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Miswak *(Salvadora persica)* Roots as Antibacterial Agent and a Potential Food Bio Preservative

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Abstract: ***Bio-preservation, the use of natural antimicrobial compounds, a safe and ecological approach to increase the shelf life and enhance food safety, has gained increasing attention in recent years. The antibacterial activity of aqueous root extracts of Salvadora persica L. were evaluated on the microbial growth of different bacterial strains by determining inhibition ratio. In addition, these aqueous extracts were tested as natural preservative agents in chicken burger by estimating the total bacterial count and sensory quality characteristics of products. The extracts were prepared by soaking miswak's root in distilled water (10g/100ml, w/v) for 24 and 48 hrs. All tested extracts exhibited effectiveness for preventing growth of some spoilage bacteria, but the 48 hrs extract showed the strongest inhibitory effects against the bacterial growth of (Streptococus mitis, Streptococcus salivarius, Streptococcus mutans, Staphylococcus aureus, Bacillus sabtilis, Pseudomonas earuginosa, E. coli, Salmonella typhimurium,and Candida albicans). The inhibition ratios were ranged from 50-100% depending on bacterial cultivar. Also, total viable plate count and coliform group count were lower than control sample. Moreover, the organoleptic results of chicken burger products revealed that there are no significant differences between samples in all sensory attributes. This study proves the effectiveness of miswak as antibacterial ingredient and a bio preservative agent and recommends its potential employing in acceptable meat processed products where spoilage is caused mainly by microbial activity.***

Keywords: Miswak, chicken products, natural preservative, bio-preservation, Antibacterial activity.

1. Introduction

Food quality deterioration due to a wide range of physical, chemical, enzymatic and microbiological reactions. The various forms of spoilage and food poisoning caused by micro-organisms are preventable to a large degree by a number of preservation techniques, most of which act by preventing or slowing microbial growth. When preservation fails, the consequences range from extreme hazard, *e.g.* if any toxinogenic micro-organisms are not controlled, to relatively trivial loss of quality such as loss of color or flavor. The most serious forms of quality deterioration include those due to micro-organisms, following the survival and/or growth of infectious pathogenic bacteria or the growth of toxinogenic ones [1]. Preservatives are natural or synthetic substances that are added to fruits, vegetables, prepared food items, cosmetics and pharmaceuticals in order to increase their shelf life and maintain their quality and safety by inhibiting, retarding or arresting their fermentation, acidification, microbial contamination and decomposition [2].

In the recent years, consumers have become more concerned about the processed food they eat. Synthetic preservatives, which have been used in foods for decades, may lead to negative health consequences [3]. Besides, the use of synthetic compounds have significant drawbacks, such as increasing cost, handling hazards, concerns about residues on food and threat to human environment [4]. Therefore, there has been increasing interest to replace synthetic preservatives with natural, effective and nontoxic compounds. Those are, in the first place, extracts and essential oils (EOs) of spices and herbs [5].

Miswak (*Salvadora persica* L.) is a desert plant which grows from north-western India to Africa [6]. Leaves make good fodder and are rich in minerals, the leaves are readily consumed by goats and cattle and the fodder is available during the dry season, the fresh leaves are eaten as salad [7]. Branches and roots of *Salvadora persica* are widely used as a tooth cleaning stick [8]**.** Miswak contains important phyto-constituents such as vitamin C, salvadorine, salvadourea, alkaloids, trimethylamine, cyanogenic glycosides, tannins, saponins and salts mostly as chlorides [9]**,** in addition to sulphur [10]**,** organic sulphur compounds [11] and lignan glycosides [12]. Pharmacological studies indicated that *S. persica* L. plant possess anti-microbial, anti-plaque, aphrodisiac, alexiteric, analgesic, anti-inflammatory, anti-pyretic, astringent, diuretic and bitter stomachic activities [13], [14]**,** anticonvulsant ,and Antiulcer activity [15]**,** [16], hypoglycemic effect and it reduced body weight [17]. Miswak has great medicinal use in the treatment of nose troubles, piles, scabies, leucoderma, scurvy, gonorrhea, spleen disorders, boils, sores, gum disease, stomachache and toothache, to treat hook worm, venereal diseases, for teeth cleaning, in rheumatism, cough and asthma, to lower cholesterol plasma levels, reestablishment of the components of gastric mucosa and as a laxative [18].

