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ENHANCE THE QUALITATIVE SENSORY CHARACTERISTICS AND ANTIMICROBIAL ACTIVITY OF BOVINE MILK BY USING (*Hibiscus sabdariffa)*

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ABSTRACT

Bovine milk is one of the richest nutrients that contain minerals and vitamins that enhance immunity, especially in children, but because many children do not want to drink the raw milk, therefore this study aimed to enhance the sensory characteristics of raw milk by using hibiscus plant extract, which is characterized by red color and distinctive flavor as well as studying the effect of aqueous extract of *Hibiscus sabdariffa* on inhibiting the growth of microorganisms, by using three concentrations of the aqueous extract (0.5, 1.0 and 1.5%), where the statistical results showed a significant difference ($P \leq 0.05$) between the concentrations in color, texture and general acceptance, and the best results appeared when using a concentration of 1.0%, while the results showed an insignificant difference in flavor values with different concentrations of the extract added to milk, Because the hibiscus plant, it is considered an acidic plant, which affected the degree of acceptance of the product. As for the inhibition of the growth of microorganisms, the results showed that the growth of microorganisms was inhibited after keeping in refrigeration for 24 hours, where the growth of the total number of bacteria was inhibited to (6×10^{-7}), while we note a decrease in colon bacteria to (2×10^{-6}) either Fecal coliform bacteria, the result showed a decrease in numbers to (2×10^{-4}), then was noticed a slight decrease in numbers continuously after incubation for 7 days in the refrigerator, where the results showed a decrease in the total number of bacteria (1×10^{-7}) and coliform bacteria (1×10^{-6}) while for fecal coliform was (1×10^{-4}).

Keywords: *Hibiscus sabdariffa*, antimicrobial activity, sensory evaluation.

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تعزيز الصفات الحسية والفعالية الضد مايكروبية للحليب البقري باستعمال نبات الكركديه (*Hibiscus sabdariffa*) .

دعاء عادل قاسم

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الخلاصة

يعد حليب الابقار من اغنى العناصر الغذائية التي تحوي على المعادن والفيتامينات والتي تعزز المناعة وخصوصا لدى الاطفال ولكن بسبب عدم رغبه العديد من الاطفال بشرب الحليب الخام هدفت هذه الدراسة الى تحسين الصفات الحسية للحليب الخام باستعمال مستخلص نبات الكركدية والذي يتصف باللون الاحمر والطعم المميز فضلا عن دراسة تأثير المستخلص المائي للكركدية في تثبيط نمو الاحياء المجهرية، وذلك باستعمال ثلاث تراكيز من المستخلص المائي (0.5 و1.0 و1.5%)، حيث اظهرت النتائج الاحصائية وجود فرق معنوي (P≤0.05) بين التراكيز في اللون والقوام والتقبل العام وكانت افضل النتائج ظهرت عند استعمال تركيز 1.0%، بينما أظهرت النتائج اختلاف طفيف في قيم النكهة بتركيزات مختلفة من المستخلص المضاف إلى الحليب بسبب نبات الكركدية يعتبر من النباتات الحمضية مما أثر على درجة قبول المنتج، أما بالنسبة لتثبيط نمو الأحياء المجهرية أظهرت النتائج ثبيط في نمو الأحياء المجهرية بعد الحفظ في التبريد لمدة 24 ساعة حيث تم تثبيط في نمو العدد الكلي للبكتيريا الى (6×10^{-7}) بينما نلاحظ انخفاض بكتريا القولون الى (2×10^{-6})، اما بكتريا القولون البرازية اظهرت النتيجة انخفاض بالاعداد الى (2×10^{-4}) ثم لوحظ انخفاض طفيف في الأعداد بشكل مستمر بعد الحضانة لمدة 7 أيام في الثلاجة، حيث أظهرت النتائج انخفاضا في العدد الإجمالي للبكتريا (1×10^{-7}) والبكتريا القولونية (1×10^{-6}) بينما بالنسبة لبكتريا القولون البرازية كانت (1×10^{-4}).

الكلمات المفتاحية: نبات الكركدية، الفعالية الميكروبية، التقييم الحسي.

