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Basil seed gum edible films incorporated with *Artemisia sieberi* and *Achillea santolina* essential oils: Physical, antibacterial, and antioxidant properties

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Abstract

Active edible films were prepared by incorporating *Artemisia sieberi* and *Achillea santolina* essential oils (EOs) in basil seed gum (BSG) films. The results showed an increase in opacity, contact angel, and water vapor permeability of the films by the addition of EOs, whereas the moisture content, water absorption capacity, and water solubility declined. The presence of EOs significantly improved the DPPH and ABTS radical scavenging activities of BSG films. The agar diffusion disc method was carried out to evaluate the antibacterial activity of the films against *Staphylococcus aureus*, *Bacillus cereus*, *Listeria monocytogenes*, *Shigella dysenteriae*, *Salmonella Typhi*, and *Pseudomonas aeruginosa*. BSG film containing *A. santolina* + *A. sieberi* (1:2 v/v) demonstrated the highest antibacterial activity against the selected bacteria. It can be concluded that BSG film loaded with *A. santolina* + *A. sieberi* (1:2 v/v) EOs have the potential to be used as an active edible film and improve the shelf life of food products by delaying the oxidation and the microbial growth.

Novelty impact statement

- Active edible films were prepared by incorporation of *Artemisia sieberi* and *Achillea santolina* essential oils (EO) into basil seed gum (BSG) films.
- EOs significantly increased the opacity, water contact angel, and water vapor permeability of BSG films.
- Films incorporated with the mixture of EOs (*A. santolina* + *A. sieberi* (1:2 v/v)) showed the highest antibacterial and antioxidant activities.
- BSG films that contained these EOs have the potential to be used as active packaging to increase the shelf life of food products.

1 | INTRODUCTION

Packaging is very essential to promote the safety of the food products and facilitate their shipping and marketability. Petroleum-based plastics are the major materials used in the food packaging sector. However, due to the global concerns regarding the negative effects of plastics on the environment or the presence of micro-plastics in food, the demand for developing new edible/biodegradable films has increased (Hashemi & Khaneghah, 2017). Proteins and polysaccharides are generally recognized as safe

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(GRAS) for using in human and animal foods and the edible films and coatings manufactured from these biopolymers exhibit considerable mechanical and barriers properties (Khodaei et al., 2020). Moreover, these film-forming materials can be used as carriers for delivering active ingredients such as flavors, colorants, probiotics, and essential oils on the surface of food products (Khodaei & Hamidi-Esfahani, 2019).

Basil (*Ociumum basilicum* L.) is an aromatic and the pharmaceutical plant which is widely grown in some regions of Iran and India, and it is broadly used in traditional medicine to heal the colic ulcer, dyspepsia, diarrhea, and inflammations (Khazaei et al., 2014). During the soaking of basil seeds in water, the outer pericarp of the seeds swells and the viscous mass of basil seed gum (BSG) forms. BSG is composed of hydrophobic (glucomannan and highly branched arabinogalactan) and hydrophilic (xylan) fractions (Gahruie et al., 2017; Hashemi et al., 2017). BSG is a biodegradable, heat resistant, and hydrophilic biopolymer with a considerable rheological properties that makes it an excellent candidate as a natural hydrocolloid in food industry (Hosseini-Parvar et al., 2010; Karimi & Kenari, 2016). The film-forming properties of BSG have been confirmed in previous studies (Gahruie et al., 2017; Hashemi & Khaneghah, 2017; Hashemi et al., 2017).

Essential oils are natural biocompounds extracted from plants, and they have a wide application in the food industry as a flavoring and antimicrobial agents (Hashemi et al., 2017; Tavakolpour et al., 2017). However, the direct incorporation of EOs into food systems due to their possible negative effects on the organoleptic properties of the food or their interactions with the food components and also the low water solubility of EOs is limited (Gahruie et al., 2017). Therefore, some new methods such as encapsulation, emulsion, and using edible films have been proposed to protect the EOs during the processing and storage and also reduce the negative organoleptic effects of these oils on food. Chemical composition, the specific parts of the plant used, methods of extraction, harvesting season are some of the factors influencing the biological activities of essential oils (Vitoratos et al., 2013).

Achillea santolina is one of the 115 known species of *Achillea* L. (*Asteraceae*) genus that it is widely distributed in the northern regions of Europe and Asia (Al-Snafi, 2013; Ebadi, 2006). Antimicrobial, anti-inflammatory, antidiabetic, antioxidant, and cardiovascular activities are some of the pharmacological properties attributed to essential oils from *A. santolina* (Al-Snafi, 2013).

Artemisia sieberi (*Artemisia herba-alba*) is another medicinal plant from *Compositae* family growing in Spain, North Africa, and the Middle East. This herbal plant is shown to have considerable antimicrobial activity, and it is commonly used in Iran as a herbal remedies for treating colds, coughing, intestinal disturbances, intestinal parasites infection, and wound healing (Mahboubi & Farzin, 2009).

The effect of incorporation of essential oils on the physicochemical properties of edible films has been reported in various studies. However, to the authors' knowledge, there are no reports on the active edible films loaded with EOs from *A. santolina* and *A. sieberi*. Thus, the main aim of this research was to formulate active BSG-based films containing different ratios of *A. santolina* and *A. sieberi* EOs and evaluate the physical, antioxidant, and antibacterial properties of the prepared films.

