Journal of Food Science and Technology (Iran)

Homepage:www.fsct.modares.ir

Scientific Research

Antioxidant and antibacterial activity of coriander essential oil nanoemulsion in *Aloe vera* extract

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ARTICLE INFO

Article History:
Received:2024/5/27
Accepted:2024/7/20

Keywords:

Nanoemulsion,

Total flavonoid content,

Total phenolic content,

Food additive.

DOI: 10.22034/FSCT.22.158.119.

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ABSTRACT

This study was done as a response to recent demands for developing and introducing new natural preservatives and quality enhancers for application in food industry. The emulsion produced by ultrasonic method, was consisted of coriander essential oil nano-droplets dispersed in Aloe vera extract. The two ingredients were tested individually and proved to have antioxidant and antibacterial activities. Total phenolic and flavonoid content of nanoemulsion estimated 33.53 mg GAE/g and 657.33 mg QE/g nanoemulsion respectively. DPPH and ABTS radical scavenging evaluated about 73.70 and 71.30 percent at 300 mg/mL concentration of nanoemulsion. Four methods (disk diffusion agar, well diffusion agar, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)) were used to evaluating antimicrobial effect of prepared nanoemulsion. Similar to disk diffusion agar method, the results of well diffusion agar method showed the inhibition zones with diameters less than 10 mm. Based on MIC and MBC results, the nanoemulsion had an inhibitory effect on all subjected pathogens with MBC of 512 mg/ml against *Staphylococcus aureus* and *Bacillus cereus* while higher concentrations than 512 mg/mL were required for other pathogens. Overall results of four antibacterial tests indicated a stronger effect of nanoemulsion on the Gram-positive pathogens than the Gram-negative bacteria used in this study. Prepared coriander essential oil nanoemulsion in *Aloe vera* extract can be used as an antioxidant preservative with high performance in the food industry yet it is required to do more tests on this nanoemulsion to reach the valid effective concentrations.

1- Introduction

Growing the awareness of consumers about the ingredients used in food products and their negative perspective of chemical preservatives and additives has created the need to investigate and introduce plant extracts and essential oils as new anti-corruption agents in the production of these products. Plant extracts and essential oils, with significant amounts of bioactive substances and with many properties such as anti-microbial and anti-oxidation, can use as natural additives with many benefits for human health and also have a positive effect on the flavor of food. However, the physicochemical characteristics of these extracts and aromatic oils, do not allow them to be used in their raw form. The volatility of essential oils and its negative impact on organoleptic properties of the products can be one of the reasons for this limitation.in recent years, the solution that has attracted the attention of food experts seems to be the transforming these compounds into emulsion forms. Emulsions are systems consisting of two insoluble phases such as water and oil in present of a stabilizing agent, which are produced in two forms: water in oil and oil in water (w/o and o/w, respectively). Due to their hydrophobic properties, essential oils are usually unable to dissolve in foods and also are not easy for intestinal absorption, completely. As a result, through emulsification process, spherical drops of essential oil with a size of less than 100 nm will be uniformly dispersed in the continuous aqueous phase and a nanoemulsion with milder organoleptic properties and suitable solubility for use in various products will be obtained and the volatile active compounds in the essential oil and extract will be stabilized [1, 2 & 3]. The green *Aloe vera* plant with bladed and juicy leaves, which grows mainly in the Middle East and the Mediterranean, has been used as a healing miracle since ancient times. The colorless fillet inside the plant's leaf contains about 98% water and a wide range of biological compounds such as enzymes, proteins, organic acids, vitamins, minerals and anthraquinones. Many properties such as antimicrobial, anticancer, antioxidant and antidiabetic activity have been seen from this plant, which has led to its use in various fields such as health, medicine and food industry. The high fiber content of *Aloe vera* helps to reducing weight and stabilizing blood glucose and also insulin level at human body. The gel obtained from the fillet inside the leaf, in addition to its preservation and health properties, can also play another effective role in food industry; The structural polysaccharides of *Aloe vera* (glucomannan and also known as acemannan) have turned the gel extracted from the plant leaves into a mucilage that can be used in emulsification process [4 & 5].

