks:

A- Green Coffee beans with willow drink

Ground green coffee (10 gm) was put in cup and boiled water was poured then the mixture was left to be extracted for about 10 min and then the mixture was filtered the both through a fine sieve; the healthy green coffee drink is ready to drink.

B- Lemon ginger drink with willow leaves powder

About 2 gm of lemon and ginger were put in 200 ml water at (80 °C to 1 min) then 2.5 gm. sugar was added according to Lee *et al.* (2008). with some modifications.

Green coffee drink with willow powder

Different drinks were prepared as follows:

GC1: Green coffee alone as a control

GC2: Green coffee with (lemon + ginger) fortified with willow leaves powder (2.5%).

GC3: Green coffee with (lemon + ginger) fortified with willow leaves powder (5%).

GC4: Green coffee with (lemon + ginger) fortified with willow leaves powder (7.5%).

Lemon ginger drink with willow powder added

Lemon with ginger powder alone as a control

G2: Willow powder was added to lemon with ginger by 0.1% to make a 2 gm packet (0.2 gm willow powder + 1.8 gm lemon with ginger)

G3: Willow powder was added to lemon with ginger 0.2% to make a packet of 2 g (0.4 gm of willow powder to lemon with ginger).

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G4: Willow powder was added to lemon with ginger 0.3% to make a 2 gm packet (0.6 gm willow powder + 1.4 lemons with ginger).

Preparation of willow leaves extract

The young leaves were directly extracted with hot water (maceration process) where 7 g of fresh leaves 100 ml distilled boiled water(100^{o}C) for 20 min, then it was filtered through a sterilized Miracloth and centrifuged at 15,000 rpm for 15 min) according to El-Shemy *et al.* (2003).

Chemical analyses

1. Determination of gross chemical composition

The moisture and ash contents of willow leaves *(Salix safsaf L.)* were determined according to A.O.A.C. (2012), however, Crude fibers content was determined according to A.O.A.C. (2005).

2. Determination of total phenolic compounds (TPC) willow leaves

The concentration of total phenolic compounds in the methanolic extract (1:10) was determined by using Folin-ciocalteu's reagent according to Singleton and Rossi (1965). Calibration curve was prepared using Gallic acid as standard for TPC which was expressed as milligrams of Gallic acid equivalent (GAE) per gram on dry weight basis.

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3. Determination of total flavonoid of willow leaves.

The calorimetrically method as described by Barros *et al.* (2010) was used to determine total flavonoid content. Calibration curve was prepared using quercetin as standard for total flavonoid contents were expressed as mg quercetin equivalent (QE) per gram g on dry weight basis.

3. Determination of total carotenoids, chlorophyll a and chlorophyll b of willow leaves

Total carotenoids, chlorophyll a (chl. a) and chlorophyll b (chl. b) pre mg /L were determined as described by Ranganna (1977).

4. Separation and identification of phenolic components of the willow leaves extracts by HPLC:

HPLC Agilent 1200 series equipped with quaternary pump, auto sampler, column compartments ET at 35°C, wavelength detector set at 330nm, 280nm for detection of flavonoid compounds and phenolic compounds, degasser, column used for fractionation Zorbax OD. 4.6x250mm and the flow rate of mobile phase during run was 1 ml/min.

a HPLC analysis of phenolic compounds

The phenolic compounds of willow leave methanolic extract were fractionated and identified by HPLC according to the method described by Goupy *et al*. (1999).

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b HPLC analysis of flavonoid compounds

Flavonoid compounds of willow leave methanolic extract according to the method described by Mattila *et al*. (2000).

c HPLC analysis of Iso flavanone compounds

Isoflavanone compounds of willow leaves extracts were determined, and identified by HPLC as mentioned before at the same conditions except that of detector which set at 254 nm (Mantovani *et al.,* 2011).

5. Determination of radical scavenging activity in willow leaves The free radical scavenging activity of each extract was assessed by the discolorationof a methanolic solution of 2, 2-diphenyl-1- picrylhydrazyl (DPPH) radical (violet color) according to the method of Brand-Williams *et al*. (1995).

