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Effect of Bioactive Compounds of Sting Nettle *(Urtica Diocia* L.*)* Leaves on Shelf Life of the Salty Biscuits

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ABSTRACT

Stinging nettle *(Urtica dioica)* is an edible plant, well-known for its nutraceutical properties. Stinging nettle leaves are typically rich in fibers, minerals and vitamins, as well study was to evaluate the sensory properties and the rheological properties of wheat salted biscuits after adding of dried nettle leaves to flour wheat at different concentrations of 5,10 and 15 % antioxidant compounds, i.e., phenols and flavonoid. along with the control sample. Also, changes in the microbial load of biscuits added to nettle leaves during storage periods (1,3,6 and zero time) were investigated. Showed that total bacterial counts were not detected at all directly after processing with all used concentration increased gradually with time. At 5%, and 10% concentration, total bacterial count appeared a month later zero time. During storage period, total bacterial count appeared and a month later with (3.08 and 2.30 log cfu/g) and increased gradually to reach (3.34 and 2.77 log cfu/g) respectively, while 6 months later of storage, total bacterial counts increased gradually reaching (5.50, 4.91 and 4.56 log cfu/g) with concentrations 5%,10% and 15% respectively compared to control. It means that, 15% can be enough as preservation treatment for biscuits. concentration with 15% had higher effect, where total bacterial counts appeared after 3 months (2.30 log cfu/g) and increased to (4.65 log cfu/g) after 6 months of storage. fungi was not detected (ND) through increasing time of storage due to the low moisture in biscuits samples and reducing the numbers of fungies in the various concentrations of nettle leaf. The antimicrobial activity of ethanolic and methanolic extracts of nettle they demonstrated no effects against yeast-like fungi *(Candida albicans)*. Although all extracts exhibited an antimicrobial activity against all tested bacteria with inhibition zones ranging from 10.0±0.58 to 12.7±0.67 mm., they were more effective against gram-positive bacteria *(Bacillus cereus and Staphylococcus aureus)* than against gram gram-negative bacteria *(Salmonella typhimurium and Escherichia coli).*

1. Introduction

Nettles (*Urtica diocia L.*), or stinging nettle, is a perennial plant growing in temperate and tropical wasteland areas around the world. It grows 2 to 4 meters high and produces pointed leaves and white to yellowish flowers, and it belongs to the family Urticaceaea (Kregiel et al., 2018). The rubric name Urtica comes from the Latin verb urere, meaning ‘to burn,’ because of these surcharging hairs. The species name dioica means ‘two houses’ because the plant usually contains either male or female flowers. Nettle has a well-given character for giving a savage sting when the skin touches the hairs and bristles on the leaves and stems. They have also been used for centuries in traditional medicine and have been accepted as a healing plant because of their considerable effects on human health in

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many countries all over the world (Adhikari et al., 2016). They are used as a diuretic, antihypertensive, anti-diabetic, hemostatic, anti-asthenia, antianemic, antispasmodic, antirheumatic, and as a remedy for headaches and chills (Bnouham et al., 2002). Nettle is also used to treat spleen, renal, and dermal disorders (Borges Botelho et al., 2014). The whole plant is used as a diuretic, antihypertensive, anti-diabetic, hemostatic, anti-asthenia, antianemic, antispasmodic, antirheumatic, and as a remedy for headaches and chills (Belščak-Cvitanović et al., 2015). The presence of precious biologically important composites such as proteins, vitamins, phenolic factors, macro- and microelements, tannins, flavonoids, sterols, adipose acids, carotenoids, and chlorophylls contributes to the application of stinging nettle in different ways (Nica et al., 2012; Kopyt'ko et al., 2012). Therefore, nettle is used as a dietary supplement as well as for the production of functional foods. However, it is still an underrated plan (Man et al., 2019). Stinging nettle is one such raw material. Thus far, it has been successfully used as a functional ingredient in bread (Đurovi´c et al., 2020) or as a component of a herbal mixture added to wheat bread baked without salt (Wójcik et al., 2021). It has also been used to coagulate milk proteins in the cheese-making process (Fiol et al., 2016), and concentrated and lyophilized stinging nettle extract was used to manufacture chocolates (Belšˇcak-Cvitanovi´c et al., 2015). In order to enhance the activity of bioactive compounds contained in nettle, attempts have been made to nanocapsulate its extracts, and the research findings indicate the possibility of. In this study, nettle leaf powder was added to salted biscuit dough to improve the nutritional intake of minerals, fiber, protein, and antioxidant compounds, as well as increase the shelf life of the biscuit. the obtained nanocomposites in various food matrices (Rutakhli et al., 2019 and Delfanian and Sahari, 2020).