2 | MATERIALS AND METHODS

2.1 | Plant materials

Artemisia sieberi and *Achillea santolina* (14% MC d.b.) leaves were purchased from a local market in Shiraz city, Fars province, Iran. Basil seeds were purchased from a local market in Fasa city, Fars province, Iran.

2.2 | Essential oils extraction

Approximately, 50 g of each plant was hydrodistillated for 3–3.5 hr by an all-glass Clevenger tool. About, 550-ml distilled water was utilized (Pharmacopoeia, 1980). The obtained EOs were dried using anhydrous sodium sulfate and stored in sealed vials at 4°C. The EO yield was determined based on the volume of essential oil relative to the dry weight of the plant. The yield of extraction for *A. sieberi* and *A. santolina* EOs were 3.6% and 3.2% (v/w), respectively.

2.3 | Essential oil analysis

GC-MS (6890N, Agilent Technologies, Palo Alto, CA, USA) was applied for EOs analysis. An HP-5MS capillary column (30-m length, 0.25-mm internal diameter, and 0.25-μm film thickness) with a split ratio of 1:30 and helium as a carrier gas (1.3 ml/min) were used. The injector was set at 265°C, and the EOs were diluted with n-hexane (1:10 v/v). Oven temperature was regulated to 60°C for 6 min, accordingly enhanced to 280°C at 3°C/min. Semi-quantitative data were obtained from FID area percentages without the use of correction factors. Retention indices (RI) were determined by using retention times of n-alkanes (C6–C24) and the compounds were recognized by comparing their mass spectral fragmentation patterns with those of similar compounds from the database (Wiley/NBS library) (Hashemi & Khaneghah, 2017).

2.4 | Bacterial strains

Staphylococcus aureus PTCC 1784, *Bacillus cereus* PTTC 1015, *Listeria monocytogenes* PTCC 1299, *Shigella dysenteriae* PTCC 1188, *Salmonella Typhi* PTCC 1609, and *Pseudomonas aeruginosa* PTCC 1430 were obtained from the culture collection at Iran Institute of Industrial and Scientific Research (Tehran, Iran). Each lyophilized pathogen was reactivated in Soybean casein digest medium (Oxoid,

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UK) at 25–37°C for 20 hr. All microbial strains were grown in broth medium three times prior to the tests.

2.5 | Extraction of basil seed gum

After sieving, basil seeds (Esfahan variety; 13% MC d.b.) were washed with ethanol (75% w/v) for 10 min, and then ethanol was eliminated from seeds using filtering and drying. Basil seeds were steeped in distilled water (12:1 v/w) at 37°C for 7 hr. Subsequently, seed–water slurry was stirred using a blender at 1,000 rpm for 20 min to separate the gum layer. Final extraction of BSG from the seeds was done by using a cheese cloth filter and the insoluble residue if any was filtered out, exit a concentration of 18% (w/w). The drying process of the extracted gum was carried out by vacuum oven at 50°C (Hashemi & Khaneghah, 2017).

2.6 | Preparation of film solution

An aqueous hydrocolloid solution containing 6% basil seed gum and proper amount of glycerol (30% w/w) was mixed and heated to 36 ± 1°C under continuous stirring at 500 rpm for 20 min. Then, Tween-20 (15% v/v) was added and the solution mixed for 15 min. The control film was named "F1" which is BSG film. The EOs-loaded films were prepared by the addition of 3% (v/v) of the oils to the film forming solutions (F2: BSG film incorporated with *A. santolina* EO; F3: BSG film incorporated with *A. sieberi* EO; F4: BSG film incorporated with *A. santolina* EO + *A. sieberi* EO (1:1 v/v); F5: BSG film incorporated with *A. santolina* EO + *A. sieberi* EO (2:1 v/v)); F6: BSG film incorporated with *A. santolina* EO + *A. sieberi* EO (1:2 v/v). Afterward, the solutions were degassed by a vacuum pump and the films were cast by pouring the film forming solutions onto petri dishes followed by drying at 35°C for 48 hr. Then, the dried films were peeled off from the petri dishes and conditioned at ambient temperature and 53% RH in chambers containing saturated solutions of $Mg(NO_3)_2$ for 5 days prior the tests.

2.7 | Film thickness

The thickness of the films was measured using a digital micrometer (Mitutoyo, Tokyo, Japan). Determinations were made at five different locations for each film.

2.8 | Opacity of films

The opacity of films analyzed according to the method by Khodaei et al. (2020). The films were prepared into rectangular pieces (10 × 40 mm), and the absorbance was read at 550 nm using a Cary 60 UV-VIS spectrophotometer (Agilent Technologies, Santa Clara, CA, USA). The opacity of the films was measured using the equation below where higher opacity values indicates lower transparency (Kanmani & Lim, 2013):

$$\text{Opacity} = \frac{A_{550}}{\text{thickness}}.$$

2.9 | Swelling index

Approximately, 0.0001 g of the film's cuts left in petri dishes and 35 ml of distilled water was added. Then, they were stored at relative humidity of 50%–55% and 25°C ± 1°C for 24 hr. Afterward, excess water was removed from the films using filter paper and the mass of the film was determined using a microbalance (Mayachiew & Devahastin, 2010). The swelling degree was calculated by the percentage increase in weight of film samples relative to the initial weight of the film.

2.10 | Water contact angle measurements

Approximately, 5 μl of water drop were placed on the surface of films (5.0 × 5.0 cm), and the contact angles were recorded using an optical goniometer (Kruss G10, Germany; RH: 30%, 25°C). The angle of the tangent to the basis of the droplet was calculated (Ojagh et al., 2010).