Salvadora persica and plant-derived products can also be used as antimicrobial agents; alcoholic and aqueous extracts have strong antibacterial activity on *Streptococcus mutans, Lactobacillus acidophilus, Aggregati bacter, actinomycete mcomitans, Porphyromonas gingivalis*, and *Haemophilus influenzae* [19]**,** and *Candida albicans* [20]. Furthermore, antifungal, antibacterial, and antioxidant activities of this plant have also been reported by [21]**,** [22]. Although, this

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plant has very important activities (antifungal, antibacterial, and antioxidant) concerning food safety and preservation it had not received much attention as a food bio- preservative agent. Therefore, the present work explores the possibility of using the aqueous root extract of this plant as antibacterial and a natural food preservative agent as well as its effects on chicken burger chemical composition, microbial profile and organoleptic characteristics.

2. Materials and Methods

2.1 Plant Material

Dried roots of *S. persica* were purchased from a local market in Jazan city, KSA, were cut in 2012-2013 season.

Aqueous Miswak Root Extracts (AMREs) **Preparation**
Air dried roots of miswak were cut into small pieces and grounded with a grinding machine into powder. Two quantities, each ten grams of powdered dried root miswak (dry weight) were macerated in 100ml of sterile de-ionized water (ratios 10%, w/w) in sterile screw capped bottles at 40 ^{o}C for 24hr to obtain (AMRE 24) and for 48 hr to yield (AMRE 48). The extracts were centrifuged at 3000 rpm for 15min. Then, supernatants were sterilized by passing through filter paper (0.45µm pore size). And then stored at 4 ^{o}C until used within one week [23]. 2-column illustrations may cross the gap). If your figure has two parts, include the labels "(a)" and "(b)".

2.2 Microrganisms

A total of nine microbial strains including *Staphylococcus mitis, Streptococcus salivarius, Streptococcus mutans, Staphylococcus aureus, Bacillus sabtilis, Pseudomonas earuginosa, E. coli, Salmonella typhimurium* and *Candida albicans,* were used in the presented study. These strains were obtained from the culture collection of Department of Microbiology, Faculty of Pharmacy, Tanta University, Egypt.

2.3 Determination of the Minimum Inhibitory Concentrations of Miswak Extracts

The minimum inhibitory concentrations (MICs) of the aqueous miswak extracts were determined by the agar diffusion method [24]. Agar plates containing medium composed of double strength Muller Hinton plus one of the following concentrations of each extract (12.5%, 25% and 50%). Sterile de-ionized distilled water was used to adjust the final concentrations. The tested bacterial strains (10^4/spot) were inoculated onto the surfaces of the agar plates by using the multipoint inculcator. The plates were then incubated at 37^{o}C for 24hr before defining the MICs.

2.4 Chicken Burger Preparation

Fresh chicken burger samples were prepared as described by [25]. Basal constituents of chicken burger recipe were as follow: minced chicken meat included fat 71.5%, fresh onion (finely ground) 7%, whole egg (blended) 5%, toasted bread crust powder 5%, rehydrated extruded soy 10%, sodium chloride 1.5 % and 1% spices mixture. The mixture consisted of the cardamom powder 2%, clove 8%, cubeb 20.26%, laurel leaf 9.5%, cinnamon 19.8%, white pepper 20.44% and rosemary 20%. The blend was divided into seven portions: one of these portions used as control without any more addition, and three portions treated with 12.5%, 25% and 50% of aqueous miswak extract (soaking for 24hrs), and the other three portions treated with 12.5%, 25% and 50% of aqueous miswak extract (soaking for 48hrs). The chilled minced chicken meat thoroughly mixed with other ingredients. The mixture was shaped to burger 10cm diameter, 7.8mm thickness with average weight 50g. The burger was emplaced into right plastic plates and wrapped with polyethylene sheet. The burger was stored in deep freezer at -18 ^{o}C.

