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Phytochemical Content of Hawthorn (*Crataegus elbursensis*) Fruit Extract and Release Behavior from Biopolymer Matrices

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ABSTRACT

Hawthorn (*Crataegus elbursensis*) is extensively utilized in both pharmaceutical and food-related applications due to its abundance of bioactive compounds. However, its application is restrained due to its relative instability. Microencapsulation is a good approach for overcoming this challenge. In the present study, the total phenolic, flavonoid, and anthocyanin contents of the hydroalcoholic hawthorn fruit extract (HFE) were investigated, and its antioxidant capacity was evaluated by the DPPH free radical scavenging method. The HFE was encapsulated with combined hydrocolloids (Sage-Basil, Sage-Balangu, and Balangu-Basil), and the *in vitro* release profile of the HFE was examined. The results showed the total phenol and anthocyanin content of HFE to be 17.9 ± 3.78 mg GAE/g DW and 10.6 ± 0.1 mg cyanidin/g DW, respectively. Additionally, the highest amount of anthocyanin release was observed in the SIF (pH 7.4), with a maximum release value of 98.16 ± 3.23% for Sage-Balangu after 48 h. In general, the results showed that combined hydrocolloid carriers, especially Sage-Balangu, can effectively encapsulate and control the release of HFE for medicinal purposes.

Keywords: Antioxidant activity, Anthocyanin, Hawthorn fruit, Hydrocolloids, *In vitro* release.

INTRODUCTION

The high importance of utilizing herbal plants in the food and health industries, combined with their widespread availability worldwide, has made investigating their properties highly valuable (Veleshkolaii *et al.*, 2024). The hawthorn (*Crataegus elbursensis*) is a genus of the *Rosaceae* family that can reach a height of 3 meters and sometimes more. This plant grows mainly in forests and groves and is abundantly found in the highlands of Mazandaran, Iran (Cui *et al.*, 2024). Hawthorn fruits are consumed fresh or processed into juice and jams. This fruit contains flavonoids, proanthocyanins, and some phenolic acids. Hawthorn has a special place in the pharmaceutical industry due to the presence of certain types of bioflavonoids such as anthocyanins (Wang *et al.*, 2018). Studies have shown that hawthorn fruit extract (HFE) effectively lowers blood pressure, prevents heart attacks, reduces serum cholesterol, and

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improves heart function, which may be attributed to anthocyanins (Shzahrani, 2024; Zhu *et al.*, 2019). Anthocyanins also exhibit a range of biological activities, such as protective effects on pancreas and liver cells, anti-neuronal disorder effects, anti-inflammatory, anti-cancer, anti-bacterial, antiviral, and antioxidant activity (Xue *et al.*, 2024). The utilization of antioxidants in foods is one of the most effective ways to protect human cell components against the danger of free radicals (Alipour Kakroudi *et al.*, 2021; Chaudhary *et al.*, 2023). Anthocyanins may react with each other or amino acids and proteins to form brown polymers. The presence of some metals also causes adverse reactions and the destruction of anthocyanins. Further, these compounds are sensitive to various acid and heat treatments, so they need a stabilizing method for effective activity (Liu *et al.*, 2018). One of the ways to prevent the destruction of anthocyanins is to microencapsulate them. Microencapsulation is a process in which the fine particles of a substance are coated with various compounds to maintain their bioactive properties. Coating of materials is mainly done to prevent the occurrence of chemical reactions between the active substance and the environment, prevent side effects of the active material, prolong the storage time, Prevents degradation at high pH, increases bioavailability, and control release (Multisona *et al.*, 2025; Waqar *et al.*, 2026). Microencapsulation is essential to overcome bioactive compounds' physicochemical instability or premature release (Ghanbari Hassan Kiadeh *et al.*, 2024). Applying hydrocolloids as a coating and promising drug delivery carriers is an attractive option to increase the stability of anthocyanins.