INTRODUCTION

Milk is a raw material for many lactic products (such as pasteurized milk, sterilized, etc.). The quality of these products depends primarily on the raw milk characteristics used in their manufacture, such as physical, qualitative, and microbial characteristics **(Whitney 2006)**. Among the qualities of raw milk of good quality is that it has a slight sweet taste and natural aroma and contains a low number of somatic cells (such as white cells). The high microbial contamination of the milk reduces the duration of preserving the processed dairy products, which produces products with poor flavor, and thus affects the low cost-effectiveness of the products in addition to the risk and spread of diseases **(Carey *et al*., 2005)**.

The reason for the human use of medicinal plants in the prevention and treatment of diseases since the beginning of human civilizations and for thousands of years is the urgent need to reveal new antimicrobials with various chemical compositions and valuable work mechanisms because there are cases of an increase in the occurrence of repeated, varied and new diseases and the other big reason is the increased resistance to antibiotics Which is used continuously, and to obtain natural treatments to strengthen immunity, scientists resorted to new research to overcome the resistance of microbes to antibiotics **(Nasciment *et al*.,2000)**.

Cultivation of medicinal and aromatic plants and herbs in most parts of the earth and its uses varied and was used in the form of whole herbs or powders or other form, due to its pharmacological efficacy and speed of recovery of diseases without complications **(Akindahunsi *et al*., 2003)**.

These plants occupy a distinctive and large place in the global agricultural production because they contain natural chemicals of benefit and great importance in their physiological effect and their therapeutic activity taking into account their few side effects on human and animal health in comparison with chemically manufactured drugs such as antibiotics that affect the consumer's health negatively, As a result of the deposition of its residues in food products,

which negatively affects human health and the occurrence of diseases that were not previously known **(David *et al*., 2015)**.

Fortification of cow's milk and products with some nutrients has increased in recent years due to the increase in milk consumption and its importance from the nutritional and health characters, as adding these products to milk increases the nutritional value of the meal and makes it more beneficial from a health point of view and eliminating the smell, which is one of the most important problems when drinking cow's milk, especially in children **(Elwood *et al*., 2010)**.

Many types of research have dealt with the use of fruits such as oranges, bananas, and strawberries, as well as the addition of chocolate, as well as the use of some medicinal plants and herbs as a flavoring and fortifying substances for milk, which is available in the market in the form of glass or cardboard containers, which have gained great popularity in the global markets, especially with children **(To EK 2012)**.

One of the medicinal plants that are widely used in treatments and food is the hibiscus plant (Hibiscus sabdariffa). The various plant parts of it, which include seeds, leaves, and fruits, are used in the pharmaceutical and textile industries, as well as in various food industries as an acidic drink sweetened with sugar and used to resist colds in the winter as well as an intestinal disinfectant and depressant for blood pressure and temperatures **(Norhaizan *et al*., 2010)**, it is considered a sedative for the nerves and tonic for the heart, and it is considered an anti-bacterial, fungi, antioxidant, tumor, and inflammatory, as well as a de-worming **(Tolulope 2007)**.

Hibiscus flowers are distinguished by the wonderful red color and contain organic acids such as citric acid and hydroxy citric acid, as well as contain flavonoids, glycosides, and phenols, and are a rich source of fiber and vitamins such as vitamin C and mineral elements **(Jaroni*et al*., 2012)**. Due to the lack of studies on the importance of using the hibiscus plant and adding it to milk and its products, this study aimed to know the effect of using hibiscus as an antibacterial and to improve the characteristics and sensory properties of cow's milk.

MATERIALS AND METHODS

Sample collection

Twenty-five of bovine raw milk samples were collected from different areas of Baghdad city and the samples were placed in sterile test tubes. The samples were transferred to the Microbiology Laboratory of the Market Research and Consumer Protection Center/the University of Baghdad. Serial dilution was prepared using peptone water. The total number counts of bacteria were estimated using the nutrient agar medium, after incubation for 24 hours at 37ºC Nutrient agar medium, Coliform and fecal coliform counts bacteria were estimated using MacConkey Agar medium and VRBA medium. The plates were incubated at 37ºC for 48 hours and the bacterial colonies were counted **(Quinn *et al*., 2011)**.