Coriander plant also known as *Coriander sativum*, with its special aroma and flavor, has been used as a part of food additives with the purpose of preserving food and maintaining the human health. The therapeutic use of this plant, in addition to influencing on the sensory properties of food, is one of the main important reasons for its popularity. The essential oil obtained from different parts of coriander, especially from its seed, can be used as an additive without causing undesirable effects in medicines and food products. The colorless coriander essential oil with its strong and sharp aroma can have various influences on health matters, including preventing colon cancer, diabetes, indigestion, flatulence, dealing against oral transmitted microorganisms, and also creating an oxidative balance in the human body. The volatile short chains of the essential oil have limited its application in the food industry. The lack of solubility of the essential oil as well as its low absorption in human guts, causes the low efficiency and waste of its essential compounds and bioactive properties. This essential oil is generally recognized as safe by the US Food and Drug Administration, but it's application limited to a certain standard amounts [6 & 7].

In the present study, a novel nanoemulsion of coriander seeds essential oil in the aqueous extract of *Aloe vera* plant introduced and its potential as a new preservative were investigated. For this matter, total phenolic and flavonoid content in the nanoemulsion was measured using colorimetric methods. These two compounds have many antimicrobial and antioxidant properties. Antioxidant potential of the produced emulsion is evaluated by two methods, ABTS and DPPH assays (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) and 2,2-diphenyl-1-picrylhydrazyl, respectively). And finally, the antimicrobial effect of this combonation on several types of oral transmitted pathogenic bacteria, causing diseases such as diarrhea, vomiting, and intestinal inflammation was tested using four methods and their results were analyzed. the pathogenic bacteria used in this study, were included: *Salmonella typhi* ،

Listeria monocytogenes ،*Staphylococcus aureus* ، *Bacillus cereus* ،*Shigella dysenteriae* and *Escherichia coli*.

2- Material and methods

Aloe vera leaves were purchased directly from the shops, Mollasani, Khuzestan, Iran. and coriander essential oil was purchased from the GiyahKala refinery, Iran. The experiments were carried out in the laboratories of Department of Food Science and Technology, Agricultural Sciences and Natural Resources University of Khuzestan, Mollasani, Iran.

2-1- Extraction method for *Aloe vera* leaves

Green leaves of the plant were washed and their surface were gently cleaned with a brush as the first step to obtaining the extract. Then, to separate the thick yellow liquid present in Aloe vera, the head and base were cut and the leaf was placed vertically in distilled water for 60 minutes. After that, the blades and one side of the green latex were separated with a knife and the colorless sticky Aloe vera fillet (mucilage) was extracted. The mucilage were stirred for 3 minutes in a domestic blender so a uniform and homogeneous gel was obtained, and the final extract was prepared by passing this gel through a cotton cloth (such as muslin) to filter the suspended particles and polysaccharides from extract [8].

2-2- producing the Nanoemulsion

Certain concentrations of essential oil and emulsifier (1% essential oil and 1% Tween 80) were used to prepare nanoemulsion. After adding specific amounts of extract (continuous aqueous phase), essential oil (dispersed oil phase) and emulsifier, the ultra-turrax device was used to homogenize the mixture. The final nano-scale emulsion fabricated using an ultrasound device and was tested to check its characteristics [5].

2-3- Determining total phenolic content of nanoemulsion

The folin–ciocalteu assay (colorimetry) was performed and the total amount reported as mg of gallic acid per gram of nanoemulsion. First, a certain concentration of emulsion was prepared and 0.5 ml of it was added to folin solution (10%). The obtained solution was well stirred and after 6 minutes, 2 ml of sodium carbonate (7.5%) was added to. After 30 minutes of incubating the final solution at room temperature in a dark place, the absorbance of the solution was determined by a spectrophotometer (WPA, England) at a wavelength of 765 nm and measured by placing in the formula obtained from the gallic acid standard curve [9].