Balangu Shirazi *(Lallemantia royleana*), a plant of the *Labiatae* family, is a unique flowering plant that contains approximately 220 genera and 4000 species. This plant is native to Iran, Pakistan, India, and Afghanistan (Khan *et al.*, 2019). The seeds mucilage of this plant contains 76.74% carbohydrates, 1.29% crude fiber, 9.92% ash, 9.48% moisture, and 3.86% protein (Behbahani and Fooladi, 2018). Balango seed gum has a high molecular weight and good internal viscosity, making it relatively flexible, and when it dissolves in water, it creates a sticky and turbid liquid. Most studies have shown that the protein moieties of hydrocolloids play a basic role in gum surface activity and its emulsifying properties (Amini, 2019). It has also been found that polysaccharides isolated from balango seeds have significant antioxidant properties (Farhan *et al.*, 2022).

Basil (*Ocimum basilicum*) is a member of the mint family. This herbal plant is found in wide areas of the world, especially in the tropics of Asia and Africa (Nazir and Wani, 2021). Basil seeds are a source of high amounts of hydrocolloids with specific rheological properties. Basil seed gum is a distinctive hydrocolloid categorized as an anionic polysaccharide

(Farahmandfar and Naji-Tabasi, 2020). This seed contains 43.50% carbohydrates, 36.30% total fiber, and 9.40% protein (Nazir and Wani, 2021). Galactose, glucose, and mannose are the main constituent sugars, and potassium (K) is the main ion in this gum (Armijo-Montes *et al.*, 2026).

Sage (*Salvia macrosiphon*) seeds also contain a high amount of hydrocolloids; as soon as they are in water, a thick layer forms around them. Sage seed mucilage exhibits exceptional mucoadhesive properties essential for targeted drug delivery (Ojha *et al.*, 2024). Studies have shown that Sage seed gum has significant biological activities due to the presence of flavonoids and antioxidant compounds. Due to the production of abundant mucilage, this seed is traditionally used in the formula of four seeds, which is used to relieve an itchy throat and cough (Razavi *et al.*, 2014).

Therefore, this study aimed to (i) evaluate the bioactive compound profile and antioxidant capacity of HFE, (ii) microencapsulate the anthocyanin-rich HFE using natural hydrocolloids derived from sage, Balangu, and basil seeds (in combination) to enhance its stability, and (iii) investigate the effects of different hydrocolloid matrices on the controlled release behavior of anthocyanins under simulated gastrointestinal conditions (pH 1.2 and pH 7.4).

MATERIALS AND METHODS

Materials

Hawthorn fruits were collected from the heights of the Chelav region in Amol, Iran. Dialysis membrane (cut-off 12 kDa) was purchased from Sigma-Aldrich, USA. Methanol, ethanol, sodium carbonate (Na_2CO_3), and hydrochloric acid were purchased from Scharlau, Spain. All reagents used were of analytical grade.

Preparation of the hawthorn fruit extract (HFE)

Hawthorn fruits were dried in an oven at 40 °C for 48 h. In order to prepare the hydroalcoholic extract, 10 g of powdered fruit was added to 100 mL of 70% ethanol and then stirred at 120 rpm for 24 h using a magnetic stirrer. The solution was filtered via Whatman No. 1 filter paper, and then the solvent was removed from the extract using a rotary evaporator (RV 10 Digital V_C made in Germany) at 40°C under reduced pressure (120 mbar). Finally, the resulting extract was stored at 4 °C until use (Ghanbari Hassan Kiadeh *et al.*, 2021).

Determination of the total phenol

Folin–Ciocalteu reagent was used to determine the amount of total phenol in the HFE. Initially, 20 µL of the extract solution (1 mg/mL) inside the test tube was mixed with 1.160 mL of deionized water and Folin-Ciocalteu reagent (100 µL). After 1 min, 300 µL of Na_2CO_3 solution (20% w/v) was added to the test tube. After shaking, the test tubes were placed in a water bath (40 °C), and after 30 min, their absorbance was measured with a UV–Vis spectrophotometer at 760 nm (Rahaiee *et al.*, 2025). Also, the calibration curve of gallic acid in different concentrations (1, 2.5, 10, 25, 50, 100, and 200 µg/ml) was prepared, and the results were reported as mg gallic acid equivalents per gram dry weight of raw material (mg GAE/ g DW).