Nettle is a raw material not only of high health-promoting potential. It is rich in chlorophyll and metallic elements and can be used alongside vegetable powders and concentrates as a natural coloring component.

2. Materials and Methods

Materials

Plant materials

The nettle samples (*Urtica diocia L.*) were obtained from the Faculty of Agriculture, Cairo University, Giza, Governorate. The biscuit ingredients (wheat flour, butter, eggs, salt, and baking powder) were purchased from one of the markets near the Food Technology Research Institute in Giza, Government. 2.1.2. Bacterial and fungal strains aggregated from five strains from gram-positive bacteria, gram-negative bacteria, and incentive-such fungi (Salmonella typhimurium NCTC 12023/ATCC 14028, Escherichia coli ATCC 25922, Bacillus cereus ATCC 33018, Staphylococcus aureus ATCC 25923, and Candida albicans CAIM-22) were provided by the Microbiological Resources Center (MIRCEN), Faculty of Agriculture, Ain Shams University, Cairo, Egypt.The fungal strain was maintained on potato dextrose agar, whereas bacterial strains were cultivated on nutrient agar.

Methods

Preparation of raw materials

Nettle leaves were thoroughly washed with running water under the tap. When done, the nettle was dried in an air oven (Type: VENTICELL55- Artikel Nr. 000721/10000 - Temperaturberech: 250°C – Anschlub:230V AC 50/60Hz–Leistungaufn.:1250W Germany) at 40°C and passed through a sieve of 250 µm for 24 hours. (Akubor and Owuse 2020). Dried dry nettle leaves were crushed using an electric grinder (Type: Moulinex MFP626, 220V, 50-60Hz, 1000W, 250 ml, France), passed through 250 µm to obtain a uniform size, packaged in polyethylene bags, and stored in a desiccator until used for further analysis (Salama et al., 2022).

Production of biscuits

The biscuits were prepared using the straight dough method as described by (Akubor 2016). The ingredients used were 20% buter, 10% homogenized whole egg, 2% salt, and 0.5% baking powder. The ingredients were weighed and thoroughly mixed manually. The wet ingredients were first mixed in a mixing bowl at an evenly low speed for

5 minutes. The dry ingredients were added, and the mixing was continued at a high speed until a uniform texture was obtained. Using an electric perineum (RBSFOODMIXERPRO, Cuizimate, Thailand) at low (80 rpm, for 1 min) and moderate speeds (100 rpm, for 10 min), use wheat flour to make biscuits, adding dry nettle leaves in proportions of 5, 10, and 15% to the wheat flour. The dough samples were then manually kneaded, molded, shaped, and placed on the baking tray. and baked in a thermostatically controlled baking oven at 170°C for 20 minutes. The biscuits produced were cooled to ambient temperature (32 oC) and packaged in polyethylene bags prior to analysis. The 100% wheat flour biscuit served as the control (Thliza et al., 2021).

Formula of crackers

Control = 100% wheat flour

A = 5% addition of nettle leaves + wheat flour

B = 10% addition of nettle leaves + wheat flour

C = 15% addition of nettle leaves + wheat flour

Methods of analysis

All analyses of crude protein, fat, fibers, and ash content were done according to the Association of Analytical Chemists Methods (A.O.A.C. 2000). carbohydrates = 100 (% crude protein + % crude fat + % ash + % crude fibers) were estimated by difference. humidity and titratable acidity were determined according to (A.O.A.C. 2012).