7. Antimicrobial activity of the willow leaves extracts

Bacterial strains

The disk diffusion method is used to evaluate antimicrobial activity of willow leaves extract. The plant extract (50 mg) were re-dissolved in 3 ml of distilled water, sterilized through Millipore filter (0.22 mm) then loaded over sterile filter paper discs (8 mm in diameter) to obtain final concentration of 10 mg/disc.

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Ten ml of Mueller-Hilton agar medium were poured into sterile Petri dishes (as a basal layer) followed with 15 ml of seeded medium previously inoculated with bacterial or mold suspension (100 ml of medium/1 ml of 10^7 (CFU) to attain 10^5 CFU/ml of medium. Sterile filter paper discs loaded with plant extract concentration of (10 mg/ml) were placed on the top of Mueller-Hilton agar plates. Filter paper discs loaded with 5 mg of Ciproflaxin was used as a positive control. The plates were kept in the fridge at 5 °C for 2 h. to permit plant extracts diffusion then incubated at 37°C for 24 h. The presence of inhibition zones was measured by Ruler, recorded and considered as indication for antibacterial activity (Mostafa *et al.,* 2018).

7. Determination of minimum inhibitory concentrations (MIC's) of the effective willow leaves extract

MIC is defined as the lowest concentration of the antimicrobial agent that inhibits the microbial growth after 24 h. of incubation. The most effective plant extracts which exhibiting a strong antibacterial activity at 10 mg/ml was manipulated to determine their MIC using disk diffusion method and evaluate their efficiency in controlling bacterial strains causing food poisoning diseases. Different concentrations of the effective plant extract (5, 10.0 and 15.0 mg/ml) were prepared separately by dissolving 50 mg in 5ml of distilled water, sterilized through Millipore filter and loaded their requisite amount over sterilized filter paper discs (8 mm in diameter).

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Mueller-Hilton agar was poured into sterile Petri dishes and seeded with bacterial suspensions of the pathogenic strains. The loaded filter paper discs with different concentrations of the effective plant extract were placed on the top of the Mueller-Hilton agar plates. Then incubated at 37°C for 24 h. The inhibition zones were measured by Ruler and recorded against the concentrations of the effective willow leaves extracts (Mostafa *et al.*, 2018).

a) Bacterial strains inoculation

Bacterial strains were inoculated into Mueller Hinton broth (Difco) and incubated at 37°C for 24 h. The cultures were subjected to three successive 24 hr., transferred before use. All cultures were adjusted to 10^6 CFU per ml prior to use.

b) Disc diffusion assay

Twenty milliliter of Muller Hinton agar were placed into 10 ml petri dishes and 100 µl of the active cultures were spread over the plate using a sterile glass spreader in order to get a uniform microbial growth for all plates (Bauer *et al.*, 1966).

8. Sensory evaluation of healthy drinks samples

Ten panelists were chosen from the staff of Food Tech. Res. Inst., Giza, were asked to score the drinks by 10 points for their taste, color, odor, and overall acceptability according to the method of Lindley *et al.* (1993).

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9. Statistical analysis

Data were subjected to the convenient statistical analysis method. Where, mean and standard error (SE) were calculated. Data were analyzed using two way-classifications ANOVA as described by Snedecor and Chochran (1980) followed by Duncan's multiple comparison tests to find the statistical significant difference between the ten treated groups.

RESULTS AND DISCUSSION

Table (1) shows the chemical composition of willow leaves, it was observed that moisture content has the highest value in sun dried operation (7.26%) while the lowest content was recorded in vacuum drying (5.48%). Ash content exhibited significant variation among drying methods. Crude fibers content in all samples recorded 1.5, 2.04 and 2.23% in oven, vacuum and sun drying, respectively.

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Table (1): Proximate composition of dried willow leaves (on dry weight basis).