Hibiscus extracts preparation

Dried hibiscus flowers were obtained from local markets and an aqueous extract was prepared by placing 100 mL of distilled water in a sterile glass Flask and adding 10 g of hibiscus plant to it. The extract was heated at a temperature of 60ºC for an hour and after filtering the extract with filter paper; the extract was centrifuged for ten minutes at 5000 cycles per minute. The filtrate was concentrated by using an oven at a temperature of 45ºC, to evaporate the water and obtain a dry powder from the aqueous extract, three concentrations of the dried powder were made, which are 0.5, 1 and 1.5% in addition to the control without hibiscus powder**(Parekh & Chanda, 2007)**.

Sensory evaluation

Sensory evaluation of raw milk fortified with concentrations prepared in advance of the hibiscus plant was conducted by 20 evaluators from the College of Veterinary Medicine, University of Baghdad, and the data was recorded according to the evaluation form by using the preference balance from 1-5 in which 5 = Excellent, 4 = Very good, 3 = Good, 2 = Acceptable and 1 = not acceptable, Where the evaluation was based on color, flavor, texture, and appearance or (general acceptance) **(Fasoyiro *et al*., 2005)**.

Antimicrobial activity of hibiscus extracts on raw milk

The hibiscus powder that was prepared was added to the raw milk to prepare the following concentrations 0.5, 1 and 1.5% to obtain 100 mL of prepared milk mixed with hibiscus. After that, the samples were stored for 7 days at a refrigerator 4ºC and the tests of the inhibitory effect of hibiscus on the total number of bacteria, coliform, and fecal coliform bacteria were performed. The test was done on the first day and the 7th day of the production period **(Balouiri *et al*., 2016)**.

The statistical analysis

The statistical program of SAS – **Statistical Analysis System (2012)** was used in data analysis to study the effect of different parameters on the studied traits, Least significant difference–LSD test (Analysis of Variation-ANOVA) was used to significant compare between means n this study.

RESULTS AND DISSUASIONS

Bacteriological test

The results in (Table 1) indicate the presence of a large number of microorganisms per one ml of raw milk, where the results range from (3×10^{-6}) to (5×10^{-8}),As well as the results indicate a large number of coliform bacteria and fecal coliform bacteria, where the number of coliform bacteria ranges from (1×10^{-3}) to (3×10^{-6}), while the fecal coliform ranges from (2×10^{-2}) to (8×10^{-4}) as these numbers are much higher than the acceptable or good limits according to the **(Iraqi standard of microbial limits, 2003)**.

Table (1): The total count of bacteria, coliform bacteria and fecal coliform bacteria

Test No.	Total bacterial	Coliform bacteria	Fecal coliform bacteria
1	3×10-^{7}	2×10^{-5}	5×10^{-3}
2	5×10^{-7}	4×10^{-4}	3×10^{-3}
3	2×10^{-7}	5×10^{-5}	2×10^{-4}
4	3×10^{-8}	3×10^{-6}	1×10^{-4}
5	3×10^{-7}	3×10^{-6}	7×10^{-3}
6	7×10^{-6}	1×10^{-3}	5×10^{-2}
7	1×10^{-7}	4×10^{-5}	8×10^{-3}
8	5×10^{-8}	2×10^{-5}	1×10^{-4}
9	6×10^{-7}	5×10^{-5}	3×10^{-3}
10	1×10^{-8}	7×10^{-4}	1×10^{-4}
11	2×10^{-8}	3×10^{-6}	4×10^{-4}
12	5×10^{-7}	6×10^{-4}	6×10^{-3}
13	3×10^{-8}	1×10^{-5}	4×10^{-4}
14	6×10^{-7}	7×10^{-3}	6×10^{-3}
15	1×10^{-7}	2×10^{-5}	2×10^{-3}
16	3×10^{-6}	5×10^{-4}	2×10^{-2}
17	7×10^{-7}	3×10^{-5}	8×10^{-4}