Determination of the total flavonoids

The aluminum chloride colorimetric method determined the amount of flavonoids in the HFE. Briefly, 1500 µL of the extract (1 mg/mL) was added to 1500 µL of aluminum chloride (20% w/v). The resulting mixture was stored for 40 min at room temperature in the dark, and then the absorbance was read at a wavelength of 415 nm (Rahaiee *et al.*, 2025). The standard curve of quercetin was also prepared in different concentrations (1, 2.5, 10, 25, 50, 100, and 200 µg/ml), and the results were reported as mg quercetin equivalents per gram dry weight of raw material (mg QE/ g DW).

Measuring total anthocyanin content (TAC)

In order to determine the total anthocyanin content (TAC), 1 mL of HFE (20 mg/mL) was diluted to 10 mL with potassium chloride–hydrochloric acid buffer (pH 1.0) and sodium acetate–hydrochloric acid buffer (pH 4.5), respectively. Then, the solution was subjected to centrifugation at 6000 rpm for 30 min. Subsequently, the supernatant was removed, and its absorbance was measured at the wavelengths of 520 and 700 nm (Yan *et al.*, 2023). The TAC was calculated using the following formulas:

$$A = (A_{520} - A_{700})_{\text{pH 1.0}} - (A_{520} - A_{700})_{\text{pH 4.5}} \quad (1)$$

$$C\ (\text{mg/L}) = (A \times MW \times DF \times 1000) / (\varepsilon \times I) \quad (2)$$

$$TAC\ (\text{mg cyanidin/g}) = (C \times V) / M \quad (3)$$

Where A represents the absorbance; C represents the anthocyanin concentration; DF is the dilution factor of solution; MW is the molecular weight of cyanidin-3-O-glucoside (449.2 g/mol); 1000 is factor for conversion from g to mg; ε is the molar absorptivity of cyanidin-3-

O-glucoside (26,900 L/mol/cm); *I* is the optical path length (1 cm); *TAC* is the total anthocyanin content; *V* is total volume of extract (L); *M* is the initial weight of dry extract (g).

Antioxidant activity of hawthorn extract (HFE) using the DPPH method

To measure the activity of the extract to scavenge the DPPH free radical, 1 mL of different concentrations of the extract solution (100, 200, 300, 400, 500 µg/mL) was added to 1 mL of 50 µM methanolic DPPH solution, and then the final volume was made up to 4000 µL with methanol. Then, the solution was stirred at 25 °C for 30 min in the dark, and the change in absorbance of the samples was read at 517 nm (Mazaraie *et al.*, 2018). Finally, the free radical scavenging capacity (%) was calculated using the following formula:

$$\text{DPPH free radical scavenging (\%)} = \frac{A_D - A_S}{A_D} \times 100 \quad (2)$$

A_D and A_s are the absorbance of the DPPH solution and the sample solution, respectively.

Hawthorn fruit extract (HFE) microencapsulation

Natural hydrocolloids of Sage, Balangu, and Basil seeds were used for microencapsulation. In this way, each of these hydrocolloids (1% w/v) was prepared separately in a water bath (70 °C) under constant stirring (120 rpm) and then placed at 4 °C for 12 h to complete rehydration. Then, 1:1 ratios of hydrocolloids were prepared, and the dry extract (20% w/v) was added and stirred at 10,000 rpm for 4 min in a high shear mixer (T18 Basic Ultra Turrax, IKA, Germany). Eventually, the combined hydrocolloids containing the extract were dried in a freeze dryer (OPERON, Korea) at -40 °C for 48 h (Cai *et al.*, 2019).

Field emission scanning electron microscopic (FE-SEM) analysis

The morphology, size, and distribution of the prepared hydrocolloid microcapsules were investigated using a field-emission scanning electron microscope (MIRA 3, TESCAN).