Determination of minerals

The mineral content of iron (Fe), zinc (Zn), calcium (Ca), potassium (K), and manganese (Mn) was determined using an Agilent Technologies (model 4210 MPAES) atomic absorption spectrophotometer according to the method of A.O.A.C. (2011).

Determination of Total Antioxidant Activity

Total antioxidant content (TAC) was estimated by using a 2,2-diphenyl-1-picrylhydrazil (DPPH) assay to measure the free radical scavenging capacity (Sidaoui et al., 2015).

Determination of Total Phenolic Percentage

Total Phenolic Content (TP) of Extracts was determined using the Folin-Ciocalteu reagent as described previously by (Ghaima et al., 2013). The TP of the excerpts was expressed as milligrams of gallic acid fellow (GAE) per 100 g sample.

Determination of Total Flavonoid Percentage

Total flavonoid content (TF) was measured using AlCl3, a colorimetric method A.O.A.C. (2006). The absorbance was measured at 510 nm using the spectrophotometer. The TF of the excerpts was expressed as milligrams of catechin fellow (GAE) per 100 g sample.

Rheological properties

Farinograph test

Farinograph tests were carried out on wheat flour and its mixtures of nettle leaves were added to it in different proportions (5, 10, and 15) in the following constant flour weight procedure according to method A.A.C.C. (2002), in the rheology laboratory of the Food Technology Research Institute in Cairo, Egypt.

Extensograph test

Extensograph parameters were measured with a Brabender Extensograph (Brabender OHG) according to A.A.C.C. (2002) in the rheology laboratory of the Food Technology Research Institute in Cairo, Egypt. Extensograph measurements included elasticity (B.U.), extensibility (mm), proportional number, and energy (cn2).

Preparation of powder

The upstanding corridor of U. Dioica was shade -dried at room temperature for ten days and also ground to a fine greasepaint using an electronic blender. Extraction was done from one gram of obtained powder using 10 mL of alcohol (ethanol or methanol, Merck, Germany) and distilled water solution (8:2 v/v), centrifugation at 3000 rpm for 15 minutes, and harvesting the supernatant. This process was repeated three times, and solvents were evaporated by placing the yielded materials at room temperature for seven days (Riehemann. et al., 1999; Seyyednejad. et al., 2001).

Antimicrobial assay

Antimicrobial activities were assessed with the agar well-diffusion method (Perez and Paul, 1990). In brief, 1ml of fresh bacterial or fungi culture was pipetted in the center of a sterile Petri dish. Also, 15 ml of nutrient agar medium for bacterial strains or potato dextrose agar medium for fungal strains was poured into the Petri dish containing the inoculum and mixed well. A sterile cylinder (8 mm in periphery) was used to make wells into the solidifying agar plates containing inoculums. After solidification, 100 µl of the diluted stock solutions (extracts) were added to the wells. Wells containing reference antibiotics (tetracycline for bacteria and Nystatin for fungi) served as positive controls (50 µg/ml). The incubation period was either 24 hours for bacteria or 48 hours for incentives. Antimicrobial exertion was determined by measuring the zone of inhibition (including the well periphery) after the incubation period.

Microbiological analysis

To determine microbial counts, three replicas from each treatment and control were aseptically opened. Ten grams from each sample were transferred to a sterilized glass bottle containing 90 ml of sterile physiological saline (0.85% NaCl). The bottle was shaken to homogenize the sample, and then serial 10-fold dilutions were prepared. Appropriate serial dilutions were duplicate plated (pour plate method) with Plate Count agar medium (APHA, 1985) for the total bacterial count. Then the plates were incubated at 37 oC for 24-48 hours. Yeast mold agar (Jong and Edwars, 1991) was used for counting yeast and molds at 25 oC for 5 days.