18	3×10^{-8}	1×10^{-6}	2×10^{-4}
19	6×10^{-6}	5×10^{-4}	3×10^{-2}
20	2×10^{-8}	2×10^{-6}	6×10^{-4}
21	1×10^{-7}	7×10^{-5}	3×10^{-4}
22	5×10^{-6}	2×10^{-3}	5×10^{-2}
23	3×10^{-7}	3×10^{-4}	7×10^{-2}
24	2×10^{-7}	1×10^{-5}	6×10^{-4}
25	4×10^{-7}	2×10^{-5}	3×10^{-4}

The reason for the high number of microorganisms in raw milk is due to the lack of cleanliness of the workers in the field, the lack of cleanliness of the milking tools in addition to the mismanagement in the field as well as the period between the milking process and the laboratory examination that affects the increase in the number of bacteria **(Pal *et al*., 2016)**

The high number of bacteria in milk affects the quality of the products manufactured from it, especially when manufacturing products that are not subjected to high heat treatments in order to eliminate the microorganisms in it, such as sterile milk. For this reason, most milk products are exposed to rapid spoilage and remain in violation of the standard specifications **(Banik *et al*., 2014)**.

The effect of hibiscus extract on the sensory properties of raw milk

The results of the effect of hibiscus extract on the values of color, general acceptance, and texture, with different concentrations of the added extract, (Table 2) showed a significant difference in color, texture, and general acceptance, The results of adding hibiscus extract to the product showed a positive effect on the acceptance of the product, and the best result was obtained when adding the extract at a concentration of 1.0%, and the highest values of the average values of color, texture, and general acceptance were 4.35, 3.75 and 4.1, respectively.

While the results showed an insignificant difference in flavor values with different concentrations of the extract added to milk. The addition of hibiscus extract to the raw milk led to a decrease in the flavor levels of the milk sample, the percentage of decrease increased with the increase of the addition rate from 0.5 to 1.0%, as the flavor decreased from 3.25 to 3.15.

After adding the concentration of 1.5%, the percentage of decrease increased to 3.1 due to the high acidity ratio in which the milk samples increased, since the hibiscus plant is considered an acidic plant, which affected the degree of acceptance of the product **(Aurelio *et al*., 2007; Al-Qazzaz*et al*., 2012)**

Table (2): The effect of different concentrations of hibiscus extract on the sensory properties of raw milk

Treatment	**Mean ± SE**			**Appearance or (general acceptance)**
	Color	**Flavor**	**Texture**	
Control	4.15 ±0.27	3.45 ±0.17	3.15 ±0.13 c	3.55 ±0.16 b
A1 (0.5%)	4.25 ±0.33	3.25 ±0.12	3.65 ±0.23 ab	3.85 ±0.24 ab
A2 (1.0%)	4.45 ±0.33	3.15 ±0.16	3.75 ±0.24 a	4.10 ±0.25 a
A3 (1.5%)	4.35 ±0.42	3.10 ±0.09	3.30 ±0.21 bc	3.90 ±0.18 a
LSD value	0.492 *	0.366 NS	0.428 *	0.427 *
Means having with the different letters in same column differed significantly. * (P≤0.05).				

Antimicrobial activity of hibiscus extracts on raw milk

It is noted in (Table 1) that the total number of microorganisms was in the highest percentage in raw milk before adding hibiscus extract, but after adding the extract, it was

notice a decrease in the number of microorganisms after preservation with cooling for 24 hours, as the results showed in (Table 3), the highest percentage of numbers of microorganisms (6×10^{-7}) While in coliform bacteria, we notice the decrease to (2×10^{-6}) while fecal coliform bacteria were (2×10^{-4}), then was noticed a slight decrease in numbers continuously after incubation for 7 days in the refrigerator, where the results showed in the (Table 4) a decrease in the total number of bacteria (1×10^{-7}) and coliform bacteria (1×10^{-6}), while for fecal coliform was (1×10^{-4})

Table (3): shows the results of the total bacterial, coliform bacteria and fecal coliform bacteria count after 24 hr of preservation in the refrigerator.