***In vitro* release study**

The release of anthocyanin pigment from the encapsulated extract was assessed in enzyme-free simulated gastric fluid (SGF, pH 1.2) and enzyme-free simulated intestinal fluid (SIF, pH 7.4) (Cai *et al.*, 2019). For this purpose, 40 mg of lyophilized microcapsules were placed into a wet dialysis membrane (MWCO 12 kDa, Sigma-Aldrich Co., USA) having 2 mL of SGF or SIF buffer. The dialysis membrane was incubated in a compartment comprising 60 mL of buffer at 37 °C under stirring (120 rpm). Finally, at distinct time points, 2 mL of solution was taken and filtered with syringe filters (0.22 µm) and then centrifuged at 6000 rpm for 30 min.

To evaluate the protective effect of microencapsulation, the release behavior of non-encapsulated (free) extract was also assessed under identical conditions. The cumulative amount of anthocyanin released was determined by a UV/Vis spectrophotometer at wavelengths of 510 and 700 nm using the equation below:

$$\text{Cumulative release (\%)} = \frac{C_{\text{outside dialysis membrane}} \times 60\ \text{mL}}{C_{\text{inside dialysis membrane}} \times 2\ \text{mL}} \times 100 \quad (3)$$

Statistical analysis

All analyses were performed in triplicate (n = 3), and results are presented as means ± SD. Statistical evaluation was performed using a one-way analysis of variance (ANOVA) followed by Duncan's test. The *P*-value <0.05 was considered significant. Data analysis was done using SPSS 26 and GraphPad Prism 9 software.

RESULTS AND DISCUSSION

Determination of phenolic compounds

The content of phenolic compounds (total phenol, total flavonoid, and total anthocyanin) of HFE was determined by *in vitro* assays. According to the obtained results, the extract's total content of phenol and flavonoids was measured as 17.9 ± 3.78 mg GAE/ g DW and 9.75 ± 0.44 mg QE/ g DW, respectively. The antioxidant activity of polyphenols and flavonoids in biological systems is attributed to neutralizing free radicals, chelating transition metals, suppressing oxygen molecules through the decomposition of peroxides, reducing hydrogen oxidation, and activating antioxidant enzymes (Alahyane *et al.*, 2026). Studies have shown that different geographical locations, fruit harvesting time, extract preparation conditions, and the method used to determine phenolic compounds have a significant effect on their content (Alirezalu *et al.*, 2020; Sadeghi Mahoonak *et al.*, 2014). In the study of Alsobh et al. (2024), extraction of HFE by heat, microwave, and ultrasonic using methanol, ethanol, and isopropanol solvents was compared. The results of this study showed that ultrasonic extraction yielded the highest total phenol content of 49.14 ± 0.38 mg GAE/ g DW, where methanol was the superior solvent compared to ethanol and isopropanol. Also, the highest total flavonoid content of 18.38 ± 0.19 mg QE/ g DW was reported using the ultrasonic method (Alsobh *et al.*, 2024).

Also, the amount of total anthocyanin was measured as 10.6 ± 0.1 mg cyanidin/ g DW of the extract. Studies have shown that the contents of anthocyanins can be changed under the influence of factors such as light, pH, enzymes, and fermentation products (Liu *et al.*, 2016). It has also been found that the extraction method has a significant difference in the amount of this pigment. In the study of Alami et al. (2014) on the investigation of the phenolic compounds

of the hawthorn fruit acetonic extract, the amount of total anthocyanin was found to be 0.86 ± 0.1 mg cyanidin/ g DW of the extract, which is much weaker than the results of the present study (Alami and Ghorbani, 2014). This difference can be caused by the higher polarity of the hydroalcoholic extract in the extraction of anthocyanin compounds.