Sensory evaluation

The sensory characteristics of the control biscuit samples and the different addition ratios 5, 10, and 15 were evaluated in terms of color, taste, smell, texture, crispness, appearance, and general acceptance. The evaluation was carried out by 10 food tech judges. Rec. Using a 10-point pleasure scale. Samples receiving an overall quality score of 7 or above were considered palatable. according to the method of (Al-Marazeeq and Angor 2017).

Statistical analysis

one-way analysis of variance (ANOVA), with a multiple range significant difference (LSD) test, and standard error (SE) ($p < 0.05$) were carried out using SPSS according to (Sarmento and Costa 2019).

3. Results and Discussions

Proximate composition of dried nettle leaves

The chemical analysis of nettle leaves powder was carried out on dry weight basis and the results are presented in Table 1. stinging nettle powder showed higher amount of carbohydrates content 40.86% while recording the protein of 30.59%. Also found that total ash content of nettle. Dietary fiber not estimated record 16.25% this may be caused nettle is rich in minerals. The result in the same table showed that the crude fiber contents in nettle powder record 8.80%, it may note in this table that the moisture and fat content are less in the chemical composition of dry nettle leaves, as percentages were recorded 3.81% and 3.50% respectively. From the table of the chemical composition of the nettle plant, it is clear that it has a high nutritional value that makes it a candidate plant for use in the food industry, the results are in harmony with (Shonte et al., 2020)

Table 1. Chemical composition of dry nettle leaves powder

Parameters (%)	Nettle
Moisture	3.81±0.36
protein	30.59±2.48
Fat	3.50±0.05
Ash	16.25±0.04
Crude fiber	8.80±0.35
Carbohydrates	40.86±0.01

*Chemical composition was calculated based on the dry weight basis. Data are presented as means ± SDM (n=3)

Content of mineral elements of dry nettle leaves

Stinging nettle is rich in minerals, as shown in Table 2. Current studies showed the nettle powder contained ash content, which is much higher than conventional cereals. It contains nettle powder at a high rate of Mg, Ca, and K (858.98, 673.56, and 641.93 ppm), respectively, while the leaf powder recorded a good percentage of Fe and Na (67.71 and 30.44 ppm). It was the lowest content of the elements Mn and Zn (12.47 and 7.50 ppm) nettle powder probably is one of the richest sources of minerals among the plant foods this is evident from the study of the mineral content of nettle leaf powder these results are in symmetry with (Adhikari et al. 2016)

Table 2. Mineral content of dry nettle leaves powder

Nettle	Mineral elements (ppm)
Ca	673.56±3.16
K	641.93±3.80
Mg	858.98±12.72
Na	30.44±1.82
Fe	67.71±2.36
Mn	12.47±0.70
Zn	7.50±0.61

Antioxidant content of dry nettle leaves

The results of content and antioxidant content determined in the leaves of nettle are presented in Table 3. It was noticed from the table that the highest percentage was recorded for the total phenolic content of nettle powder, TP (206.00 mg GAE/100 g), followed by the total antioxidant content, TA (64.27%), and the lowest total flavonoids, TF (25.54 mg CAT/100 g). These results were recorded by Otles and Yalcin (2012), and it has been reported that the natural phenolic compounds play an important role in cancer prevention and treatment. Phenolic compounds from medicinal herbs and dietary plants include phenolic acids, flavonoids, tannins, and others. The colorful bioactivities of phenolic composites are responsible for their chemopreventive properties, e.g., antioxidant, anticarcinogenic, antimutagenic, and anti-inflammatory.