Test No.	Total bacterial	Coliform bacteria	Fecal coliform bacteria
1	5×10^{-6}	4×10^{-4}	1×10^{-3}
2	6×10^{-6}	1×10^{-4}	1×10^{-3}
3	5×10^{-6}	2×10^{-5}	4×10^{-3}
4	6×10^{-7}	1×10^{-6}	5×10^{-3}
5	1×10^{-7}	2×10^{-6}	2×10^{-3}
6	1×10^{-6}	5×10^{-2}	1×10^{-2}
7	6×10^{-6}	1×10^{-5}	3×10^{-3}
8	4×10^{-7}	1×10^{-5}	6×10^{-3}
9	1×10^{-7}	2×10^{-5}	1×10^{-3}
10	4×10^{-7}	3×10^{-4}	4×10^{-3}
11	6×10^{-7}	1×10^{-6}	2×10^{-4}
12	1×10^{-7}	2×10^{-4}	3×10^{-3}
13	5×10^{-7}	4×10^{-4}	1×10^{-3}
14	1×10^{-7}	2×10^{-3}	6×10^{-3}
15	5×10^{-6}	5×10^{-4}	1×10^{-3}
16	6×10^{-5}	2×10^{-4}	1×10^{-2}
17	2×10^{-7}	1×10^{-5}	2×10^{-4}
18	5×10^{-7}	4×10^{-5}	1×10^{-4}
19	2×10^{-6}	2×10^{-4}	1×10^{-2}
20	6×10^{-7}	4×10^{-5}	2×10^{-4}
21	5×10^{-6}	3×10^{-5}	1×10^{-4}
22	1×10^{-6}	1×10^{-3}	2×10^{-2}
23	4×10^{-6}	1×10^{-4}	2×10^{-2}
24	6×10^{-6}	4×10^{-4}	2×10^{-4}
25	3×10^{-6}	1×10^{-5}	2×10^{-3}

Table (4): shows the results of the total bacterial, coliform bacteria and fecal coliform bacteria count after 7 days of preservation in the refrigerator.

Test No.	Total bacterial	Coliform bacteria	Fecal coliform bacteria
1	2×10^{-6}	1×10^{-4}	1×10^{-3}
2	3×10^{-6}	3×10^{-3}	5×10^{-2}
3	2×10^{-6}	1×10^{-5}	1×10^{-3}
4	1×10^{-7}	5×10^{-5}	2×10^{-3}
5	7×10^{-6}	1×10^{-6}	1×10^{-3}
6	5×10^{-5}	2×10^{-2}	4×10^{-1}
7	3×10^{-6}	1×10^{-5}	1×10^{-3}
8	1×10^{-7}	6×10^{-4}	2×10^{-3}
9	7×10^{-6}	1×10^{-5}	1×10^{-3}
10	6×10^{-6}	1×10^{-4}	1×10^{-3}
11	1×10^{-7}	4×10^{-5}	1×10^{-4}

12	5×10^{-6}	1×10^{-4}	1×10^{-3}
13	1×10^{-7}	1×10^{-4}	4×10^{-2}
14	8×10^{-6}	1×10^{-3}	2×10^{-3}
15	1×10^{-6}	2×10^{-4}	1×10^{-3}
16	2×10^{-5}	5×10^{-3}	1×10^{-2}
17	8×10^{-6}	5×10^{-4}	1×10^{-3}
18	1×10^{-7}	2×10^{-5}	1×10^{-4}
19	8×10^{-5}	1×10^{-4}	6×10^{-1}
20	7×10^{-6}	2×10^{-5}	6×10^{-3}
21	1×10^{-6}	2×10^{-5}	6×10^{-3}
22	7×10^{-5}	6×10^{-2}	4×10^{-1}
23	1×10^{-6}	4×10^{-3}	2×10^{-2}
24	2×10^{-6}	1×10^{-4}	5×10^{-3}
25	6×10^{-5}	5×10^{-4}	1×10^{-3}

The results of the study showed that the effectiveness of the aqueous extract of the hibiscus plant has shown that its effect against microorganisms appeared after incubation for 7 days, meaning that the rate of bacterial activity decreases after continuous incubation by cooling, and this is consistent with the rapid changes that occur as a result of adding the extract.