2.5 Cooking Methods

Frozen burgers were thawed at 5 ^{o}C in refrigerator and cooked by fraying individually in little amount of sunflower oil at 165^{o}C for 5min/side according to method described by **Cannel *et al*. (1989)** [26].

2.6 Hunter Color Values

Objective evaluation of chicken burgers surface color Hunter (a*, b* and L*) parameters was measured using a spectro-colorimeter (tristimulus color machine) with the CIE lab color scale (Hunter, Lab Scan XE- Reston VA, USA) in the reflection mode. The instrument was standardized (at each time) with white tile of Hunter Lab Color Standard (LX No. 16379): X= 72.26, Y= 81.94 and Z= 88.14 (L*= 92.46; a*= -0.86; b*= -0.16) as reported by [27].

2.7 Gross Chemical Composition Determination

The gross chemical components, moisture, crude protein, crude fat and ash content for chicken burgers were determined according to the standard methods of the [28]**.** Where, the total carbohydrates content was calculated by difference as follows: total carbohydrates content (%, on dry weight basis) = 100 - (crude protein% + crude fat% + ash%).

2.8 Sensory Evaluation for Chicken Burgers

Chicken burgers containing aqueous miswak extract with different concentrations were subjected to sensory evaluation by ten trained panelists from the staff members of the national research center, Giza, Egypt. According to the procedure of [29]**,** the sensory evaluation was carried out for color, taste, odor, texture and overall acceptability of produced burgers.

2.9 Statistical Analysis

The obtained results of sensory evaluation were statistically analyzed using SPSS statistical package (Version 9.05) according to [30]**.** Analysis of variance (ANOVA), Duncan's multiple range test and least significant difference (LSD) was chosen to determine any significant difference among various treatments at $p \leq 0.05$.

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3. Results and Discussion

3.1 Antibacterial activity of aqueous miswak root extracts (AMREs)

Data in table 1 exhibited the antimicrobial activity of aqueous miswak root extracts (AMRE 24) and (AMRE 48). The obtained results demonstrated that (AMRE 48) was more effective than (AMRE 24) to all examined microorganisms.

Table 1: The percentages of minimal inhibitory concentrations (MIC) for aqueous miswak root extracts (AMRE 24) and (AMRE 48).

Types of microorganisms	*% of Inhibition for (AMRE 24) at different concentrations*			*% of Inhibition for (AMRE 48) at different concentrations*		
	12.5%	25%	50%	12.5%	25%	50%
Streptococus mitis	10	80	100	50	100	100
Streptococcus salivarius	10	80	100	50	100	100
Streptococcus mutans	10	80	100	50	100	100
Staphylococcus aureus	10	70	100	80	100	100
Bacillus sabtilis	-	40	50	-	90	90
Pseudomonas earuginosa	-	-	-	-	-	50
E. coli	-	60	80	90	100	100
Salmonella typhimurium	-	60	80	60	90	100
Candida albicans	-	60	60	-	80	80

The highest growth inhibition was obtained for *Streptococcus* strains with a ratio of inhibition 10, 80 and 100% at 12.5, 25 and 50% concentration of (AMRE 24). While, inhibition ratios were 50, 100 and 100% at 12.5, 25 and 50% concentration of (AMRE 48), respectively. There was no inhibitory effect at 12.5% concentration of (AMRE 24) for *Bacillus sabtilis, E. coli, Salmonella typhimurium* and *Candida albicans.* The most resistant bacterial strain was *Pseudomonas earuginosa* which was not affected with all treatments except 50% of (AMRE 48) exhibiting 50% growth inhibition. *Bacillus sabtilis* and *Candida albicans* showed resistance at 12.5% concentration of both (AMRE 24) and (AMRE 48). These results are in harmony with those concerning the essential oils of *S. persica* which have a considerable effect on several aerobic bacteria as reported by [18], [21], [31], [32]. Also, it was reported that the volatile oil of Jordanian *S. persica* stems exhibited potent antibacterial activity against both Gram-positive and Gram-negative bacteria [18]. The current and reported results clearly showed that both water soluble and fat soluble constituents of miswak have antibacterial activity. These antimicrobial properties of *S. persica* may be attributed to various chemicals in the extracts such as sodium chloride, potassium chloride, vitamin C, salvadourea, salvadorine, saponins, silica sulfate compounds, isothiocyanate, tannins, tannic acid, benzyl isothiocyanate, alkaloids, terpenoids, oleic, linoleic and stearic acids, chloride, sulphate, thiocyanate, nitrate and resin [33], cyanogenic and lignin glycosides [12].