2-4- Determining total flavonoid content of nanoemulsion

Total flavonoid content of the nanoemulsion was also measured by colorimetry method using a spectrophotometer. Standard solution of quercetin was prepared and standard curve obtained using different concentrations of this solution, the total flavonoid in the emulsion was measured by placing at the standard curve equation and reported as mg quercetin per gram of nanoemulsion. To perform this test, 75 µl of sodium nitrite solution (5%) was added to the emulsion sample using a sampler and after stirring, incubated for 6 minutes. 150 µl of Aluminum chloride solution (10%) was added to the mixture with a sampler and incubated again for 5 minutes. Finally, by adding 1 ml of sodium hydroxide (1 M), the absorbance of the final solution was immediately measured at the wavelength of 510 nm, and the total amount of flavonoid was reported accordingly [10].

2-5- DPPH assay

Evaluating of the percentage of DPPH free radical scavenging of different concentrations of the emulsion done using spectroscopy, following hojjati *et al.* [11] method with a slight change. First, different concentrations of emulsion were prepared (50, 100, 150, 200, 250 and 300 mg/ml). Next step, a solution of DPPH powder (methanol as the solvent) was prepared which its absorbance at the wavelength of 517 nm was about 0.7 (control). Different emulsion dilutions were mixed with methanol solution in tubes (1:1 v/v) and their absorbance recorded after incubating for 30 minutes in a dark environment. The percentage of free radical scavenging was calculated using equation 1.

$$[(A_{control}-A_{sample})/A_{control}] \times 100$$

Equation 1

2-6- ABTS assay

In this test, the percentage of free radical scavenging against $ABTS^{+}$ was calculated and reported by following the Kaparakou *et al*. [12] method with some changes. First, different dilutions of emulsion (50, 100, 150, 200, 250 and 300 mg/ml) were

prepared. Control solution was obtained by mixing certain amount of $ABTS^{+}$ with distilled water and added to a 2.45 mM solution of potassium persulfate with a ratio of 1:2. Control solution was placed in a dark place for 16 to 24 hours to activate the free radicals on the next day, methanol was added to the solution until its absorbance reached about 0.7 at the wavelength of 734 nm. Finally, different dilutions of the emulsion were mixed with the control solution at a ratio of 1:1 and their absorption were recorded after 6 minutes. The percentage of radical scavenging related to different concentrations was determined and reported using equation 1.

2-7- Estimating the inhibitory potential of nanoemulsion against pathogens

Determination of antimicrobial activity of the nanoemulsion was performed against 6 pathogenic bacteria, including *Escherichia coli* ATCC 12435, *Shigella dysenteriae* ATCC 13313, *Salmonella typhi* ATCC 65154 (gram negative bacteria), *Staphylococcus aureus* ATCC 14154, *Listeria monocytogenes* ATCC 19115 and *Bacillus cereus* ATCC 10876 (gram positive bacteria). For this purpose, 4 techniques were used: disc diffusion agar, well diffusion agar, minimum inhibitory concentration and minimum bactericidal concentration.

2-7-1- Preparing foodborne pathogenic bacteria

To perform microbial tests, fresh cultures of pathogenic bacteria were needed. Standard suspensions of the included bacteria were prepared using 0.5 McFarland standard (1.5 x 108 CFU/mL). This standard suspension was prepared using a spectrophotometer at a wavelength of 625 nm at an absorbance between 0.08 and 0.13 [13].