DPPH Radical Scavenging Activity

The antioxidant activity of HFE was evaluated by the DPPH method. DPPH free radicals are widely used to investigate the scavenging activity of bioactive compounds. Studies have shown that the activity of free radicals in the body causes numerous complications, including immune system weakness and cancer. Free radicals can also have negative effects on nutritional value, sensory characteristics, and shelf life of foods by decomposing proteins, vitamins, and pigments such as anthocyanins (Lorenzo *et al.*, 2018). The inhibition percentage of DPPH radicals by different concentrations of extract is shown in Figure 1. Based on the obtained results, it was found that the scavenging activity of the extract is significantly concentration-dependent. The highest scavenging rate of 79.81% was recorded at 500 µg/ml. This can be due to the increase in the number of hydroxyl groups (–OH) in the reaction solution and the possibility of hydrogen bonding to free radicals in higher concentrations of phenolic compounds (Zhang *et al.*, 2009). These results are largely consistent with the findings of Moghaddam et al (2022) (Moghaddam and Mehdizade, 2022). In general, the pronounced antioxidant capacity of HFE can be ascribed to its content of bioactive compounds such as phenols and flavonoids (Alirezalu et al., 2020). In a study conducted by Zheng et al. (2018) on the antioxidant capacity of Chinese hawthorn (*Crataegus pinnatifida*), it was found that the free radical scavenging capacity was significantly positively correlated with the release rate of total phenols and flavonoids content, highlighting the main role of these compounds in antioxidant activities (Zheng *et al.*, 2018). Among them, spiraeoside, chlorogenic acid, rutin, isoquercetin, quercetin, and procyanidin were proposed as the main compounds with strong radical scavenging activity (Bahri-Sahloul *et al.*, 2009). While the DPPH assay provides strong evidence for free radical scavenging activity, multi-method validation (e.g., ABTS or FRAP) will be necessary to confirm the antioxidant capacity of these compounds.

FE-SEM analysis

Field emission scanning electron microscopy (FE-SEM) was used to evaluate the morphology and surface topography of combined hydrocolloids (Sage-Basil, Sage-Balangu, and Balangu-Basil with 1:1 ratios) containing HFE. As shown in Figure 2, it was found that

the combined hydrocolloid carrier Sage-Balangu has a more regular structure than the other carriers. The formation of a continuous and compact structure without any pores and phase separation can indicate the high compatibility between Sage and Balangu hydrocolloids (Fig. 2A). This compression can be caused by the hydrophilic nature of hydrocolloids and the associative interaction between them (Kan *et al.*, 2019). This polymer network is expected to reduce the diffusivity of encapsulated bioactive compounds, thereby providing enhanced protection against environmental stressors such as low pH, oxygen, and enzymatic degradation. Such dense architectural integration has been mechanistically linked to enhanced interaction between biopolymer functional groups (Yiasmin *et al.*, 2026). Furthermore, the associative interactions create local concentration gradients that promote the formation of tightly bound domains, a phenomenon well-documented in protein-polysaccharide complex systems where electrostatic complementarity drives network densification (Zhang *et al.*, 2021).

In vitro release study

The *in vitro* release profile of anthocyanin from hydrocolloid carriers was measured in enzyme-free simulated gastric fluid (SGF, pH 1.2) and enzyme-free simulated intestinal fluid (SIF, pH 7.4) for 48 h at 37 °C. The results showed that the release profile of anthocyanin from the carriers prepared at two pH levels is different. Generally, the amount of anthocyanin released from hydrocolloid carriers in the SIF was much higher than in the SGF. It was also found that the release rate of anthocyanin from all hydrocolloid carriers was faster at the beginning of the incubation period, but the rate decreased during the process. This can be due to the rapid release of anthocyanin attached to the surface of the carriers at the beginning of the profile. The second step is related to the drug released due to the disintegration of the polymer network (Huang and Zhou, 2019). As shown in Figure 3, the rate of release of anthocyanin from the hydrocolloid of Sage-Balangu in the SGF was slower than other carriers, so that after 48 h, only 9.86 ± 2.45% Anthocyanin was released into the medium. This reduced release at acidic pH can be attributed to the contracted and compact structure of the hydrocolloid matrix under these conditions. While in the SIF (pH 7.4), the highest percentage of release belonged to the same hydrocolloid (Sage-Balangu), so after 48 h, 98.16 ± 3.23% of anthocyanin was released into the medium (Fig. 3). This pH-dependent release behavior is a desirable feature for targeted delivery of bioactive compounds to the intestine, where absorption predominantly occurs. Also, the free extract exhibited rapid and extensive release in both fluids, with approximately 90.5 ± 4.1% and 92.5 ± 2.7% of anthocyanins released within the first 12 h in