Table 3. Determination of total antioxidant content (TA), total phenolic, (TP) and total flavonoids (TF) of dry nettle leaves powder

Parameters	Nettle
TA (%)	64.27±2.05
TP (mg GAE /100g)	206.00±2.02
TF (mg CAT /100g)	25.54±5.10

Rheological properties

Farinograph test

All measured values of the rheological properties (Farinograph and Extensograph) of wheat flour to which dry nettle leaves are added are shown in Table 4. Water absorption gradually increased with the addition of dry nettle leaves; it increased from 61.5% for the control wheat flour sample to 62.5%, 63.0%,63.0 64.5%, respectively, for wheat flour supplemented with 5%, 10%, and 15% dry nettle leaves. It is well known that an increase in fiber content in a dough causes an increase in water absorption (Abdel-rahman 2014). The hydroxyl groups of the fiber structure cause an interaction between water and hydrogen bonds, and this illustrated the increase in the tendency of flour to hold water (Amir et al., 2015). Likewise, dough development time increased and reached 3.0 min for adding 15% dry nettle leaves as compared to the corresponding control (1.5 min). On the other hand, dough stability time (min) was shortened in the dough in the percentage of addition 15% because the existence of fiber particles resulted in a disruption of the starch-gluten network and thus a decrease in dough stability time, while this value increased and reached its maximum in the dough with 5% and 10% addition compared with the corresponding control (Abdel-Ghafor et al. 2013). The dough stability is better when the time is longer (Mehfooz et al., 2018). They demonstrated that the high concentration of dry nettle leaves mixed with white flour led to a progressive elevation in water immersion, dough stability time, and appearance time. The degree of softening (B.U.) increased with the addition of dry nettle leaves and reached its maximum in the dough blending at 15%. (El-Taib et al., 2018) support our findings when they found that the degree of weakening (BU) was raised progressively by the increasing ratios of dry nettle leaf addition, and it can be attributed to lower wheat gluten content in the dough (El-Basyouny et al., 2019)

Extensograph test

The Extensograph provides information about the viscoelastic behavior of dough (Sharma et al., et al., 2016). This equipment measures dough extensibility and resistance to elasticity. A combination of good resistance and good extensibility results is needed for dough properties (Rizk et al., 2016). The data is shown in Table 4. illustrated that the elasticity of the dough was reduced with the increase in dry nettle leaves, and the same phenomenon was observed in the extensibility. These data are on the same line with (El-Taib et al., 2018), who mentioned that the dough extensibility is the ability of the dough to extend or stretch; it depends on the gliadin protein in the dough. showed a pronounced retard as the percentage of dry nettle leaves increased the extensibility of the dough. Mixtures that have an increased distance before rupture (high extensibility) whereas the increasing force demanded to rupture the dough (high maximum resistance to elasticity) (Sullivan et al., 2010) also stated that the elasticity of the dough lowered with the addition of dry nettle leaves. It can be noticed that the proportional number (P.N.) increased from 2.40 for the control sample to 3.16, 3.86, and 3.66, respectively, at levels 5, 10, and 15% of dry nettle leaves. These data were in agreement with (Aly et al., 2021), who demonstrated that the proportional number increased as the levels of dry nettle leaves increased. Concerning the dough energy (cm^2), the values were reduced by increasing the proportion of dry nettle leaves, suggesting that the incorporation of dry nettle leaves makes the dough more suitable for making biscuits (Wang et al., 2015).

Table 4. Farinograph and Extensograph parameters of biscuit dough containing dry nettle leaves

Sample	Farinograph					Extensograph			
	Water absorption (%)	Arrival time (min)	Dough Development (min)	Stability (min)	Degree of softening (B.U)	Elasticity (B.U)	Extensibility (mm)	P. N	Energy (cn^2)
Control	61.5	1.0	1.5	2.5	50	320	95	2.40	64
5%	62.5	1.5	2.0	10.0	60	300	125	3.16	46
10%	63.0	1.5	2.0	10.0	60	270	85	3.86	43
15%	64.5	1.5	3.0	9.5	80	250	70	3.77	32