2.11 | Moisture content

Moisture content of the films was calculated by determination of weight loss of films in an oven at 90°C for 24 hr (Hashemi & Khaneghah, 2017).

2.12 | Water vapor permeability, water absorption capacity, and water solubility

The water vapor permeability of the films was recorded gravimetrically using the ASTM E96-00 method with some modification. The film samples were kept in glass penetration cups with silica gel and the cells were hold in desiccator by means of distilled water at 30°C. The cups were weighed at interval of 1 hr through 24 hr time and water vapor permeability (WVP) of the films were determined (Khodaei, Oltrogge, et al., 2020). The water absorption capacity and solubility of the films in water was measured according to the technique of Sadegh-Hassani and Nafchi (2014).

2.13 | Antimicrobial activity

A suspension (0.1 ml of 6 log CFU/ml) of each pathogen was spread on the petri dishes containing Soybean casein digest medium. The films discs (10-mm diameter) were aseptically placed on the center of mediums. The petri dishes were kept at 25°C–37°C and the

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diameters of the inhibition zones were calculated using caliper after 24 hr of incubation (Hashemi & Khaneghah, 2017).

2.14 | Antioxidant assays

2.14.1 | DPPH assay of films

Approximately, 3 ml of methanol were added to 25 mg of each film. Afterward, 2.8 ml of films' extracted solution were added to 0.2 ml of 1-mM DPPH (Sigma-Aldrich) in methanol. After adequate shaking of the solutions in dark condition, the absorbance at 517 nm was read using Cary 60 UV-VIS spectrophotometer (Agilent Technologies, Santa Clara, CA, USA) (Siripatrawan & Harte, 2010).

2.14.2 | ABTS assay of films

Approximately, 150 μg of each film sample was blended with 2,850 μl of ABTS (Sigma-Aldrich) solution and kept at room temperature for 2 hr. Then, the absorbance of the solutions was read at 734 nm (Cary 60 UV-VIS spectrophotometer, Agilent Technologies, USA), using methanol as a blank (Arnao et al., 2001).

2.14.3 | FRAP assay of films

Approximately, 150 μg of the film samples were mixed with 2,850 μl of FRAP (Sigma-Aldrich) solution and held for 30 min in dark condition. The absorbance was read at 593 nm (Cary 60 UV-VIS spectrophotometer, Agilent Technologies, USA). The absorbance of blank was obtained by replacing FeCl3 from FRAP solution with distilled water (Benzie & Strain, 1996).

2.15 | Statistical analysis

Statistical analyses were performed with ANOVA and significant differences at $p < .05$ were performed by Duncan's multiple range tests using SPSS package program. All measurements were made by triplicate and the data are presented as mean ± standard deviation of each treatment.

3 | RESULTS AND DISCUSSION

3.1 | Essential oils composition

The chemical composition of *A. sieberi* and *A. santolina* EOs were carried out by GC-MS, and the results are listed in Table 1. As can be seen, a total of 21 components were determined in the EO of *A. sieberi* and the principal component observed was 1,8-cineole (48.4%), followed by camphor (30.5%) and camphene (8.9%). Other studies have also shown that the EOs of *A. sieberi* from the northern regions of Iran including Tehran and Semnan were contained camphor, 1,8-Cineole as the major constituents (Sefidkon et al., 2002; Weyerstahl et al., 1993) while the samples from northern east part of Iran such as Khorasan province contained α-thujone, β-thujone, and camphor as the major compounds (Farzaneh et al., 2006). Khosravi et al. (2011) reported that β-thujone (23%), camphor (19.5%), α-thujone (15%), Verbenol (9.7%), 5-dien-8-ol (6.4%), and Davanone (5.8%) were the major components in the oil of *A. sieberi* from Qom province in Iran.

TABLE 1 Essential oil composition of *A. sieberi*

Component	Retention index	%
tricyclene	921	0.4
α-pinene	933	1.5
camphene	948	8.6
Sabinene	969	1.1
β-pinene	973	1.2
Dehydro-1,8-cineol	988	0.5
α-terpinene	1,014	0.1
p-cymene	1,022	1.8
1,8-cineole	1,028	48.4
cis-arbusculone	1,050	0.4
γ-terpinene	1,054	0.3
Cis-sabinene hydrate	1,067	0.4
Trans-arbusclone	1,068	0.1
chrysanthenone	1,025	0.5
Camphor	1,143	30.5
Cis-chrysanthenol	1,161	0.3
pinocarvone	1,162	1.2
Terpinen-4-ol	1,174	1.2
α-terpineol	1,186	0.7
Myrtenol	1,193	0.2

However, the EO from *A. santolina* exhibited more complex composition (48 identified components) and as it is shown in Table 2 the main components were camphor (18%), isoborneol (13.12%), 1,8-cineole (12.44%), Caryophyllene oxide (7.3%), β-eudesmol (4.72%), and terpinen-4-ol (4.27%). Different studies have shown that the EO composition of *A. santolina* depends on the region and extraction techniques. Motavalizadehkakhky et al. (2013) reported that 1,8-cineole, camphor, terpinene-4-ol, fragranol, fragranyl acetate, α-terpinyl acetate, caryophyllene oxide, and α-muurolol were the principal compounds observed in the oils of *A. santolina* collected from Khorasan province of Iran. 1,8-cineole, camphor, 4-terpineol, and trans-carveol were the major compounds of the EO of *A. santolina* collected from Jordan (Bader et al., 2003). Ahmed et al. (2020) reported that the EO of *A. santolina* from Jordan was rich in monoterpenoids with the eucalyptol as the principal component. In another study, 1-methyl 1-vinyl 2-isopropenylcyclobutane (28.23%) and 1,8-Cineole