2-7-2- Disc diffusion agar assay

Measuring the diameter of inhibition zones, first the blank disc was soaked inside the nanoemulsion for 15 minutes. 100 µl of standard suspensions were transferred on Müller-Hinton Agar using spread plate method. Then, on each petri dish, a disk containing emulsion, a blank disk (negative control) and a gentamicin antibiotic disk (positive control) were placed at certain distances from each other and the Petri dish wall. After 24 hours of incubation at 37°C, inhibition zones were measured and reported at millimeter scale [14].

2-7-3- Well diffusion agar

Based on this method, after using spread plate method for bacterial suspensions on the MHA, wells with specific distances from each other and the Petri dish wall were cut on the agar using sterile glass pasteur pipette and the bottoms were closed with semi-solid (milten) agar. Then, 20 µl of the nanoemulsion was transferred into the well using a sampler. The same amount of sterile distilled water (control) was poured into other well. After the incubation time, the diameters of the inhibition zones around the wells were measured [15].

2-7-4- Minimum inhibitory concentration

The serial dilution method was used to perform this microbial test. First, the initial concentration was prepared mixing Müller-Hinton broth culture medium, nanoemulsion and 5 ml of dimethylsulfoxide. Other sequel concentrations obtained by adding 5 mL of MHB to each one of them. 100 µl of each concentration was taken with a sampler and transferred into the wells of a 96-well plate. Then 20 µl of the prepared suspensions was added to each dilution so that each row contained different concentrations of the essential oil containing bacteria. A positive and a negative control was considered in each row. The negative control was the mixture without antibacterial agent and the positive control was the mixture without bacteria. After incubation for 24 hours at 37°C, the 96-well plate was removed from the incubator to add color reagent to the houses. A 5% solution of 2,3,5-Triphenyl-tetrazolium chloride (TTC) as color reagent was added (20 µl) to the and transferred to the incubator again for 30 minutes. The first well without preserving red color was selected as the minimum concentration that inhibited bacterial growth [16].

2-5-7- Minimum bactericidal concentration

To determine the minimum bactericidal concentration of the nanoemulsion, 100 µl of colorless wells (no bacterial growth) were taken and cultured on the MHA. 24 hours after incubating, Petri dishes were removed and the concentrations that were free of bacterial growth were selected and reported as MBC [16].

2-8- Statistical analysis

The average of data obtained from the experiments with 3 repetitions were analyzed and results reported using analysis of variance (one-way Anova) at a significance level of 5% in Duncan's test. Therefore, SPSS (26 version) was used.

3- results and discussion

3-1- Antioxidant activity of nanoemulsion

Phenolic compounds, among the important plant bioactive substances, include a wide range of phytochemicals that are divided into different categories. High amounts of these substances will increase the antioxidant potential by scavenging free radicals through different mechanisms. Ultrasound method has been used in many researches as a way to uprising and improve the phenolic content of essential oils and plant extracts. In fact, ultrasound processing through creating a shock and breaking the cell wall causes a better release of biological compounds from inside the cell and increases their accessibility and performance [17, 18 & 19].

Total phenolic content of the nanoemulsion was determined as 33.53 ± 1.57 mg GA/ g nanoemulsion. Results showed a total flavonoid content equal to 657.33 ± 6.6 mg QE/g nanoemulsion. Antioxidant potential of the produced nanoemulsion was investigated; Based on the obtained results, very high ability to inhibit action against ABTS and DPPH free radicals was observed which are reported in Table 1.

Table 1. The antioxidant activity (DPPH & ABTS) of nanoemulsion

Concentration (mg/mL)	Radical scavenging effect (%)	
	DPPH	ABTS
50	57.33 ± 0.42^{A}	49.31 ± 0.29^{A}
100	60.16 ± 0.37^{B}	51.94 ± 0.11^{B}
150	66.18 ± 0.25^{C}	55.79 ± 0.15^{C}
200	68.40 ± 0.90^{D}	67.61 ± 0.27^{D}
250	72.53 ± 0.21^{E}	69.02 ± 0.15^{E}
300	73.70 ± 0.33^{F}	71.30 ± 0.07^{F}

Numbers in the table are the mean of 3 replicates ± standard deviation. Different letters in each column show a significant difference (5%) between the data in each column.