Chemical Composition	Drying methods		
	Oven drying	Vacuum drying	Sun dried
Moisture content (%)	5.95^{ab}±1.21	5.48^{b}±0.59	7.26^{a}±0.17
Ash content (%)	0.42^{b}±0.32	1.22^{a}±0.11	0.34^{b}±0.11
Crude fiber (%)	1.5^{b}±0.43	2.04^{ab}±0.02	2.23^{a}±0.20
Chlorophyll A(mg/L)	3.12^{b}±0.03	1.52^{c}±0.00	3.42^{a}±0.02
Chlorophyll B (mg/L)	12.64^{b}±0.09	5.88^{c}±0.00	14.19^{a}±0.00
Carotenoids(mg/g)	8.27^{a}±0.00	8.64^{a}±0.011	6.98^{a}±1.50

Value are mean (n=3) ±SE Means in the same raw with different superscript are significantly different ($p \leq 0.05$)

Chlorophyll A, B, and total carotenoids were determined in all samples results indicated that the highest values were Chlorophyll A (3.42mg/l) was in sun dried leaves, however, it was the lowest (1.52 mg/l) in vacuum dried. On the other hand, the table showed that the higher content (14.19 mg/l) at sun dried, while, the lowest content was noticed at vacuum drying in Chlorophyll B. No significant ($p < 0.05$) differences were recorded in total carotenoids content of all samples, where the contents were 8.64, 8.27 and 6.98 mg/l g in vacuum, oven and sun dried samples, respectively.

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Table (2) Bio active compounds of willow leaves

Treatments	DPPH	Total phenol (mg/g)	Total flavonoid s	Tannins (mg/L)
Oven drying	89.85 [a] ±0.99	114.53 [a] ±1.98	26.66[a] ±2.37	19.33[b]±2.29
Vacuum drying	89.36 [a]±.35	93.61 [b]±7.29	24.43[a]±1.46	27.47[a]±0.87
Sun dried	89.50 [a]±1.99	88.17 [b]±9.54	14.85[b]±0.53	26.14[a]±1.39

Value are mean ± (n=3) Means column with different letters are significantly different ($p \leq 0.05$)

Results in Table (2) revealed the high decrement of antioxidant activity and antioxidant content (Total phenolic and flavonoids) in willow oven dried comparing with other samples. Table (2) showed that the oven dried willow had the highest amount of total phenolic (114.53mg/g), subsequently, antioxidant activity ($p \leq 0.05$) (89.85), followed by willow vacuum (93.61mg/g) in total phenolic compounds. The lowest value was recorded in sun dried (88.17mg/g) with non-significant differences.

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On the other hand, no significant ($p \leq 0.05$) differences were found between oven drying and vacuum drying samples (26.6 and 24.42) however, the lowest content was recorded for sun dried sample (14.85) While, the contents of tannins in the samples were recorded the nearest value in vacuum and sun dried (27.47 and 26.14) and the lowest content of tannins that record in oven drying. These results are in agreement with those of Harbourne *et al*.(2009) who found that oven drying at 30 °C had no significant effect on the phenolic constituents (e.g. total phenols, salicylates, querctin), although, increasing the drying temperature to 70 °C resulted in an increment in the drying rate of both herbs it also led to the loss of some phenolic compounds. The results indicated that S. alba extract have good free radical scavenging activity and it can be used as a radical inhibitor or scavenger, acting possibly as a primary antioxidant. However, the results from total phenolics analysis and the antioxidative assay were observed. Polyphenolic compounds are known to have antioxidant activity and it is likely that the activity of the extract is due to these compounds (Djeridane *et al.,* 2006).

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Table (3). The phenolic profiles by using HPLC in dried willow leaves (*Salix safsaf)* extract with different drying methods, (mg/100g).

Components (mg/100g)	Drying methods		
	Oven	Sun	Vacuum
Pyrogallol	1035.86	279.09	1293.95
Gallic acid	4.43	3.51	2.24
3-Hydroxy tyrosol	26.79	5.98	29.62
Catechol	116.12	74.79	162.46
4-Amino banzoic acid	45.93	14.21	43.05
Catechin	185.92	83.21	185.00
Cholrogenic acid	1384.19	355.53	1105.62
P-OH-Benzoic acid	144.57	67.24	173.67
Caffeic acid	135.61	64.62	66.88
Vanillic acid	75.58	0.00	39.24
Caffeine	1676.80	1110	2451.64
Ferulic acid	334.19	391.27	258.97
Ellagic	3542.40	3966.96	4800.09
Oleuropein	590.86	429.82	107.94
Coumarin	0.00	69.36	156.53

The chromatograms of the phenolic compounds from dried willow leaves with different drying techniques, showed different profiles (Table 3).