SGF and SIF, respectively. This indicates that free anthocyanins are not protected from rapid release and environmental degradation. Studies have shown that edible biopolymers are superior to synthetic matrices in terms of biocompatibility, biodegradability, and ease of regulation (Paul *et al.*, 2026). Also, some natural biopolymers such as protein-starch complex matrices, which are widely used in the food and pharmaceutical industries, usually require chemical crosslinking agents to achieve pH responsiveness (Sohrabi *et al.*, 2026). However, the combined hydrocolloids used in the present study achieve pH responsiveness through the swelling properties of the native polysaccharide, which can profoundly reduce the need for synthetic modifiers. Therefore, it can be concluded that Sage-Balangu hydrocolloid, due to its protective role in edible coatings and its ability to encapsulate and allow controlled release of bioactive compounds, can be a suitable coating for the protection of anthocyanin or other bioactive compounds of hawthorn fruit in acidic conditions and their controlled release in neutral and alkaline conditions.

CONCLUSIONS

In the present study, the hydroalcoholic extract of hawthorn fruit, rich in phenolic and flavonoid compounds, showed significant and dose-dependent antioxidant activity (via DPPH free radical scavenging). Microencapsulation within a combined hydrocolloid matrix, particularly the Sage-Balangu formulation, significantly improved the stability of anthocyanin-rich bioactive compounds under acidic conditions (pH 1.2) and enabled controlled release at neutral and alkaline pH (7.4). Such a finding highlights its unique potential as a dual drug delivery carrier. Also, the results of FE-SEM analysis showed that this combined hydrocolloid carrier has a more regular structure than other carriers. These findings demonstrate that the encapsulated hawthorn extract, with its potent antioxidant properties, offers a viable plant-based alternative to synthetic antioxidants for potential applications in food and pharmaceutical systems.

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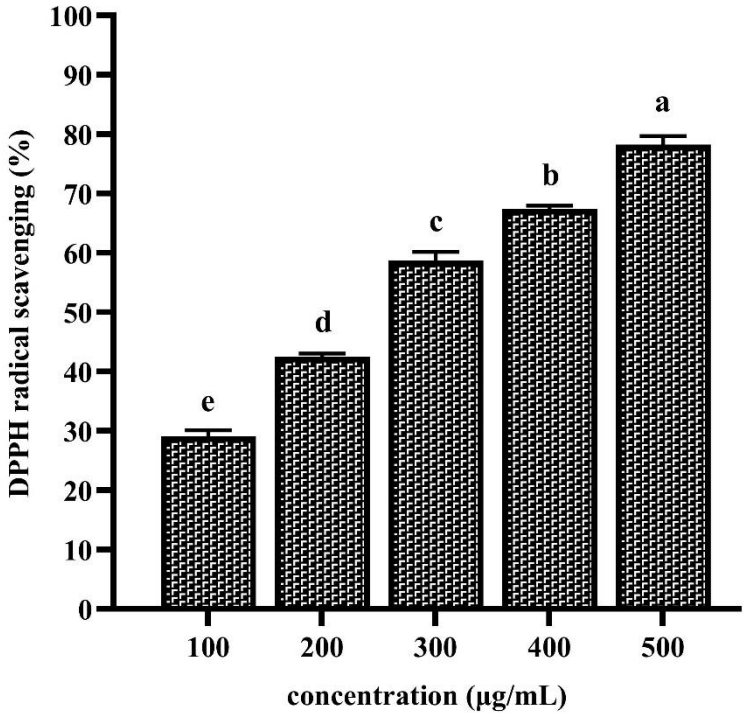

Fig. 1. DPPH radical scavenging activity of HFE. (Columns with different letters indicate significant differences at P-value <0.05).