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TABLE 2 Essential oil composition of *A. santolina*

Component	Retention index	%
α-thujene	923	0.10
α-pinene	930	2.13
Exo-2-norborneol	944	1.49
Sebinene	970	0.17
β-pinene	972	0.37
α-terpinene	1,014	0.92
p-cymene	1,022	0.71
1,8-cineole	1,031	12.44
γ-terpinene	1,057	1.79
Cis-linalool oxide	1,071	0.22
terpinolene	1,086	0.73
α-terpinolene	1,097	1.34
Linalool	1,099	0.66
Cis-linalool	1,102	1.18
Cis-p-menth-2-en-1-ol	1,119	0.19
α-campholenal	1,124	1.13
Camphor	1,146	18.0
dill ether	1,157	0.51
pinocarvone	1,160	0.63
Isoborneol	1,169	13.12
Trans-pinocamphone	1,173	0.53
terpinen-4-ol	1,179	4.27
1-phenyl ethyl acetate	1,184	0.22
α-terpinoel	1,191	1.62
Mytenol	1,195	0.50
Verbenol	1,206	0.50
trans-carveol	1,218	0.60
isobornyformate	1,226	0.29
carvotanacetone	1,241	0.2
trans-carvone oxide	1,255	0.30
cis-chrysanthenyl acetate	1,260	0.62
isobornyl acetate	1,284	0.60
Thymol acetate	1,293	0.65
α-thymol acetate	1,302	0.2
cis-carvyl acetate	1,337	2.0
Eugenol	1,355	0.11
Cis-carvyl acetate	1,362	0.12
e-jasmone	1,395	0.15
(z)-caryophyllene	1,418	0.30
Neryl acetone	1,451	0.20
1-mthoxy-naphthalene	1,475	0.20
germacrene B	1,481	0.13
(E)-β-Ionone	1,484	0.15
cis-eudesma-6,11-diene	1,486	0.22
bicyclogermancrene	1,496	0.25
Caryophyllene oxide	1,585	7.30
Humulene epoxide	1,609	0.42
epoxy-allo-alloarmadendrene	1,633	0.61
z-methyl jasmonate	1,647	0.27
β-eudesmol	1,653	4.72
α-caryophylla-4(14),8(15)-dien-5.ol	1,672	1.27
n-nonadecane	1,900	0.15
n-eicosane	2,000	0.12
n-octadecane	2,082	0.13
n-heneicosane	2,100	0.21
n-docosane	2,200	0.15
n-pentacosane	2,500	0.30

(13.34%), and Grandisol (10.49%) reported as the major constitutes of *A. santolina* samples collected from Egypt (Ali & Abd El-Moaty, 2017). Fragranyl acetate (27.3%), Fragranol (8.2%), cis-Thujone (8.4%), 1,8-Cineole (7.1%), and Camphor (6.7%) were the principle constituents of *A. santolina* from Egypt (Almadiy et al., 2016). Camphor, 1,8-cineole, and α-terpineol were the major constituents of EOs extracted from flowers, leaves, and stems of *A. santolina* from Algeria (Berramdane et al., 2018).

3.2 | Physical properties of the BSG-based films containing different EOs

The effect of incorporation of *A. sieberi* and *A. santolina* EOs separately or in different combination ratios on the physical properties BSG films are presented in Table 3. The thickness of the films ranged from 0.07 to 0.08 mm the results showed that it was not influenced by the addition of EOs ($p \leq .05$). This observation is in consistence with our previous study that the increase in oregano EO in BSG films did not alter the thickness of the films (Hashemi & Khaneghah, 2017). Other studies have also confirmed that the incorporation of EOs had no influence on the thickness of gelatin films (Martucci et al., 2015; Moradi et al., 2016).

The transparency is another important factor of edible films since it has a direct effect on the marketability, appearance, and acceptance of the final product by the consumers (Hashemi & Khaneghah, 2017). As can be seen in Table 3, incorporation of EOs into BSG-based films significantly increased the opacity compared to the control film ($p \leq .05$). The lowest opacity value (2.23) observed for control BSG film (F1), followed by BSG film containing *A. santolina* (F2) with the opacity value of 2.86. Comparing the effect of both EOs on the opacity of the films revealed the greater effect of the oils of *A. sieberi* on the opacity of the film. This observation can be a result of the more complex composition of the EO from *A. sieberi*. The EO from *A. santolina* is composed of two major components and the

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TABLE 3 Physical properties of BSG films incorporated with different ratios of *A. santolina* and *A. sieberi* EOs