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The leaves from oven willow powder contained in indicated the pyrogalol (1035.86 mg/100g), gallic acid (4.43 mg/100g), 3-Hydroxy-Tyrosol (26.79 mg/100g), 4 amino-benzoic acid (45.93 mg/100g), catechin (185.92 mg/100g), chlorogenic acid (1384.19mg/100g), caffeic acid (135.61), vanillic acid(75.85mg/100g),caffeine (1676.80mg/100g)ferulic acid (334.19mg/100g), ellagic acid (3542.40 mg/100gm),oleuropein (590.86). Ellagic acid, Caffeine and chlorogenic acid acid were the major phenolic compounds in the oven dried willow leaves.In sun dried willow leaves, HPLC-chromatograms showed that it contained pyrogalol (279.09 mg/100g), Gallic acid (3.51mg/100g) 3-Hydroxy tyrosol (5.98 mg/100g) catechol (74.79mg/100g) 4-Amino benzoic (14.21 mg/100g), chlorogenic (355.53mg/100g), ρ-OH-Benzoic (67.24 mg/100g) catechein (83.21 mg/100g), Caffeic acid (64.62mg/100g), Caffeine(1110mg/100g).Ellagic acid (3966.96 mg/100g) Oleuropein (429.82mg/100g), ferulic acid (391.27 mg/100g), coumarin (69.36 mg/100g). Ellagic, Caffeine and Oleuropein were the major phenolic compounds in the sun dried willow leaves. Also, ellagic , caffeine and pyrogallol were the major phenolic compounds in the vacuum drying willow powder.

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Table (4) The flavonoids profiles by using HPLC in dried willow leaves *(Salix safsaf)*extract with different drying methods(mg/100g).

Components (mg/100g)	Drying methods		
	Oven	Sun	Vacuum
Rutin	684.64	28.48	22.00
Naringin	587.34	195.42	285.79
Rosmarinic	75.80	25.82	62.67
Quercitin	637.21	54.69	200.72
Apigenin7 Glucose	203.42	47.24	0.00
Quercetrin	1110.38	57.85	177.17
Naringinen	10.2	9.79	11.82
Kaempferol	38.95	0.00	11.99
Apegenin	8.113	0.94	1.90

The chromatograms of the flavonoid compounds from willow leaves with, oven drying , sun-dried and vacuum drying showed different profiles. The oven dried willow leaves contained in Table (4) indicated the presence of oven drying powders were identified 9 compounds, quercetrin, rutin and quercitin were the major compounds in flavonoid the oven drying willow leaves.

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On the other hand, the sun dried leaves the major flavonoid compounds: naringin, quercetrin and quercitin were the major flavonoid compounds. In vacuum drying leaves, the major compounds were naringin, quercitin and quercetrin.

Table (5) the Isoflavanone compounds in sample of willow leaves *(Salix safsaf)* extract and different methods, oven, sun and vacuum by HPLC.

constituents (mg/100g)	Drying methods		
	Oven	Sun	Vacuum
Isorhamtine	139.52	16.62	32.64
Daidazein	45.20	90.63	0.003
Genistein	43.02	15.44	7.65
Isoformentin	3.41	0.11	1.008
Biochainin	0.48	0.012	0.03

The isoflavone compounds of dried willow leaves processed with different methods were determined. Data in Table (4) showed that isoflavone present in all dried willow leaves. It was found that the major compounds of willow leaves as isorhamtine (139.52), followed diadazein (45.20) and genistein (43.02) in oven drying leaves, however in sun dried, diadazein (90.63), isorhamtine (16.62). Meanwhile vacuum drying, Isoformentin (1.008).