3.2 Color characteristics of chicken burger batches affected by the incorporation of aqueous miswak root extract

A color characteristic is considered as a major criterion that affects the other quality criteria of the final chicken burger product. Therefore, the effect of the incorporation of aqueous miswak root extract at all experimented levels on color characteristics; Hunter values of whiteness (L), redness (a) and yellowness (b), values for the chicken burger, and the obtained results were recorded as in table 2. As shown in Table 2, the color of all fortified chicken burger had slightly higher L (whiteness) and Yellow (b) values, when compared with the control. Concurrently, increasing the (AMREs) to chicken burger batches, led to slightly lower values of redness (a) in all fortified samples, indicating that the formation of the lighter color of chicken burgers was in linear relation with increasing addition level of (AMREs).This may be due to the association between the enhancement of antioxidant amount and the increasing of (AMREs) [21].

Table 2: Color characteristics of chicken burger batches affected by the incorporation of aqueous miswak root extracts (AMREs)

Incorporation treatment	*L*	*A*	*b*
Control	41.37	17.88	14.22
12.5% of (AMRE 24)	44.15	14.76	15.87
25% of (AMRE 24)	46.85	12.96	17.11
50% of (AMRE 24)	49.34	11.02	19.69
12.5% of (AMRE 48)	45.00	13.67	16.54
25% of (AMRE 48)	49.99	11.21	18.88
50% of (AMRE 48)	55.28	10.42	21.73

*Where: (AMRE 24) = aqueous miswak root extract prepared by maceration for 24hr. (AMRE 48) = aqueous miswak root extract prepared by maceration for 48hr.

3.3 Determination of Gross Chemical Composition of Chicken Burger Products:

The percentage of gross chemical components; moisture, crude protein, crude fat, ash and carbohydrates content of chicken burger samples treated with (AMREs) levels were determined and the obtained results are tabulated in Table 3. The obtained data (Table 3) showed a linear relationship between chemical composition values and the increasing of aqueous miswak root extract concentrations. Crude protein, crude fats, and carbohydrates content of the treated chicken burger were decreased. On the other hand, moisture and ash were increased. This may due to the high content of aqueous miswak extract of minerals such as sodium chloride, potassium chloride and silica sulfate and low content of other components as crude protein, crude fats and carbohydrates as reported by [33].

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Table 3: Chemical composition of cooked chicken burger batches treated with aqueous miswak root extracts (AMREs)

Incorporation treatment	*Component value %*				
	Moisture %	*Crude Protein* %*	*Crude Fat* %*	*Ash* %*	*Carbohydrates* %*
Control	63.25	62.18	21.14	9.02	7.66
12.5% of (AMRE 24)	63.51	61.64	20.65	10.10	7.61
25% of (AMRE 24)	63.78	61.07	20.21	11.14	7.58
50% of (AMRE 24)	63.89	60.35	19.94	12.18	7.53
12.5% of (AMRE 48)	64.01	61.13	20.62	10.88	7.37
25% of (AMRE 48)	64.11	60.62	20.15	12.12	7.11
50% of (AMRE 48)	64.05	60.05	19.58	13.22	7.15

*On dry weight basis; **the values represent the means of triplicate determinations.*Where: (AMRE 24) = aqueous miswak root extract prepared by maceration for 24hr. (AMRE 48) = aqueous miswak root extract prepared by maceration for 48hr.