Films	Thickness (mm)	Opacity	Swelling index (%)	Contact angle measurements (°)	Moisture content (%)	Water vapor permeability ($\times 10^{-11}$ g/msPa)	Water absorption capacity (g/g dried film)	Water solubility (%)
F1	0.07 ± 0^{a}	2.23 ± 0.03^{c}	37 ± 5^{a}	50.1 ± 0.9^{c}	17.88 ± 0.07^{a}	4.29 ± 0.6^{d}	3.21 ± 0.04^{a}	35.6 ± 0.8^{a}
F2	0.07 ± 0^{a}	2.86 ± 0.06^{b}	43 ± 4^{a}	57.9 ± 1.6^{b}	17.63 ± 0.08^{b}	4.56 ± 0.07^{c}	3.05 ± 0.06^{b}	29.4 ± 0.7^{b}
F3	0.08 ± 0^{a}	3.05 ± 0.04^{a}	46 ± 6^{a}	63.1 ± 1.7^{a}	17.44 ± 0.06^{c}	4.77 ± 0.06^{a}	2.91 ± 0.05^{c}	24.3 ± 0.9^{c}
F4	0.08 ± 0^{a}	2.93 ± 0.09^{ab}	45 ± 3^{a}	60.4 ± 1.8^{ab}	17.58 ± 0.09^{b}	4.67 ± 0.02^{b}	2.99 ± 0.05^{bc}	27.1 ± 0.7^{d}
F5	0.07 ± 0^{a}	2.90 ± 0.05^{ab}	44 ± 5^{a}	58.6 ± 1.2^{b}	17.55 ± 0.05^{b}	4.70 ± 0.04^{ab}	3.01 ± 0.04^{bc}	28.3 ± 0.6^{bd}
F6	0.07 ± 0^{a}	3.02 ± 0.04^{a}	45 ± 3^{a}	61.2 ± 1.9^{ab}	17.50 ± 0.05^{bc}	4.73 ± 0.06^{ab}	2.96 ± 0.05^{c}	25.5 ± 0.5^{c}

Note: Values are represented as means ± standard deviations. Means within a column with different lowercase letters are significantly different at $p < .05$. F1: BSG film; F2: BSG film incorporated with *A. santolina* EO; F3: BSG film incorporated with *A. sieberi* EO; F4: BSG film incorporated with *A. santolina* EO + *A. sieberi* EO (1:1 v/v); F5: BSG film incorporated with *A. santolina* EO + *A. sieberi* EO (2:1 v/v); F6: BSG film incorporated with *A. santolina* EO + *A. sieberi* EO (1:2 v/v).

interactions between the components in *A. sieberi* EO can influence the color characteristics of the oil. So that films loaded with the EO of *A. sieberi* (F3), and EOs of *A. santolina* EO + *A. sieberi* (1:2 v/v) (F6) exhibited the highest opacity values.

The water contact angel (WCA) is another important index showing the hydrophobic characterization of the film's surface (Phan et al., 2005). The results show a high WCA for control BSG film (50.1°) that it represents the hydrophobic nature of the BSG and loading EOs into the films significantly increased the WCA of the films' surface ($p \leq .05$). Higher WCA for the films containing EOs from *A. santolina* and *A. sieberi* can be due to the hydrophobic nature of the oils. EOs by covering the functional and hydrophilic groups on the surface of the film, decrease the surface water wettability (Bahram et al., 2014). Comparing the WCA of the films contained different ratios of EOs revealed a significant difference between the hydrophobicity of the oils from *A. seiberi* and *A. santolina* and the film containing higher ratio of *A. seiberi* (F3) exhibited the highest WCA (63.1°).

Swelling index (SI) % for the different films varied from 37% to 46% and incorporation of oils in different ratios into the BSG films had no influence on the SI of the films. A similar observation has been reported by Valderrama et al. (2015) that the incorporation of rosemary and thyme EOs had no effect on the SI of the chitosan edible films.

The moisture content (MC) % of the films represent the possible interaction of EOs and the biopolymers that may influence the affinity of the films to water molecules (Khodaei, Hamidi-Esfahani, et al., 2020). As shown in Table 3, the MC of the films significantly influenced by the addition of the EOs and their combinations ($p \leq .05$). The highest MC (17.88%) observed in control film (F1) and it reduced by the incorporation of EOs in films. So that the lowest MC value observed in F3 film (17.44%) that it can explain the higher hydrophobic nature of oils from *A. sieberi* compared to the oils of *A. santolina*. Hashemi et al. (2017) also reported an increase in the MC of BSG films by the addition of oregano EO.

Water vapor permeability (WVP) is another important physical property of the films that gives useful information about the mechanism of transferring water molecules from the surface of the films or the possible interactions between the polymers and EO (Bertuzzi et al., 2007). Control BSG film (F1) showed the lowest WVP among the other treatments (4.29×10^{-11} g/msPa) and the addition of EOs into films, separately or in combined forms, significantly increased the WVP of the films. The higher WVP in the BSG films loaded with the oils can be explained by the destabilization of the matrix of biopolymer which results in increased diffusion rate of water molecules through the film surface (Reyes-Chaparro et al., 2015). The film contained the oils of *A. sieberi* (F3) exhibited the highest WVP (4.77×10^{-11} g/msPa). Bonilla et al. (2012) also reported that the presence of thyme and basil EOs increased the WVP of chitosan films.

The effect of EOs addition on the water absorption capacity (WAC) of the BSG-based films is shown in Table 3. As it is depicted, by the incorporation of EOs the WAC of the film reduced. The

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highest WAC was observed for control film (F1) and the lowest was for F3 treatment.

The water solubility (WS) % of the films is presented in Table 3. It can be seen that the addition of EOs, significantly reduced the WS of the BSG films compared to the control (F1). The lowest WS observed for the F2 and F6 films which is in correlation with other physical parameters of *A. santolina* EO loaded films. The higher hydrophobic nature of the EOs from *A. santolina* compared to *A. sieberi*, reduces the affinity of BSG with water molecules and leads in a lower WS, WVP, MC, and WAC of the film compared to other samples.