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It was found that the highest isoformantine compounds in vacuum dried leaves, while, Isorhamtine in oven drying and Daidazein in sun dried.

Effect of different concentrations of willow leaves aqueous extract (µ/ disc) against some bacteria strains

Antibacterial activity of willow leaves extract was evaluated against four types of bacterial strains. Hence, the strains of bacteria were *Bacillus subtilis, Escherichia coli, Staphylococcus aureus and Salmonella typhimurium*. It was found that with increasing the concentration of willow aqueous extract, the inhibition zone against bacterial strains increased.

Table (6). Effect of different concentrations of Willow leaves aqueous extract (µ/ disc) on the growth of some bacterial strains.

Bacterial strains	20µg/disc	40µg/disc	60µg/disc	80µg/disc	100µg/disc	Control
Salmonella Typhimurium	11.0 ^{c} ±2.0	11.67 ^{c} ±2.08	14.0 ^{bc} ±1.00	15.0 ^{bc} ±2.0	16.33 ^{b} ±3.21	41.67 ^{a} ±2.9
Bacillus subtilis	14.33 ^{d} ±3.05	15.0 ^{cd} ±3.0	16.0 ^{bcd} ±4.58	19.33 ^{bc} ±1.15	20.67 ^{b} ±.57	45.0 ^{a} ±.00
E. coli	8.67 ^{d} ±1.5	9.3 ^{d} ±1.15	14.33 ^{c} ±2.08	18.33 ^{b} ±1.52	19.33 ^{b} ±1.15	22.67 ^{a} ±1.15
Staphylococcus aureus	7.33 ^{f} ±0.57	12.0 ^{e} ±1.73	15.33 ^{d} ±.57	17.33 ^{c} ±.57	20.0 ^{b} ±1.0	45.0 ^{a} ±.0

The antibiotic Amofluxin (µ /ml) was used as a positive control to determine the antibacterial effect.

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The results in Table (6) revealed that the maximum willow leaves aqueous extract (100µg / disc) had the highest values of antibacterial activity (inhibition zone values) of all bacteria strains. Moreover, the highest inhibition zone values were achieved by concentration (100µg / disc) willow leaves aqueous extract against *Bacillus subtilis* strain, followed by *Staphylococcus auraus*. Whereas, Amofluxin (positive control µ /ml) obtained highest significant values of inhibition zone (a) as compared with other obtained by willow leaves aqueous extract (100µg / disc) of all bacteria strains (b). It was noticed that the lowest significant inhibition zone was achieved by (20 µg /disc) of willow leaves aqueous extract against *Staphylococcus aureus* (f). The explanation of Gram positive bacteria is more susceptible than Gram-negative bacteria may attribute to the differences in their cell wall structure. Gram-negative organisms are considered to be more resistant due to their outer membrane acting as a barrier to many environmental substances, including antibiotics (Kaye *et al*., 2004).

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Table (7) Effect of different concentrations of Willow leaves aqueous extract (µ/ disc) on the growth of some fungal strains.

Bacterial strains	20µg/disc	40µg/disc	60µg/disc	80µg/disc	100µg/disc	Control
Salmonella Typhimurium	11.0 ^{c} ±2.0	11.67 ^{c} ±2.08	14.0 ^{bc} ±1.00	15.0 ^{bc} ±2.0	16.33 ^{b} ±3.21	41.67 ^{a} ±2.9
Bacillus subtilis	14.33 ^{d} ±3.05	15.0 ^{cd} ±3.0	16.0 ^{bcd} ±4.58	19.33 ^{bc} ±1.15	20.67 ^{b} ±.57	45.0 ^{a} ±.00
E. coli	8.67 ^{d} ±1.5	9.3 ^{d} ±1.15	14.33 ^{c} ±2.08	18.33 ^{b} ±1.52	19.33 ^{b} ±1.15	22.67 ^{a} ±1.15
Staphylococcus aureus	7.33 ^{f} ±0.57	12.0 ^{e} ±1.73	15.33 ^{d} ±.57	17.33 ^{c} ±.57	20.0 ^{b} ±1.0	45.0 ^{a} ±.0