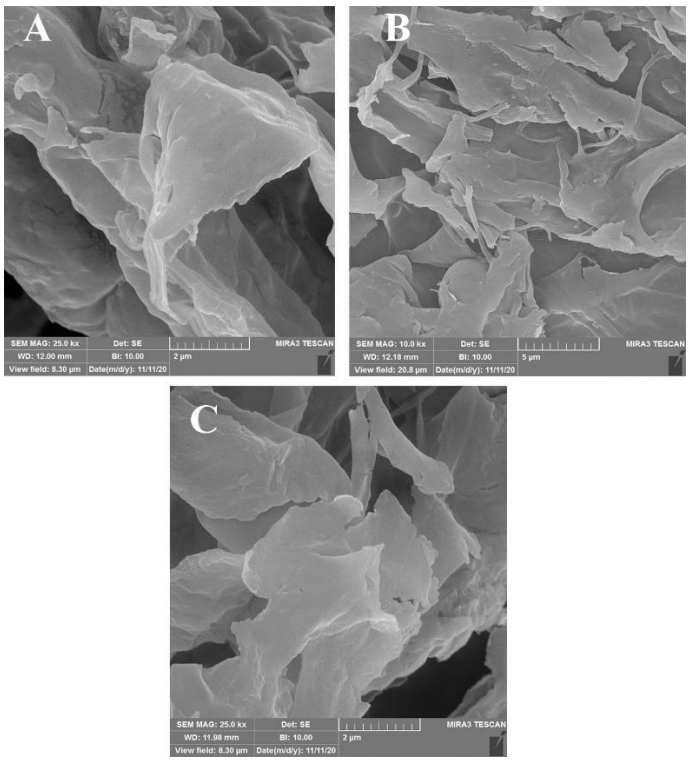

Fig. 2. FE-SEM images of combined hydrocolloids of **(A)** Sage-Balangu, **(B)** Balangu-Basil, and **(C)** Sage-Basil containing HFE.