3.3 | Antioxidant activity of films

Antioxidant characterization is one of the most important biological activities of the EOs that it is mainly attributed to the presence of phenolic compounds, terpenoids, and other volatile compounds. Studies have shown that the EOs can be used as a natural antioxidant agents into the active packaging or films to reduce the oxidation and extend the shelf life of food product (Amorati et al., 2013). The antioxidant activities of BSG-based film contained different combinations of *A. sieberi* and *A. santolina* EOs were evaluated using DPPH, ABTS, and FRAP radical scavenging assays.

DPPH radical scavenging activity of BSG-based films containing different amounts of EOs is depicted in Figure 1. Control BSG film exhibited no antioxidant activity and the addition of EOs significantly increased the antioxidant activity of the films ($p \leq .05$). DPPH value for F2 and F3 films were 0.46 and 0.61 μmol/g, respectively, that it shows the higher antioxidant activity of *A. sieberi* EO compared to the EO from *A. santolina*. Films incorporated with the different combinations of both EOs exhibited a higher antioxidant activity that may explain the possible interaction between EOs and F6 which contains *A. santolina* EO + *A. sieberi* EO (1:2 v/v) showed the highest DPPH value among the others (0.94 μmol/g film). These results are in accordance with those of Hashemi and Khaneghah (2017) and Genskowsky et al. (2015) who reported that the EOs improved the antioxidant activity of BSG and chitosan edible films, respectively.

Similar pattern for ABTS assay was observed (Figure 2) and the antioxidant activity of the BSG films increased from 0.71 μmol/g film for control film to 45.0 μmol/g film for F6. These results suggest that the incorporation a mixture of the EOs can lead in more active films to scavenging free radicals such as DPPH and ABTS.

FRAP assay has a wide application to study the antioxidant activity in plant materials (Ruiz-Navajas et al., 2013). FRAP assay also confirmed a low ability of BSG film to reduce Fe^{+3} to Fe^{+2} (0.04 μmol/g of the film) and incorporation of the EOs significantly increased the antioxidant activity of the films (Figure 3). Similar to DPPH and ABTS assays, films containing both *A. santolina* and *A. sieberi* EOs exhibited the highest FRAP values and the highest antioxidant activity was observed for F6 film (4.1 μmol/g of the film). Hashemi and Khaneghah (2017) reported that the addition of oregano EO to BSG films significantly increased the antioxidant activity compared to control films. Addition of green tea extract to the gelatin films increased the antioxidant activity compared to control film (Dou et al., 2018). Ruiz-Navajas et al. (2013) also observed that the addition of *T. moroderi* and *T. piperella* EOs increased the antioxidant activity of chitosan films.

3.4 | Antibacterial activity

The antibacterial effects of BSG film containing *A. santolina* and *A. sieberi* EOs and their combinations, against three Gram-negative bacteria (*S. Typhi*, *S. dysenteriae*, and *P. aeruginosa*) and three Gram-positive bacteria (*S. aureus*, *B. cereus*, and *L. monocytogenes*) evaluated and the results are presented in Table 4. Control BSG films (F1), showed no antimicrobial activities against the selected bacteria. Other studies have shown no antimicrobial effects for the BSG films (Hashemi & Khaneghah, 2017), zein films (Moradi et al., 2016), and gelatin films (Martucci et al., 2015). BSG film loaded with *A. santolina* EO (F2) showed the highest antibacterial activity against *B. cereus* (24.4 mm) with no inhibitory effect on the *P. aeruginosa*. F3 film, which contained EO of *A. sieberi*, showed the highest (27.5 mm) and lowest (10.2 mm) antibacterial activity

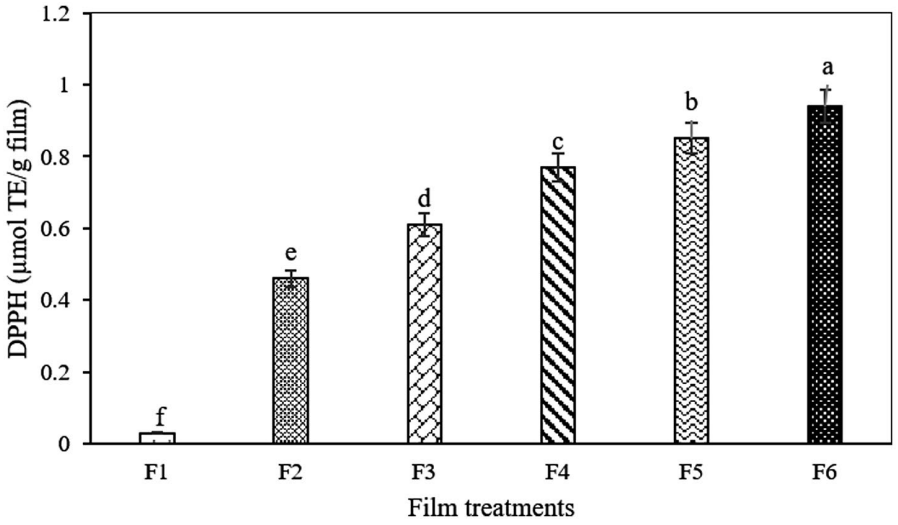


FIGURE 1 DPPH radical scavenging activity of F1: BSG film; F2: BSG film incorporated with *A. santolina* EO; F3: BSG film incorporated with *A. sieberi* EO; F4: BSG film incorporated with *A. santolina* EO + *A. sieberi* EO (1:1 v/v); F5: BSG film incorporated with *A. santolina* EO + *A. sieberi* EO (2:1 v/v); F6: BSG film incorporated with *A. santolina* EO + *A. sieberi* EO (1:2 v/v). Values represent means ±standard deviations. Each column with different lowercase letters is significantly different at $p < .05$