ND, means not detected

Data in Table (7) appeared that antifungal activity of willow leaves aqueous extract was performed against two mold strains (*Aspergillus niger* and *Penicillium spp*). The results displayed that the maximum concentration (100µg / disc) willow leaves aqueous extract achieved highly significant values of inhibition zone against *Penicillium spp* ,(a) and *Aspergillus niger* (ab). The antibiotic (µ /ml) was estimated as a positive control in determining the antifungal. Whereas, no significant differences were achieved between the values of inhibition zone of control positive and other obtained by concentration (100µg / disc) willow leaves aqueous extract against two mold strains. However, the least significant values of inhibition zone were obtained by (40 µg /disc) against *Penicillium spp.* (c) and (20 µg /disc) against Aspergillus niger (d).

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These results are in agreement with Andreu *et al.* (2018) and Hussain *et al.* (2011) found that the willow infusion inhibits the germination and the spread of fungal diseases without really killing the fungi as expected for chemical pesticide. Salix cortex has been shown to have antifungal activity against these two mold strains.

Table (8) Effect of minimum inhibitory concentration (MIC) of different concentrations of Willow leaves aqueous extract against some bacteria and fungi.

Microbe	5µg/Dic	10µg/Dic	15µg/Dic
Salmonella typhimurium	ND	6.7±1.53	10.0±1.0
Bacillus subtilis	ND	ND	11.3±0.57
E. coli	ND	ND	7.0±0.0
Staphylococcus aureus	ND	ND	ND
Penicillium spp.	ND	ND	ND
Aspergillus niger	ND	ND	ND

ND, means not detected

The results in Table (8) revealed minimum inhibitory concentration (MIC) of different concentrations of willow leaves aqueous extract and its effect on antimicrobial activity. Data in Table (8) showed that that minimum inhibitory concentration against *Salmonella typhimurium* was achieved by concentration (10µg / disc) willow leaves aqueous extract.

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Although, minimum inhibitory concentration (MIC) of willow leaves aqueous extract was concentration (15µg / disc) against *Bacillus subtilis* and *E. coli*. Indiscriminate use of antimicrobial agents and due to the emergence of resistance in pathogens, microbial populations are limiting the period of antibiotics and side effects associated with synthetic and semi-synthetic drugs limit the physicians for their use. This alarming situation requires the discovery of new biocompatible antimicrobial agents (Dafallah Bilal and Hossain, 2019). Green plants are the richest archive of agents having antimicrobial usefulness and are most cost-effective and accessible than synthetic pharmaceutical preparations that involve many side effects (Carvalho *et al.*,2018).

Table (9): Sensory evaluation of Ginger lemon with willow oven powder drink

Samples	Sensory criteria			
	Color	Taste	Odor	overall acceptability
Control	8.50_{ab}±.71	8.15^{a}±0.47	8.20^{a}±0.92	8.10^{a} ±1.37
G2	8.83^{a}±1.12	7.15^{b}±0.88	8.28^{a}±0.57	8.06^{a} ±0.95
G3	8.75^{a}±1.09	6.89^{b}±0.60	8.50^{a}±1.18	7.45^{a} ±0.83
G**4**	7.60^{b}±1.17	5.95^{c}±0.69	7.80^{a}±1.01	6.75^{a} ±0.86

Data are the mean ± SE, n=10, Mean values in the same column bearing he same superscript do not differ significantly ($p \leq 0.05$).

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Control: Ginger lemon without addition.
G2: Ginger lemon fortified with 0.1% willow leaves.
G3: Ginger lemon fortified with 0.2% willow leaves.
G4: Ginger lemon fortified with 0.3% willow leaves.