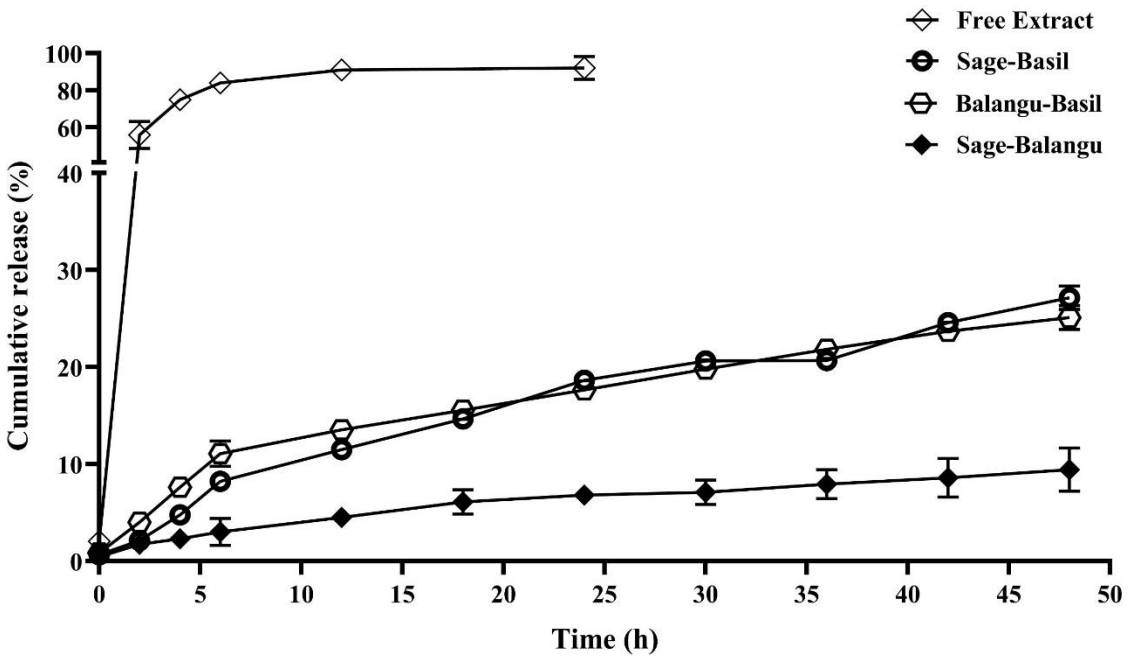

Fig. 3. *In vitro* release of anthocyanin from combined hydrocolloids in SGF (pH =1.2).

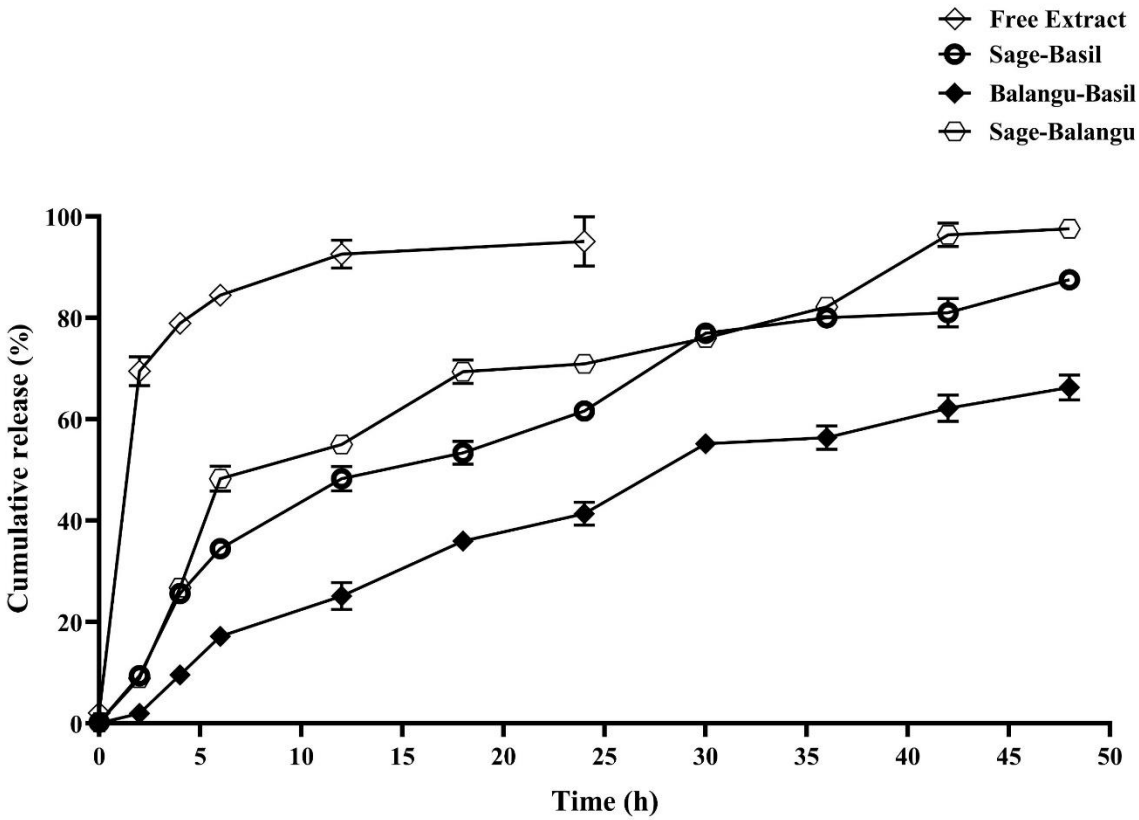

Fig. 4. *In vitro* release of anthocyanin from combined hydrocolloids in SIF (pH =7.4).
combined hydrocolloids in SIF (pH =7.4).