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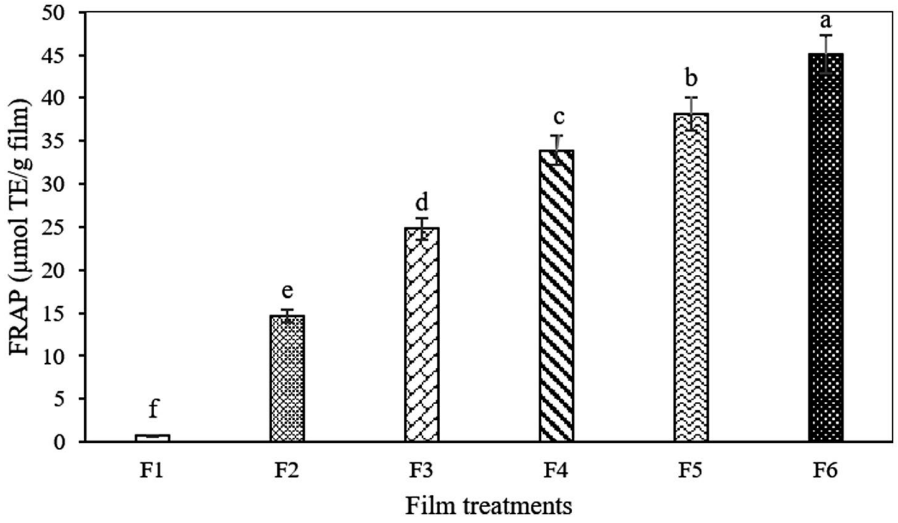


FIGURE 2 ABTS radical scavenging activity of F1: BSG film; F2: BSG film incorporated with *A. santolina* EO; F3: BSG film incorporated with *A. sieberi* EO; F4: BSG film incorporated with *A. santolina* EO + *A. sieberi* EO (1:1 v/v); F5: BSG film incorporated with *A. santolina* EO + *A. sieberi* EO (2:1 v/v); F6: BSG film incorporated with *A. santolina* EO + *A. sieberi* EO (1:2 v/v). Values represent means ±standard deviations. Each column with different lowercase letters is significantly different at $p < .05$

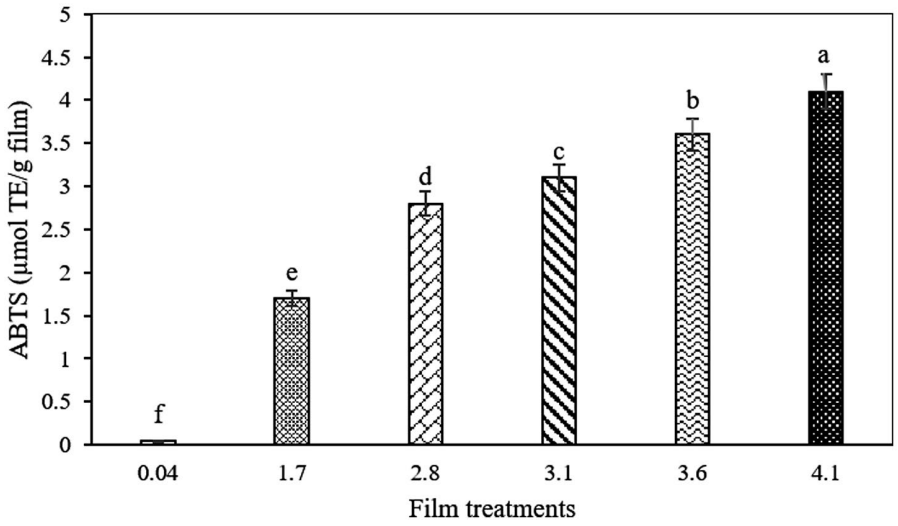


FIGURE 3 FRAP antioxidant activity of F1: BSG film; F2: BSG film incorporated with *A. santolina* EO; F3: BSG film incorporated with *A. sieberi* EO; F4: BSG film incorporated with *A. santolina* EO + *A. sieberi* EO (1:1 v/v); F5: BSG film incorporated with *A. santolina* EO + *A. sieberi* EO (2:1 v/v); F6: BSG film incorporated with *A. santolina* EO + *A. sieberi* EO (1:2 v/v). Values represent means ±standard deviations. Each column with different lowercase letters is significantly different at $p < .05$

TABLE 4 Antibacterial activity of BSG films incorporated with different ratios of *A. santolina* and *A. sieberi* EOs