The nanoemulsion produced using *Aloe vera* extract as the continuous phase and coriander essential oil as the dispersed phase showed a high ability to inhibit free radicals. This emulsion at the lowest concentration (50 mg/ml) had a free radical scavenging higher than 50% against DPPH free radical, which increased significantly to 73.70% at 300 mg/ml. Meanwhile, ABTS free radical was also inhibited in the same concentration up to 71.30%, which was almost close to DPPH scavenging capacity. Although, the inhibitory potential against $ABTS^{+}$ was less than 50% at the lowest concentration, but a sudden increase in the antioxidant capacity (more than 10%) observed at concentration level of 150 mg/ml. The high and similar antioxidant capacity of nanoemulsion in both assays can be the result of ultrasonic processing and improved performance of bioactive compounds as secondary metabolites out from the modified cell membrane of the extract, as well as the presence of coriander essential oil. Coriander essential oil with a low percentage (1% w/w) and an increasing effect on antioxidant properties was present in the *Aloe vera* extract and after the ultrasound process, it was dispersed throughout the continuous phase (extract). Dispersion of oil droplets, processing level and the effect of ultrasound on the cells at the applied frequency, can exert different influences on the characteristics of the produced nanoemulsion [20].

Aloe vera aqueous extract has been reported in many studies as the strongest extract to preventing oxidation while it's highest effect is on DPPH free radical, which is similar to the present study and according to the results reported in table 1, the effect of nanoemulsion prepared using *Aloe vera* extract as a continuous phase with a higher amount, seemed to be also more on inhibition of DPPH free radical activity. In some research, the effect of ultrasound process on various plant extracts was investigated

and reported. Based on their results, the ultrasonic process caused a significant increase ($p<0.05$) on the antioxidant property of the treated extracts compared to the pure and unprocessed extract (control). However, these results were not the same in all extracts and in some cases no change was observed [17 & 21].

Nikravan *et al.* [23] investigated and made a comparison between the antioxidant activity of essential oil and its nanoemulsion by ABTS and DPPH assays. Based on their observations, essential oil in their nanoemulsion form had a higher ability to inhibit free radicals and was able to exert antioxidant effect up to 50% at a lower concentration.

3-2- Antibacterial activity of nanoemulsion

Nanoemulsions with antimicrobial activity are actually a stable system of two insoluble liquids with the presence of a surfactant in the form of oil in water, which act on the microorganism membrane with different mechanisms. Essential oil should be used at specific concentration levels to have the maximum power against pathogenic microorganisms such as bacteria, fungi and evolved viruses. Another important factor that strongly affects this issue, is the size of the nano-particles that influence on the outer membrane and creates an electrostatic interaction between the cationic charge of the nanoparticles and the negative charge of the microorganism, which causes the rupture of the cell membrane [24 & 25].

Based on the results of studies comparing the antimicrobial properties of essential oils in both raw and emulsion forms, it was found that by changing the size of the particles to a nano-scale, the inhibitory and bactericidal potential of the antimicrobial agent will increase significantly [19].

In the former studies, essential oil in the form of nanoemulsions were produced with water, which is an inert substance without additional compounds, but reported data in this article are the result of nanoemulsions of essential oils in an extract containing different compounds. *Aloe vera* aqueous extract consists of more than 75 different compounds that undergo chemical changes during the ultrasound process and also interact with dispersed essential oil droplets [5 & 26].

The main antimicrobial agent used in the emulsion, coriander essential oil, was used in with a total amount of 1% (w/w) and was dispersed in the form of oil droplets by ultrasonication in the continuous phase (extract). The results of disc diffusion agar and well diffusion agar assays are mentioned in Tables 2 and 3, respectively.