Medicinal herbs are the basis of many therapeutically beverages used along the history. In the present, complex multidisciplinary researches are developed for complete characterization of herbal extracts leading to novel formulations with functional and medicinal uses. Medicinal beverages can take the form of infusions, decoctions, and macerations representing crude aqueous extracts or aqueous solutions prepared with dried material or liquid concentrates. However, many medicinal plants possessing immunomodulatory, immunostimulatory, antidiabetic, anticarcinogenic, antimicrobial, and antioxidant properties (Butu, 2019). Ginger lemon samples counting willow powder and hose prepared with different fortification by the oven dried powder i.e. (control, 2, 3 and 4 treatments) were evaluated for their color, taste, Oder and overall acceptability as presented in table (9). There was no significant decrease (P=0.05) in scores of color, odor and overall acceptability. While, the significant decrease in scores in Taste. Regarding to overall acceptability it could be observed that control and G2 received nears scores to control, while he lower acceptability score values of G4 that ginger lemon fortified with 0.3% willow leaves oven powder. Ginger is a flowering plant with the binomial name of Zingier Officinal from the family of Zingiberaceae, and is a part of food menus in most countries.

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From the rhizome of regular ginger root, a powder is prepared and is used as a spice from older days. Galen, a Greek physician and used ginger as the body filter. He used ginger when the body was in imbalance. Ginger is a very common condiment on a global scale that has been used in Chinese traditional medicine for more than 2,500 years for curing flu, rheumatism, neurological disorders, gum swelling, toothache, asthma, stroke, constipation, diabetic, indigestion, vomiting, cardiopathy, high blood pressure and palpitations. Oven-drying mature willow leaves at low temperature (48°C) gives practically no qualitative or quantitative differences in salicylates compared those found in fresh leaves (Julkunen Tiitto and Tahvanainen, 1989).

Table (10): Sensory evaluation of green coffee with willow oven powder drink

	Sensory criteria			
Samples	Color	Taste	Odor	overall acceptability
GC1	$8.30^{a}\pm0.82$	$7.70^{ab}\pm0.82$	$7.70^{b}\pm0.95$	$7.80^{ab}\pm0.79$
GC2	$8.30^{a}\pm1.05$	$8.05^{a}\pm0.90$	$8.40^{a}\pm0.52$	$8.30^{a}\pm0.67$
GC3	$7.30^{b}\pm0.48$	$7.00^{bc}\pm0.94$	$6.80^{c}\pm0.79$	$7.25^{b}\pm0.60$
GC4	$6.30^{c}\pm0.82$	$6.30^{c}\pm0.48$	$6.05^{d}\pm0.69$	$6.16^{c}\pm0.26$

Data are the mean ± SE, n=10, Mean values in the same column bearing he same superscript do not differ significantly ($p\leq 0.05$).

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GC1: green coffee without addition. (Control)

GC2: green coffee fortified with 2.5% willow leaves.

GC3: green coffee fortified with 5% willow leaves.

GC4: green coffee fortified with 7.5% willow leaves.

The results in Table (10) regarding showed that all treatments fortified with willow leaves oven powder observed that in general GC2, (green coffee fortified with 2.5% willow leaves oven powder) is in nearest to control (color, taste, Oder and overall acceptability), comparing the three samples with the control sample, it was found that with regard to the color of GC2 (green coffee fortified with 2.5% willow leaves), it was the closest to the control, followed by GC3 (green coffee fortified with 5% willow leaves), and the least of them in color was GC4 (green coffee fortified with 7.5% willow leaves) As for the taste, the GC1 was closer to the control sample, and with an increase in the concentration of willow leaves, the taste of the samples decreased. Regarding Odor, significant differences were noted for the three samples, and in view of the sensory acceptance of the samples, it was found that GC2 (green coffee fortified with 2.5% willow leaves) is the closest sample to the control with no significant different, followed by GC3 (green coffee fortified with 5% willow leaves) and the least of them, GC4 (green coffee fortified with 7.5% willow leaves) due to the increase in the concentration of willow leaves. Coffee and willow are known to be valuable sources of biologically active phytochemicals such as chlorogenic acid, caffeine and salicin.

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Durak *et. al. (*2015) suggested that coffee and willow contain bioactive constituents which interact with each other. The active substances found in the tested raw materials demonstrated anti-inflammatory and antioxidant activities *in vitro* and thus, may be a potential complement to the treatment of civilization diseases associated with excessive generation of reactive oxygen species.