P.N: Proportional number

Sensory evaluation of biscuits during storage at room temperature

Table 5. illustrates the sensory characteristics of biscuits containing dry nettle leaves at zero time and after storage for six months at room temperature. It is noted that the highest scores were for the control group, followed by the percentage of addition of 5%, which is close to the control scores (Rakshit and Srivastav 2022). While the percentage of addition of 10% recorded higher scores than the percentage of 15% in all sensory characteristics compared to the control during the storage periods. The taste in the percentage of addition (15%) recorded the lowest scores in evaluating the sensory characteristics of the percentages added during the storage periods (Godase et al., 2020). Dry nettle leaves contain a pungent taste, as this taste appears in the high percentage of 15% added, but this percentage was not rejected by the arbitrators, as there are consumers who desire this taste. (Mitrevski et al., 2023). Also, with regard to color, there was an evaluation of the lowest scores between the proportions added and the control group, where increasing the addition of dried nettle leaf led to a darkening of the color. However, it was not rejected by the arbitrators. Where the color was not dark to the point of rejection (Ivanisˇova et al., 2019)

Table 5. Sensory evaluation of dry nettle leaves biscuits with different addition ratios

Treatments	Color	Odor	Texture	Taste	Crispness	Appearance	Overall acceptability
				Zero time			
control	9.36±0.04	9.22±0.03	9.27±0.02	9.48±0.03	9.24±0.15	9.18±0.10	9.15±0.01
5%	9.08±0.6	9.12±0.10	9.37±0.46	9.19±0.02	9.50±0.44	9.35±0.39	9.16±0.10
10%	8.93±0.15	8.96±0.02	8.89±0.10	9.13±0.32	8.94±0.02	8.98±0.03	8.97±0.03
15%	8.88±0.15	8.94±0.18	8.87±0.20	8.90±0.03	8.90±0.10	8.87±0.20	8.88.0.15
				1 month			
control	9.25±0.05	9.17±0.02	9.22±0.03	9.43±0.01	9.19±0.16	9.06±0.44	9.12±0.14
5%	8.92±0.03	8.91±0.20	8.87±0.02	8.89±0.10	8.89±0.03	8.93±0.02	8.84±0.03
10%	8.90±0.10	8.87±0.11	8.86±0.01	8.86±0.11	8.86±0.21	8.88±0.11	8.85±0.02
15%	8.83±0.04	8.86±0.10	8.80±0.10	8.83±0.13	8.83±0.03	8.84±0.15	8.86±0.02
				3 months			
control	9.13±0.15	9.6±0.43	8.97±0.21	8.92±0.10	8.87±0.01	8.88±0.03	8.79±0.30
5%	8.88±0.15	8.92±0.02	8.84±0.04	8.83±0.15	8.86±0.20	8.85±0.15	8.75.0.16
10%	8.84±0.10	8.87±0.14	8.82±0.20	8.78±0.13	8.85±0. 02	8.83±0.16	8.74±0.30
15%	8.83±0.06	8.84±0.15	8.81±0.15	8.75±0.25	8.81±0.01	8.80±0.03	8.60±0.34
				6 months			
control	8.95±0.10	8.89±0.25	8.78±0.15	8.79±0.03	8.80±0.01	8.75±0.02	8.76±0.10
5%	8.78±0.02	8.79±0.02	8.77±0.10	8.75±0.10	8.75±0.02	8.72±0.15	8.73±0.01
10%	8.75±021	8.76±0.15	8.75±0.21	8.73±0.25	8.74 ±0.10	8.71±0.20	8.71±0.15
15%	8.69±0.01	8.75±0.10	8.70±0.01	8.70±0.02	8.73±0.15	8.65±0.01	8.69±0.10

Effect of dry nettle leaves on the total bacterial counting during storage periods