Table 2. Antimicrobial effect of nanoemulsion against pathogenic bacteria (disc diffusion agar method)

Inhibition zone (mm)	Pathogenic bacteria					
	E. coli	*S. dysenteriae*	*S. typhi*	*S. aureus*	*L. monocytogenes*	*B. cereus*
Nanoemulsion	7.20 ± 0.50^{A}	6.10 ± 0.30^{B}	8.30 ± 0.40^{C}	9.40 ± 0.60^{D}	8.80 ± 0.50^{C}	9.00 ± 0.20^{D}
Gentamicin	24.00 ± 1.00^{E}	22.67 ± 1.53^{E}	23.33 ± 0.58^{E}	24.00 ± 1.73^{E}	25.00 ± 2.00^{E}	24.00 ± 1.41^{E}

Numbers in the table are the mean of 3 replicates ± standard deviation. Similar letters show a significant difference (5%) between the data.

Table 3. Antimicrobial effect of nanoemulsion against pathogenic bacteria (well diffusion agar method)

Inhibition zone (mm)	Pathogenic bacteria					
	E. coli	*S. dysenteriae*	*S. typhi*	*S. aureus*	*L. monocytogenes*	*B. cereus*
Nanoemulsion	7.50 ± 0.40^{A}	6.60 ± 0.50^{B}	8.90 ± 0.30^{C}	9.70 ± 0.50^{C}	9.60 ± 0.25^{C}	9.60 ± 0.50^{C}

Numbers in the table are the mean of 3 replicates ± standard deviation. Similar letters show a significant difference (5%) between the data.

Based on the diameter of inhibition zones obtained from both methods, it can be said that the produced nanoemulsion does not have a high potential against considered pathogenic bacteria. The sensitivity of the bacteria to the antimicrobial agent was almost the same. Gram-positive bacteria were most sensitive to nanoemulsion. Among Gram-negative bacteria, *Salmonella typhi* showed the least and *Shigella dysenteriae* showed most resistance against the antibacterial agent. All measured inhibition zones were smaller than 10 mm. Moreover, in the disk diffusion test, a huge difference was observed between the diameter of the control disk inhibition zone and the disk containing nanoemulsion. There was no big of a difference between the inhibition zones around the disks and the wells in both tests, although the diameter of the inhibition zones was larger in the well diffusion agar test.

Nanoemulsion showed a stronger effect on gram-positive pathogenic bacteria than gram-negative bacteria, which was due to the different functions and numbers of the membrane layers of gram-positive and gram-negative bacteria, which justifies the higher sensitivity of gram-positive bacteria [27 & 28]. A similar effect also observed in other studies, which indicates the higher sensitivity of gram-positive pathogens compared to gram-negative ones [29 & 30]. Minimum concentrations of nanoemulsion that had the property to inhibit the growth and kill these pathogenic bacteria are mentioned in Table 4.

Table 4. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of nanoemulsion against pathogenic bacteria

Bacteria	MIC (mg/mL)	MBC (mg/mL)
E. coli	256	>512
S. dysenteriae	256	>512
S. typhi	256	>512
S. aureus	128	512
L. monocytogenes	128	>512
B. cereus	128	512

The growth of all three dram-positive bacteria was stopped at the concentration level of 128 mg/ml. Gram-negative bacteria also stopped their growth at the concentration of 256 mg/ml of nanoemulsion. However, the nanoemulsion at the highest concentration (512 mg/ml) showed bactericidal properties only against two pathogens, *Staphylococcus aureus* and *Bacillus cereus* bacteria.

4- Conclusions

In the present study, the antioxidant and antimicrobial properties of nanoemulsion were investigated. This substance showed a high antioxidant property based on DPPH and ABTS assays, which proves the synergism and improved effect of its constituents. Subjected emulsion had a 50% inhibitory capacity against two free radicals at a concentration of 50 mg/ml. On the other hand, the results of microbial tests showed that nanoemulsion does not have high antimicrobial activity. Finally, the nanoemulsion of coriander essential oil in aloe vera aqueous extract can act as a suitable preservative and antioxidant in the food industry; However, more tests on different concentrations of this additive are suggested.