Films	Diameter (mean and *SD*) of inhibition zone (mm) including film (10 mm)					
	S. Typhi	*S. dysenteriae*	*P. aeruginosa*	*S. aureus*	*B. cereus*	*L. monocytogenes*
F1	–	–	–	–	–	–
F2	13.7 ± 0.3^{eC}	12.1 ± 0.5^{eC}	–	20.2 ± 0.6^{eB}	24.4 ± 0.7^{eA}	21.3 ± 0.8^{dB}
F3	16.1 ± 0.5^{dC}	14.2 ± 0.3^{dD}	10.2 ± 0.6^{dE}	23.2 ± 0.7^{dB}	27.5 ± 0.8^{dA}	24.1 ± 0.6^{bB}
F4	18.2 ± 0.7^{cD}	16.3 ± 0.7^{cE}	12.5 ± 0.3^{cF}	26.1 ± 0.4^{cB}	30.4 ± 0.6^{cA}	26.1 ± 0.8^{cB}
F5	20.8 ± 0.8^{bD}	18.4 ± 0.6^{bE}	14.3 ± 0.4^{bF}	28.4 ± 0.5^{bB}	33.5 ± 0.9^{bA}	24.3 ± 0.6^{bC}
F6	23.4 ± 0.3^{aD}	20.2 ± 0.4^{aE}	16.7 ± 0.5^{aF}	31.2 ± 0.5^{aB}	35.4 ± 1.2^{aA}	27.6 ± 0.5^{aC}

Note: Values are represented as means ± standard deviations of inhibition zones. Means within a column with different lowercase letters are significantly different at $p < .05$ and means within a row with different uppercase letters are significantly different at $p < .05$. F1: BSG film; F2: BSG film incorporated with *A. santolina* EO; F3: BSG film incorporated with *A. sieberi* EO; F4: BSG film incorporated with *A. santolina* EO + *A. sieberi* EO (1:1 v/v); F5: BSG film incorporated with *A. santolina* EO + *A. sieberi* EO (2:1 v/v); F6: BSG film incorporated with *A. santolina* EO + *A. sieberi* EO (1:2 v/v).

against *B. cereus* and *P. aeruginosa*, respectively. The higher antimicrobial activity for the *A. sieberi* EO can be attributed to the more complex compositional structure and the synergistic effects between camphor, isoborneol, 1,8-cineole, Caryophyllene oxide, β-eudesmol, and terpinen-4-ol as the major components of the oil. A notable antimicrobial activity against the selected bacteria observed with the combination of both EOs in BSG films that it confirms the synergism effect of the oils of *A. sieberi* and *A. santolina*

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on the bioactive characterization of BSG films. F6 which is incorporated with *A. santolina* EO + *A. sieberi* EO (1:2 v/v) exhibited the highest antibacterial activity against all selected bacteria. The EO-loaded films showed the highest antimicrobial activity against Gram-positive bacteria than Gram-negative ($p \leq .05$). Other studies have also confirmed the higher prohibition efficiency of the EOs on the Gram-positive bacteria (Ahmad et al., 2012; Shojaee-Aliabadi et al., 2013). Almadiy et al. (2016) observed that the Gram-positive bacteria especially *S. aureus* were the most susceptible microorganisms to the antimicrobial effect of *A. sieberi* EO. The difference in the cell wall membrane of Gram-negative and Gram-positive bacteria may explain their difference in resistance to the antimicrobial agents. The hydrophobic lipopolysaccharides (LPS) in the external membrane of Gram-negative bacteria works as a blocking layer opposing the penetration of the antimicrobial components (Mirjana & Nada, 2004).

Hashemi and Khaneghah (2017) reported that the BSG films incorporated with oregano EO exhibited a higher antimicrobial activity against Gram-positive bacteria. Ruiz-Navajas et al. (2013) also observed that thymus EOs increased the inhibitory effect of chitosan films against different Gram-positive and negative bacteria.

The antibacterial properties of BSG films loaded with *A. santolina* and *A. sieberi* EOs could be a result of the presence of bioactive compounds in EOs such as camphor (Aligiannis et al., 2001; Prudent et al., 1993) and 1,8-cineole (Leung, 1980). Mahboubi and Farzin (2009) reported that the *A. sieberi* EO from the central part of Iran exhibited a high antimicrobial activity against Gram-positive bacteria and fungi and *L. monocytogenes* and *B. cereus* were reported as the most sensitive strains among others. Antibacterial activity of *A. santolina* against *S. aureus*, *P. aeruginosa*, and *Candida albicans* was confirmed by Khalil et al. (2009). Sattari et al. (2011) reported a high antimicrobial activity of *A. santolina* EO against food pathogens such as *S. aureus*, while it had an intermediate effect on *E. coli*.

4 | CONCLUSION

In this study, active edible films were prepared by incorporating *A. santolina* and *A. sieberi* essential oils (EOs) into the basil seed gum (BSG) films. The results showed that the presence of EOs had no effect on the thickness and swelling of the films. However, the opacity, water vapor permeability and water contact angle increased. Addition of EOs improved the moisture content, water absorption capacity and water solubility of the BSG films. The film contained *A. sieberi* EO exhibited a higher antioxidant and antimicrobial activities compared to *A. santolina* EO loaded film ($p \leq .05$). It can be concluded that the addition of EOs improved the antioxidant and antimicrobial activity and the incorporation of *A. santolina* + *A. sieberi* EOs (1:2 v/v) found to be the best combination showing the highest antioxidant and antibacterial activities among other films. The developed BSG active films containing a combination of *A. santolina* and *A. sieberi* EOs have a great potential to be utilized as a packaging material in order to enhance the storage life of packaged food.

CONFLICT OF INTEREST

The authors have declared no conflicts of interest for this article.

AUTHOR CONTRIBUTIONS

Seyed Mohhamad Bagher Hashemi: Conceptualization; Formal analysis; Methodology; Visualization; Writing-original draft. **Diako Khodaei:** Data curation; Formal analysis; Methodology; Project administration; Supervision; Validation; Writing-review & editing.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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