Several studies **(Camelo-Me´ndez *et al*., 2013; Alarco´n-Alonso *et al*., 2012; Yin & Chao 2008)** have reported that compounds found in the aqueous extract of the hibiscus plant such as Flavonoids and anthocyanins are responsible for their antimicrobial properties, which have proven effective against pathogenic bacteria and as an antioxidant. The substance (Flavonoids and anthocyanins) has biological activity as it is anti-microbial, a food preservative, and an appetite stimulant. Among these substances are phenols that inhibit enzymes responsible for basic reactions by interfering with proteins, which leads to protein denaturation and then the bacteria's inability to survive, It was also revealed the ability of hibiscus plant compounds to degrade the bacterial cell membrane and exit of extracellular substances, and then the death of bacterial cells **(Rukayadi *et al*. 2008 & Jung *et al*., 2013)**. In addition, a study by **(Hassan *et al*., 2016)** indicated that the aqueous extract of the hibiscus plant can be a useful agent when mixed with antibiotics to enhance the effectiveness of treatment against infection.

CONCLUSIONS

According to the aforementioned results, we note that the addition of certain concentrations of hibiscus extract led to a prolongation of its ability to be preserved for a period of up to 7 days and under refrigeration conditions. The addition of this extract improved the sensory quality of milk. As for the acidity of the product, a sugar solution can be added to the product and this leads to an increased consumer acceptance of the product. It is necessary to take into account the microbial load of the milk and the need to take care of the conditions of milk production, such as taking care of hygiene condition in the farms and cooling the milk immediately after the process of milking to prevent the increase in the number of microorganisms in the milk.

REFERENCES

1. Akindahunsi, A. A. & Olaleye, M. T. (2003). Toxicological investigation of aqueous methanolic extract of *Hibscus sabdariffa*. *Journal of Ethnopharmacology*, 2 (6), 161-164.
2. Alarcon-Alonso, J., Zamilpa, A., Aguilar, F. A., Herrera-Ruiz, M.,Tortoriello, J. & Jimenez-Ferrer, E. (2012). Pharmacological characterization of the diuretic effect of

Hibiscus sabdariffa Linn (Malvaceae) extract. *Journal of Ethnopharmacol*, 13 (9), 751–756.