5- Acknowledgement

The authors would like to express their sincere gratitude to the Vice-chancellor for Research and Technology of Agricultural Sciences and Natural Resources University of Khuzestan for supporting this study.

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مقاله علمی_پژوهشی

فعالیت آنتی‌اکسیدانی و ضدباکتریایی نانوامولسیون اسانس گشنیز در عصاره آلوئه‌ورا

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اطلاعات مقاله

تاریخ های مقاله :

تاریخ دریافت: ۱٤۰۳/۳/۷

تاریخ پذیرش: ۱٤۰۳/٤/۳۰

کلمات کلیدی:

نانوامولسیون،

فنل کل،

فلاونوئید کل،

افزودنی غذایی.

DOI:10.22034/FSCT.22.158.119.

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چکیده

پژوهش حاضر در پاسخ به نیاز جدید مصرف‌کنندگان و صنعت غذا در تولید و معرفی افزودنی‌های جدید و طبیعی برای حفظ محصولات غذایی و افزایش کیفیت آن‌ها از لحاظ مختلف انجام گرفته‌است. نانوامولسیون تولیدشده به‌روش فراصوت، شامل قطرات پراکنده اسانس گشنیز با اندازه ذرات نانو در عصاره آبی آلوئه‌ورا بود. هر دو این ترکیبات به‌صورت مجزا مورد آزمون قرار گرفته و فعالیت آنتی‌اکسیدانی و ضدمیکروبی آن‌ها ثابت شده‌است. میزان فنل و فلاونوئید کل به ترتیب ۳۳/۵۳ میلی‌گرم گالیک‌اسید در هر گرم و ٦۵۷/۳۳ میلی‌گرم کوئرستین در هر گرم بود. امولسیون تولید شده در غلظت ۳۰۰ میلی‌گرم بر میلی‌لیتر به‌ترتیب ۷۳/۷۰ و ۷۱/۳۰ درصد اثر مهاری بر رادیکال‌های آزاد DPPH و ABTS داشت. چهار آزمون میکروبی شامل دیسک دیفیوژن آگار، چاهک آگار، حداقل غلظت مهارکنندگی و حداقل غلظت کشندگی روی نانوامولسیون انجام شد. هاله‌های به‌دست آمده در آزمون دیسک دیفیوژن همگی قطر کم‌تر از ۱۰ میلی‌متر داشتند که مشابه با نتایج آزمون چاهک آگار بود. آزمون‌های حداقل غلظت مهارکنندگی و حداقل غلظت کشندگی انجام‌گرفته نیز نشان داد که این محصول دارای اثر مهاری روی تمامی باکتری‌های بیماری‌زا بود. نتایج حداقل غلظت کشندگی نشان داد که برای باکتری‌های *استافیلوکوکوس اورئوس* و *باسیلوس سرئوس* برابر با ۵۱۲ میلی‌گرم بر میلی‌لیتر بود اما برای سایر باکتری‌های بیماری‌زا بزرگتر از ۵۱۲ میلی‌گرم بر میلی‌لیتر به دست آمد. تمامی ٤ آزمون نشان‌دهنده اثر قوی‌تر روی باکتری‌های گرم‌مثبت مورداستفاده نسبت به گرم‌منفی‌ها بود. درنهایت نانوامولسیون اسانس گشنیز در عصاره آبی آلوئه‌ورا می‌تواند توانایی عملکرد به‌عنوان یک نگهدارنده و ضداکسایش مناسب وقوی را در صنعت غذا داشته باشد؛ با این حال لازم است آزمایش‌های بیشتری روی غلظت‌های مختلف این ماده افزودنی پیشنهاد می‌شود.