3.4 Microbiological tests:

The total count of bacteria, detection of microorganisms and total coliform bacteria of treated chicken burger were carried out and the obtained results are tabulated in tables 4, 5 and 6.

3.4.1 Total count of bacteria in chicken burger products:

Data in table 4 revealed that the increasing of concentration levels of both aqueous miswak root extracts in the chicken burger decreased the growth rate of total viable count in comparison to control sample. The most effective treatment was at 50% of (AMRE 48). This could be attributed to the increasing amount of antibacterial agents of miswak by increasing the extract concentration and maceration time. These results were in agreement with that reported by [21].

Table 4: Total viable plate count (log_{10} cfu/g sample) of chicken burger batches incorporated with different levels of aqueous miswak root extracts (AMREs)

Incorporation treatment	*Zero days*	*3 days*	*7 days*	*10 days*	*14 days*
Control	5.42	5.43	6.07	6.10	6.43
12.5% of (AMRE 24)	5.78	5.81	5.92	6.15	6.42
25% of (AMRE 24)	5.62	5.63	5.81	6.03	6.24
50% of (AMRE 24)	5.21	5.41	5.70	5.97	6.01
12.5% of (AMRE 48)	5.66	5.68	5.69	5.72	5.73
25% of (AMRE 48)	5.60	5.61	5.62	5.69	5.70
50% of (AMRE 48)	5.55	5.59	5.62	5.62	5.64

*Where: (AMRE 24) = aqueous miswak root extract prepared by maceration for 24hr.
(AMRE 48) = aqueous miswak root extract prepared by maceration for 48hr. **all samples were stored at 4°C

3.4.2 Detection of microorganisms in produced chicken burger:

Results in table 5 exhibits that all samples were free from microorganisms such as *E. coli, Salmonila* and *Staphylococcus aureus.* On the other hand, colifom bacteria group was detected in all samples, this may be due to a contamination of meat with coliform bacteria group by water during washing after sludge.

3.4.3 Total coliform bacteria in produced chicken burger:

The following results as per inhibition were thus obtained. All the examined aqueous miswak root extracts against coliform bacteria were found effective as bacterial suppressant. The interesting observation was that (AMRE 48) were found more effective than (AMRE 24). Furthermore, it was found that as the concentration level of (AMREs) increased the growth rate of colifom bacteria group was decreased.

Table 5: Detection of microorganisms in chicken burger incorporated with different levels of aqueous miswak root extracts (AMREs).

Incorporation treatment	*E. coli*	*Salmonila*	*Staphylococcus aureus*	*Coliform group*
Control	N.D	N.D	N.D	D
12.5% of (AMRE 24)	N.D	N.D	N.D	D
25% of (AMRE 24)	N.D	N.D	N.D	D
50% of (AMRE 24)	N.D	N.D	N.D	D
12.5% of (AMRE 48)	N.D	N.D	N.D	D
25% of (AMRE 48)	N.D	N.D	N.D	D
50% of (AMRE 48)	N.D	N.D	N.D	D

*Where: (AMRE 24) = aqueous miswak root extract prepared by maceration for 24hr.
(AMRE 48) = aqueous miswak root extract prepared by maceration for 48hr.

Table 6: Total coliform bacteria (log_{10} cfu/g sample) of chicken burger batches incorporated with different levels of either miswak stems extracts.

Incorporation treatment	*First week*	*Second week*	*Third week*
Control	2.60	3.26	5.91
12.5% of (AMRE 24)	2.71	2.98	3.85
25% of (AMRE 24)	2.53	2.77	3.37
50% of (AMRE 24)	2.44	2.65	2.97
12.5% of (AMRE 48)	2.62	2.69	2.84
25% of (AMRE 48)	2.60	2.60	2.61
50% of (AMRE 48)	2.38	2.38	2.38

*Where: (AMRE 24) = aqueous miswak root extract prepared by maceration for 24hr. (AMRE 48) = aqueous miswak root extract prepared by maceration for 48hr.