Microbial groups were counted in nettle leaves with concentrations (5%, 10%, and 15%) and control biscuit samples. The counts of microbial groups were determined directly after biscuit processing. Samples were stored at room temperature with the following microbial counts during the storage period. Data in Table 6. showed that total bacterial counts were. Gradually during the storage period. detected at all directly after processing with all concentrations used at zero time. During the storage period, the total bacterial count appeared and increased gradually. The total bacterial count appeared a month later with 3.08 and 2.30 log cfu/g and increased gradually to reach 3.34 and 2.77 log cfu/g, respectively, after 3 months of storage. 6 months later, after storage, total bacterial counts increased gradually, reaching 5.50, 4.91, and 4.56 log cfu/g, with concentrations of 5%, 10%, and 15%, respectively, compared to the control. It means that 15% can be enough as a preservation treatment for biscuits. concentration of 15% had a higher effect, where total bacterial counts appeared after 3 months (2.30 log cfu/g) and increased to 4.65 log cfu/g after 6 months of storage. It can be concluded that there are possibilities for these foods to become contaminated during production and processing. Microbial spoilage in biscuits often depends on some factors like storage temperature, humidity, storage hygiene, packaging material, moisture content in the storage area, etc. (Ahmed et al., 2020) and (Nerín, et al., 2016). As a result of the previous reasons, the bacteria that are found in dried food may come during the production process and also after storage if the packaging material is not intact or the storage condition is in poor circumstances. During the production process, there are possible chances of contamination occurring in mechanical areas of the processing line, like the hopper with feeder, grader, peeler, feed conveyor, slicer, and flavor applicator. these areas where attention should be maximized to prevent such unwanted contamination (Ostadrahimi et al., 2020).

Table 6. Effect of dry nettle leaves on the total bacterial counting (log 10 Cfu/g)

Concentration	Zero time	1 month	3 months	6 months
Control	ND	3.81	4.15	6.49
5%	ND	3.08	3.34	5.50
10%	ND	2.30	2.77	4.91
15%	ND	ND	2.30	4.65

Fungal changes in nettle biscuits

It is evident from the Table 7. that the concentrations of nettle leaf on biscuits contributed to obtaining good results. fungies was not detected (ND) through increasing time of storage due to the low moisture in biscuits samples and reducing the numbers of fungies in the various concentrations of nettle leaf according to the level of addition compared with control treatment. Control samples data showed that, the total fungi counts were not detected at zero time but increased in 1, 3 and 6 months as (2.9, 3.30 and 3.60 log cfu/g), respectively (Amir et al., 2012).

Table 7. Effect of dry nettle leaves dioica on the total fungi (log 10 Cfu/g)

concentration	**Zero time**	**1 month**	**3 months**	**6 months**
Control	ND	2.9	3.30	3.60
5%	ND	ND	ND	ND
10%	ND	ND	ND	ND
15%	ND	ND	ND	ND