3. Al-Qazzaz, M. F., Al-Jumayli, S. M. K. & Zangana, B. S. R. (2012).The effect of using Hibiscus extracts to maintain the quality and sensory characteristics for minced frozen Spent Hen Meat. *Iraqi Journal of Market Research and Consumer Protection*, 4 (2),40-58.
4. Aurelio, D., Edgardo, R. G. & Galindo, S. (2007). Thermal kinetic degradation of anthocyanins in a roselle (*Hibiscus sabdariffa* L. cv. ´Criollo´) infusion. *International Journal of Food Science and Technology*, 43(2), 322-325.
5. Balouiri, M., Sadiki, M. & Ibnsouda, S. K. (2016). Methods for in vitro evaluating antimicrobial activity: A review. *Journal of Pharmaceutical Anal*ysis, 6 (2), 71-79.
6. Banik, S.K., Das, K.K. & Uddin, M.D.A. (2014). Microbiological quality analysis of raw, pasteurized UHT milk samples collected from different locations in Bangladesh. *Stamford Journal of Microbiology*, 4 (1), 5-8.
7. Camelo-Me´ndez, G. A., Ragazzo-Sa´nchez, J. A., Jime´nez-Aparicio, A. R., Vanegas-Espinoza, P. E., Paredes-Lo´ pez, O. & Del VillarMartı´nez, A. A. (2013). Comparative study of anthocyanin and volatile compounds content of four varieties of Mexican Roselle (*Hibiscus sabdariffa*) by multivariable analysis. *Plant Foods for Human Nutrition*, 8 (3), 229–234.
8. Carey, N. R., Murphy,S. C., Zadoks, R. N. & Boor, K. J. (2005). Shelf lives of pasteurized fluid milk products in New York State: A ten-year study. *Food Protection Trends*, 5 (2), 102-113.
9. David, B., Wolfender, J. L. & Dias, D. A. (2015). The pharmaceutical industry and natural products: historical status and new trends. *Phytochemistry Reviews*, 4 (2), 299-315.
10. Elwood, P. C., Pickering, J.E. Givens, D.I. & Gallacher, J. E. (2010). The consumption of Milk and dairy foods and the incidence of vascular disease and diabetes: An overview of the evidence. *Journal of Lipid Research*, 45 (10), 925-939.
11. Fasoyiro, S. B., Babalola, S. O. & Owosibo, T. (2005). Chemical composition and sensory quality of fruit flavoured Roselle (*Hibiscus sabdariffa*) drinks. *World Journal of Agricultural Sciences*, 1(2), 161-164.
12. Hassan, T. S. S., Hassan, K., Berchová, M. M., Marie, P. & Emil, Š. (2016). In vitro synergistic effect of *Hibiscus sabdariffa* aqueous extract in combination with standard antibiotics against Helicobacter pylori clinical isolates. *Pharmaceutical Biology*, 54 (9), 1736-1740.
13. International Standard Organization ISO. (2003). *Iraqi Standards 3/2270)20-Microbiology of Food and Animal Feeding Stuffs, Horizontal Method for the Enumeration of Total Coliform and Faecal Coliform Count* .4th edition.
14. Jaroni, D. & Ravishankar, S. (2012).Bactericidal effects of Roselle (*Hibiscus sabdariffa*) against food borne pathogens.*Quality Assurance and Safety of crops and foods*, 4 (1), 33-40.
15. Jung, E., Kim, Y.& Joo, N. (2013). Physicochemical properties and antimicrobial activity of Roselle (*Hibiscus sabdariffa* L.). *Journal of the Science of Food and Agricultural*, 93 (15), 3769-3776.
16. Nascimento, G., Locatelli, J. & Freitas, P. (2000).Antibacterial activity of plant extracts and phytochemical on antibiotic resistant bacteria. *Brazilian Journal of Microbiology*, 31 (4), 246-256.
17. Norhaizan, M., Hern, F. S., Ismail, A. & Yee, C. L. (2010). Antioxidant activity in different parts of roselle (*Hibiscus sabdariffa* L.) extracts and potential exploitation of the seeds. *Food Chemistry*, 122 (4), 1055-1060.

18. Pal, M., Mulu, S. & Tekle, M. (2016). Bacterial contamination of dairy products. *Beverage and Food World*, 43 (9), 40-43.
19. Parekh, J. & Chanda.S. (2007). In vitro antimicrobial activity and analysis of some Indian medicinal plant. *Turkish Journal of Biology*, 31(4), 53-58.
20. Quinn, P. J., Markey, B. K., Leonard, F. C., Hartigan, P., Fanning, S. & Fitz, P. E. S. (2011).Veterinary microbiology and Microbial Disease. 2nd ed., UK: *Wiley – Blackwell West Sussex*, 978(1):118-302.
21. Rukayadi, Y. J., Shim, S. & Hwang, J. K. (2008). Screening of Thai medicinal plants for anticandidal activity. *Tropical Journal of Pharmaceutical Research*, 51 (4), 308-312.
22. SAS. 2012. *Statistical Analysis System, User's Guide. Statistical*. Version 9.1th ed., SAS. Inst. Inc. Cary. N.C. USA.
23. To EK. (2012). *Sensory Evaluation Ratings of Nono Dairy Milk Substitute in a Cold Chocolate Dessert*. MSc Thesis, D' you ville college, New York, USA.
24. Tolulope, O. M. (2007). Cytotoxicty and antibacterial activity of methanolic extract of *Hibiscus sabdariffa*. *Journal of Medicinal Plants Research*, 1 (1): 9-13.
25. Whitney, H. (2006). *Raw Milk Quality Testing Animal Production Factsheet Publication: AP017*. Government of Newfoundland and Labrador, Department of Natural Resources, Canada.
26. Yin, M. C. & Chao, C. Y. (2008). Anti-campylobacter, anti-aerobic and anti-oxidative effects of roselle calyx extract and protocatechuic acid in ground beef. *International Journal of Food and Microbiology*, 127(2), 73-77.