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Investigating the Impact of Surfactant and Cosolvent on the Polyphenolic Content in Arumanis Mango Leaf Extract (*Mangifera indica* L.)

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ABSTRACT: The leaf of *Mangifera indica* L. contains flavonoids and mangiferin which showed positive effects on wound healing of diabetic ulcer. The used of suitable surfactant and co-solvent is required to ensure the high solubility of the active pharmaceutical ingredient (API), thereby optimizing the efficacy. This study aims to determine the ideal surfactant and cosolvent for a thermosensitive hydrogel formula of *Mangifera indica* leaf ethanol extract. The leaf was extracted by maceration using 70% ethanol then determined for moisture content. The extract was prepared for solubility tests of flavonoid and mangiferin on surfactants (Kolliphor® EL, Tween® 20, Tween® 80, Miranol® C2M) and co-solvents (Glycerin, PEG 400, and Transcutol). Determination of total flavonoids content was carried out using Spectrophotometry UV-Vis method and mangiferin content was determined using the RP-HPLC . The moisture content in the extract was 8.590 ± 0.754%. The surfactant demonstrating the highest capability in flavonoid dissolution was Tween 20 (1863.750 ± 0.838 µgQE/g extract), followed by Tween 80, Kolliphor EL, and Miranol C2M. In the context of co-solvents, PEG 400 (1309.583 ± 0.292 µgQE/g extract) show ed superior flavonoid dissolution capability, with glycerin and Transcutol continued to decrease sequentially. Tween 20 and Tween 80 showed comparable efficacy in mangiferin dissolution, followed by Miranol C2M and Kolliphor EL. Among co-solvents, Transcutol demonstrate the highest potential for mangiferin dissolution, succeeded by PEG 400 and glycerin. This study suggests that Tween 20 is a preferred surfactant, and PEG 400 was identified as a co-solvent for use in a thermosensitive gel formula for diabetic ulcers.

Keywords: **co-solvent; extract; *Mangifera indica* L.; surfactant.**

Introduction

Ethanolic extract from Arumanis mango (*Mangifera indica* L.) leaf is effective in healing diabetic wounds because it contains compounds such as flavonoids as antioxidants and mangiferin. Antioxidant activity can protect skin cells from oxidative damage and accelerate wound healing in inflammatory conditions, thus potentially accelerating the healing of diabetic wounds. Apart from that, flavonoid compounds also have an antibacterial role which can prevent infection in wounds [1,2].

The ethanol extract of mango leaves can be formulated in hydrogel dosage form. Topical hydrogel preparations can provide fluid to necrotic wounds, exfoliate the wound, and encourage autolysis. Hydrogel is the best choice for treating dry wounds and can achieve debridement rates 50% faster than wet dressings. Hydrogel also provides hydrating, wound cooling, analgesic effects and more cost effective. However, topical preparations also have potential disadvantages, including difficulty to administer the dose accurately, difficulty in applying in narrow areas, and most patients use their hands to apply the preparation in multi-purpose containers, thereby allowing contamination of the preparation during repeated use [3]. Thermoresponsive hydrogel systems are in situ gelling systems from polymer solutions that undergo a sol-gel transition in response to changes in temperature [4]. With thermoresponsive hydrogel systems technology, the hydrogel solution will form a gel after being dropped on the wound in response to body temperature, making it easier for users when the preparation is applied to areas that are difficult to reach and can avoid contamination of the preparation because the preparation only needs to be dripped without applying. Besides that, the drip

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volume can be adjusted so that the dose can be measured more accurately.

Purified magiferin from Mangifera indica L extract has been successfully developed in the form of hydrogels [5]. The flavonoid quercetin in Pluronic thermosensitive gel formulations has been shown to be a cost-effective option for burn wound therapy [6]. However, a thermosensitive hydrogel dosage form of mango leaf extract has not yet been studied. Encapsulation of the active ingredients of extracts containing flavonoids and mangiferin in thermosensitive gel polymers is expected to improve drug loading, drug release, and drug bioavailability [7]. The active ingredients of herbal medicine have different polarity properties, so to increase the content of flavonoids and mangiferin encapsulated in polymers, surfactants, and cosolvents or those that function as cosurfactants are needed.