Antimicrobial activity

The antimicrobial activity of ethanolic and methanolic extracts of nettle appeared as a zone of inhibition for tested microorganisms. The results of the antimicrobial activity are given in Table 8. These results showed that while these extracts were effective against both kinds of bacteria (gram-positive bacteria and gram-negative bacteria), they demonstrated no effects against yeast-like fungi *(Candida albicans)*. The resistance of fungal species extracted from nettles might be attributed to their morphological structure; fungi have thicker cell walls and contain a higher percentage of chitin (Amer and Aly, 2019; Amer, 2018). Although all extracts exhibited antimicrobial activity against all tested bacteria with inhibition zones ranging from 10.0±0.58 to 12.7±0.67 mm., they were more effective against gram-positive bacteria *(Bacillus cereus and Staphylococcus aureus)* than against gram-negative bacteria *(Salmonella typhimurium and Escherichia coli)*. This behavior could be due to the differences in their cell wall structures. Gram-negative bacteria have an extra-hydrophilic outer membrane that functions as a preventive barrier. This renders the Gram-negative bacteria generally more resistant to plant extracts than the Gram-positive bacteria (Elswaby et al., 2022 and El-Rady et al., 2023). The results are consistent with numerous studies (Harrison et al., 2022; Motomedi et al., 2014 and Mzid et al., 2017) that have documented the antibacterial potentiality of Urtica dioica against Gram-positive and Gram-negative bacteria, yeast, and filamentous fungus. Solvent types can also affect the antimicrobial properties of the extracts. Solvents differ in their extraction capabilities depending on their polarity and the solute's chemical structure. Different solvent extracts have different soluble phytoconstituents in different amounts, and hence, they have different levels of antimicrobial properties. In the current report, methanol extracts showed higher activity compared with the corresponding ethanol extracts (Table 8.). This result suggests that most of the bioactive molecules in Urtica dioica are soluble in methanol. Conflicting data have been reported regarding the superiority of methanol extracts over ethanol or other extracts in nettle. Some studies have shown that methanol extracts are superior to ethanol extracts (Motamedi et al., 2014), but other studies have shown that ethyl acetate and hexane extracts have the strongest antibacterial activity against particular microorganisms (Modarresi et al., 2012). Noteworthy to remark is the fact that numerous factors contribute to variations in the antibacterial characteristics of the same solvent, making it difficult to compare the results of different experiments. For instance, environmental factors such as season, local climate and soil type, the status of plant raw material, and extraction conditions (extraction time, extraction temperature, pH, etc.) can remarkably impact the solvent property factors such as season, local climate and soil type, the status of plant raw material, and extraction conditions (extraction time, extraction temperature, pH, etc.) can remarkably impact the solvent property (Amer, 2018). In this work, *Staphylococcus aureus* was found to be the most susceptible microorganism to nettle extracts (in addition to Bacillus cereus). Especially against methicillin-resistant Staphylococcus aureus (MRSA) and vancomycin-intermediate Staphylococcus aureus (VISA). Excellent inhibition against Staphylococcus species can be a hope for the discovery of new natural antibacterial agents against coagulase-positive and coagulase-negative staphylococci (Motamedi et al., 2014).

Table 8. Antimicrobial activity of ethanolic and methanolic extracts of dry nettle leaves

Solvent extract	Zone of inhibition (mm.)				
	Staphylococcus aureus	*Bacillus cereus*	*Salmonella typhimurium*	*Escherichia coli*	*Candida albicans*
Ethanol	10.0±0.58	10.7±0.33	ND	ND	ND
Methanol	12.3±0.88	12.7±0.67	6.0±1.00	4.7±1.20	ND
Tetracycline	16.0±1.00	16.3±0.88	10.3±0.88	8.0±0.58	-
Nystatin	-	-	-	-	13.3±0.8

Values are means of triplicate determination (n=3) ± standard errors. ND, no zone of inhibition was detected. Tetracycline and nystatin served as positive controls for bacteria and fungi, respectively.

4.Conclusion

From the study of dry nettle leaves, which have a high nutritional value, they can be added to salted biscuits to improve their properties and increase their nutritional value, as the nettle contains minerals, antioxidants, proteins, and fibers.

- Adding nettle leaves to wheat flour led to a higher rate of water absorption, which made the dough soft, smooth, and easier to shape.
- bacterial load of biscuits added to nettle leaves during storage periods. The addition rate of 15% can be enough as a preservation treatment for biscuits. concentration of 15% had a higher effect, where total bacterial counts appeared after 3 months (2.30 log cfu/g) and increased to 4.65 log cfu/g after 6 months of storage. fungies were not detected (ND) through increasing storage time due to the low moisture in biscuit samples and reducing the numbers of fungies in the various concentrations of nettle leaf.
- Antimicrobial activity showed these extracts were effective against both kinds of bacteria (gram-positive bacteria and gram-negative bacteria), but they demonstrated no effects against yeast-like fungi. Although all extracts exhibited antimicrobial activity against all tested bacteria with inhibition zones ranging from 10.0±0.58 to 12.7±0.67 mm., they were more effective against gram-positive bacteria *(Bacillus cereus and Staphylococcus aureus)* than against gram-negative bacteria *(Salmonella typhimurium and Escherichia coli).* This behavior could be due to the differences in their cell wall structures.
- The nettle plant is important as it is considered a preservative in foods intended for human consumption. Therefore, it can be used as an alternative to industrial preservatives and also as an antibacterial and antifungal agent in the food industry, giving a high nutritional value to the product.

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