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الملخص العربي

إنتاج وتقييم المشروبات الوظيفية المجهزه من أوراق الصفصاف المجففه

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تنتشر أوراق الصفصاف (*Salix safsaf*) على نطاق واسع على طول نهر النيل في مصرو في السنوات الأخيرة استخدمت فى الطب التقليدي. وبالتالي ، كان الهدف من هذه الدراسة هو تقييم إنتاج المشروبات الوظيفية من أوراق الصفصاف المجففة بالطرق المختلفة ، وكذلك المستخلص المائي لها، كمضادات أكسدة طبيعية ومضادات للميكروبات وكذلك للحماية من الاكسدة. تم تجفيف أوراق الصفصاف بثلاث طرق ، بالفرن و التجفيف الشمسى والتجفيف تحت تفريغ. أعطى المستخلص الناتج بالتجفيف تحت تفريغ أعلى تركيزات من المركبات الفينوليه والفلافونويديه .تم تقدير التركيب الكيميائى لأوراق الصفصاف (على أساس الوزن الجاف) وذلك فى الثلاث طرق المختلفة. أظهرت النتائج أن الرطوبة الكلية 5.95 و 5.48 و 7.26٪ في حالة التجفيف من خلال الفرن والشمس والتجفيف بالتفريغ على التوالي بينما الرماد والألياف كانت 0.42 و 1.22 و 0.34 و 1.5 و 2.04 و 2.23٪ في الفرن والشمس والتجفيف تحت تفريغ على التوالي. و كان تقديرالكلوروفيل أ ،والكلورفيل ب 3.12 ، 12.64 ، 1.52 ، 5.88 و 3.42 ، 14.19فى حالة التجفيف بالفرن العادى ، التجفيف بالشمس و تحت تفريغ على التوالي. كانت الكاروتينات الكلية 8.27 و 8.64 و 6.98٪ في التجفيف بالفرن والتجفيف الشمسي و تحت تفريغ على التوالي. تم تقدير الفينولات الكليه ووجد ان حمض الجاليك 114.53و88.17 و 93.61 مجم مكافىء حمض جاليك / جرام في فرن حمض الغاليك والشمس والتجفيف بالتفريغ على التوالي. بينما كان مركبات الفلافونويدات الكلية querctin 26.66 و14.85 و 24.43 ملجم مكافىءكيرستين / جم من فرن querctin والشمسي والتجفيف تحت التفريغ على التوالي. والمركب الفعال من إجمالي الفينول Ellagic و Pyrogallol و Caffeine في الفرن والشمس والتجفيف بالتفريغ على التوالي. أعلى مركبات Ellagic في التجفيف بالتفريغ تليها التجفيف بالشمس والفرن وأيضًا ، كان Pyrogallol ، بينما كان الكافيين أعلى نسبة في التجفيف تحت تفريغ يليه التجفيف بالفرن. تم قياس القدرة على تثبيط النمو ضد بعض السلالات البكتيرية والفطرية المختلفةعن طريق الانتشار بالقرص بالمليمتر (مم).

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حيث تم العثور على مناطق مثبطة ضد جميع مسببات الأمراض البكتيرية المختبرة باستثناء الميكروبات الفطرية ، مما يشير إلى أن الاستخدام الفعال لمستخلص أوراق الصفصاف (Salix safsaf) كعوامل مضادة للميكروبات. من المعروف أن البن والصفصاف من المصادرالهامه القيمة للمواد الكيميائية النباتية النشطة بيولوجيًا مثل حمض الكلوروجينيك والكافيين والساليسين. مشروب الزنجبيل والليمون المدعم بـ 0.1٪ أوراق الصفصاف كان أفضل العينات للصفات الحسية يليه مشروب الزنجبيل والليمون المدعم بـ 0.2٪ أوراق الصفصاف. من ناحية أخرى ، كانت القهوة الخضراء المدعمة بنسبة 2.5٪ الصفصاف أفضل عينة مقارنة مع العينة ال control أو الغير معامله.