3.5 Sensory quality characteristics for chicken burgers as affected by the incorporation of either the S. persica stems extracts

The effect of addition of aqueous miswak root extracts (AMREs) on sensory quality characteristics, juiceness; tenderness; odor; flavor; texture; color and general acceptability of chicken burger batches, and the obtained sensory judging scores were tabulated as in table 7.

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Table 7: Influence of aqueous miswak root extracts (AMREs) on sensory characteristics of produced chicken burger batches

Incorporation treatment	*Juiceness*	*Tenderness*	*Odor*	*Flavor*	*Texture*	*Color*	*General acceptability*
Control	9.9^{a}	9.8^{a}	10.0^{a}	9.9^{a}	9.2^{a}	10.0^{a}	9.5^{a}
12.5% of (AMRE 24)	9.2^{a}	9.7^{a}	9.7^{a}	9.5^{a}	9.3^{a}	10.0^{a}	9.3^{ab}
25% of (AMRE 24)	9.4^{a}	9.6^{a}	9.4^{b}	9.2^{ab}	9.2^{a}	9.8^{ab}	9.0^{b}
50% of (AMRE 24)	9.6^{a}	9.7^{a}	8.8^{c}	8.7^{b}	9.2^{a}	9.7^{ab}	8.8^{c}
12.5% of (AMRE 48)	9.5^{a}	9.8^{a}	9.5^{ab}	9.1^{ab}	9.0^{ab}	9.6^{ab}	9.1^{b}
25% of (AMRE 48)	9.6^{a}	9.8^{a}	9.2^{b}	9.0^{b}	9.1^{ab}	9.2^{b}	8.8^{bc}
50% of (AMRE 48)	9.7^{a}	9.8^{a}	8.4^{c}	8.2^{c}	9.0^{ab}	8.8^{bc}	8.4^{c}
L.S.D**	0.99	0.62	0.53	0.71	0.24	0.35	0.22

*Mean of sensory characteristic score: Mean of each organoleptic characteristic score obtained from 10 panelists; the means within the same column having different superscripts are significantly varied (at p≤5).

*Where: (AMRE 24) = aqueous miswak root extract prepared by maceration for 24hr. (AMRE 48) = aqueous miswak root extract prepared by maceration for 48hr.

From the obtained results (Table 7), it could be illustrated that the sensory scores of the most evaluated organoleptic quality characteristics of cooked chicken burger slightly decreased or were not affected with increasing concentration level up to 50% of (AMRE 24) or 25% (AMRE 48). The produced chicken burger had good sensory quality and acceptability. On the other hand, cooked chicken burgers containing 50% of (AMRE 48) exhibited a slightly significant reduction in the judging scores of the organoleptic quality characteristics; especially odor, flavor and color. This may be attributed to the herbal flavor properties naturally present in miswak extract. From the present results for sensory evaluation of the fortified chicken burgers, it could be concluded that the 50% of (AMRE 24) or 25% (AMRE 48) may be added to the chicken burger to benefit from its preservative effect without negative impacts on the sensory quality characteristics.

4. Conclusion

Miswak has been recognized as a potential safe food and pharmaceutical ingredient. Aqueous extract of miswak's roots has good antibacterial activity and it may be added to chicken burger components at levels approximately 50% as a natural food preservative. This can be done not only with improving shelf life period of product but also without adverse effects on sensory characteristics. These current findings have shown the potential use of aqueous miswak's root extract as a food bio-preservative and a safety food additive.

5. Recommendation

The obtained results recommend the importance of developing quality standard and recommended rules for using miswak and/or miswak extracts as a safe natural food preservative. The miswak extracts could be a source with great economy impacts, if we can use it to develop new cheap food and pharmaceutical bio- preservative. Miswak not only has potential employing as a natural preservative agent in food and pharmaceutical products but also should have more attention in the field of designer foods because of its medicinal properties associated with safety use.

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