Surfactants reduce the surface tension between the nonpolar molecules of the active substance and polar molecules of the thermosensitive gel polymer. In addition, to optimize the active metabolite content in medicinal plants that are non-polar, it is necessary to add a co-solvent as a cosurfactant to help reduce surface tension by increasing the mobility of the hydrocarbon tail [7] [8]. Non-ionic surfactants, particularly Tween 20, Tween 80, and Kolliphor EL, are preferred in pharmaceutical formulations over anionic and cationic surfactants due to their higher efficacy and lower toxicity [9]. Tween 20, for instance, has demonstrated efficacy in dissolving rosemary extract without inducing irritation, as evidenced in the Hen's Egg Test—Chorio Allantoic Membrane (HET-CAM) toxicity assessment. On the other hand, Miranol C2M, an amphoteric surfactant, exhibits distinctive features, including favorable water solubility, high surface activities, a wide isoelectric range, low critical micelle concentration (CMC), high foam stability, low toxicity, low irritation, excellent biodegradability, bioactivity, and interface change, among others [10].

Transcutol has recently demonstrated efficacy as a cosolvent for improving the solubility of various poorly soluble drugs in aqueous solutions [11]. Co-solvents like polyethylene glycol 400 (PEG 400), known for their inert properties, are frequently employed to improve the solubility of drug substances [12]. Flavonoids and mangiferin, being polyphenolic compounds, prompt the selection of glycerin as co-solvents, proven to exhibit high effectiveness in the extraction process of polyphenols [13,14].

Based on this background, this research aims to determine the most appropriate surfactant and co-solvent that can be used in a standardized thermoresponsive hydrogel preparation formula of 70% ethanol extract of Arumanis mango leaves (Mangifera indica L.) which is proven to bind flavonoids and mangiferin optimally.

Methods

Materials

Mangifera indica L. leaves was collect from Magetan, East Jawa on October 2023, ethanol (Merck), toluene (Merck), Mg powder (Merck), HCl (Merck), FeCl3 (Merck), Tween 20 (PT Brataco), Tween 80 (PT Brataco), Kolliphor EL (Sigma Aldrich), Transcutol (provided by PT Rohto Laboratories Indonesia), Miranol C2M (PT Kemiko Indonesia), glycerin (PT Brataco), PEG 400 (Kimia Market), phosphoric acid (Merck), methanol HPLC (Merck), mangiferin standard (Sigma Aldrich), quercetin standard (Sigma Aldrich), AlCl3 (Merck), Na-acetate (Merck), and distilled water (PT Widatra).

Methods

Preparation of Arumanis Mango Leaves

The Arumanis mango leaves (*Mangifera indica* L.) were subjected to a rigorous cleansing with distilled water, followed by a controlled drying process at 45°C for 3-4 days in an oven (BINDER, Germany). The dried leaves exhibited a dark brown coloration, and upon manual compression, demonstrated a friable texture due to the absence of moisture. Subsequently, the leaves underwent a meticulous grinding process, and the resulting mango powder was sifted through a mesh with a standardized size of 40 [15].

Preparation of Ethanolic Extract from Arumanis Mango Leaves

Extraction was conducted using 1500 g of Arumanis mango leaf powder to a 70% ethanol solvent in a container, totaling 3600 mL. The method employed was maceration for a 24-hour duration at room temperature. Following this period, the solution was filtered using paper assisted by a Buchner funnel. Subsequently, a re-maceration process was initiated for an additional 24 hours, repeated thrice [16]. Filtrates were consolidated and subjected to evaporation using a Rotary Vacuum Evaporator (Heidolph, Germany) at 50°C, aimed at eliminating residual ethanol in the extract. The resultant solution was transferred to a porcelain dish and heated in a water bath (Memmert®, Germany) at 50°C until a concentrated extract was obtained [17]. The yield was calculated according to Equation 1 [18].

$$\text{Yield} = \frac{\text{Final weight}}{\text{Initial weigh}} \times 100\% \quad \text{..... eq.1}$$

Moisture Content Determination

The assessment followed the guidelines outlined in Indonesian Herbal Pharmacopoeia Edition II for Moisture Content determination (<83>), employing either the azeotropic or toluene distillation method. Prior to the procedure, the receiving tube and condenser were cleaned and dried. Five grams of the extract were precisely weighed and put into a flask. Afterwards, 200 mL of saturated toluene was added to the round-bottom flask, and the apparatus was meticulously assembled. The round-bottom flask was subjected to heating for a duration of 15 minutes. Once the boiling initiates, distillation was adjusted at a rate of 2 drops per second, progressively increasing to 4 drops per second. Distillation was done for 5 minutes until the receiving tube reached room temperature. In the event of water droplets adhering to the walls of the receiving tube, a rubber apparatus attached to a copper wire was utilized to rub the tube, moistened with toluene until the water droplets went down. Subsequent to this, the water volume was recorded [18]. The moisture content was calculated according to Equation 2

% Moisture content =(Water volume (ml)x 1 gram/ml)/ (Sample weight (gram)) x 100% eq.2

Solubility Test of Samples Preparation

A mass of 500 mg of concentrated Arumanis mango leaf ethanolic extract was precisely weighed and supplemented with a surfactant or co-solvent to achieve a total mass of 3 grams. The surfactants and co-solvents used in this research were presented in Table 1. The mixture underwent sonication (Elmasonic S 30H, Germany) for 30 minutes, followed by stirring using a magnetic stirrer (Thermo Fisher Scientific, USA) at a speed scale of 5 for 24 hours. Then, the mixture was centrifuged using table top centrifuge PLC-03 (Gemmy Industrial Corp., Taiwan) at a speed of 3000 rpm for 15 minutes. The resulting filtrate was obtained and stored in amber vials in cool temperature.

Determination of Total Flavonoids

The preparation of the standard curve involved weighing 10.0 mg of quercetin standard, which was subsequently placed into a 25 ml volumetric flask. Ethanol was added to the mark, resulting in a solution concentration of 400 mg/L. Concentration series were established at 5, 10, 25, 50, 75, and 100 mg/L. From each concentration, 0.5 ml was combined with 1.5 ml of ethanol, and transferred into a 5 ml volumetric flask. The standard solution was added with 0.1 ml of 10% $AlCl_3$ and 1 ml of 1M Na-acetate. Distilled water was added to reach the mark, and the solution was transferred to a cuvette. The absorbance of the standard solution was measured at a wavelength of 438 nm using a UV-Vis spectrophotometer (UV-1800 Shimadzu Corporation) [19].

For sample analysis, 0.5 ml of the sample was added with 1.5 ml of ethanol in a 5 ml volumetric flask. The sample was then added with 0.1 ml of 10% $AlCl_3$ and 1 ml of 1M Na-acetate. Distilled water was added to the mark. The sample solution was transferred to a cuvette, and its absorbance was determined at a wavelength of 438 nm [19]. The flavonoid content was calculated and expressed as quercetin equivalent (µg QE/mL) with quercetin serving as a standard.

Quantification of Mangiferin Content

The determination of mangiferin content was conducted according to the method developed by Retnaningtyas et al. (2020) with modifications [20]. High-Performance Liquid Chromatography (HPLC) (LC-2030C (-48); Shimadzu Corp., Japan) equipped with a UV detector was the selected analytical instrumen. The C18 YMC-Triart C18 column (L1), 4.6 mm x 250 nm S-5 µm, was utilized with a flow rate of 0.8 mL/min, a wavelength of 258 nm, and an injection volume of 20 µL. The mobile phase consisted of a methanol and 0.1% phosphoric acid mixture in a 31:69 ratio utilizing an isocratic technique. Preparation of the 0.1% phosphoric acid solution involved diluting concentrated phosphoric acid with distilled water

Table 1. List of surfactants and co-solvents utilized in the study

Surfactant	Co-solvents
Kolliphor EL	Glycerin
Miranol C2M	PEG 400
Tween 20	Transcutol
Tween 80	

to achieve a concentration of 0.1%. The mobile phase mixture underwent degassing for 10 minutes and was subsequently filtered through a 0.22 µm Pall membrane filter (Merck, Germany).

The standard preparation was prepared by weighing 10 mg of mangiferin standard into a dry 10 ml volumetric flask. Methanol was added up to the mark, and the solution was stirred until completely dissolved. A series of standard curve concentrations were established by withdrawing specific volumes of the stock solution and diluting them with methanol to achieve concentrations of 5, 10, 30, 40, 70, and 100 µg/ml.

Sample preparation was done by weighing 10 mg of the sample into a dry 5 ml volumetric flask, followed by the addition of 3 ml of methanol. The solution underwent sonication for 10 minutes. Subsequently, methanol was added up to the mark, and the solution was agitated until homÇogeneously mixed. The resulting solution was then filtered using a 0.22 µm PTFE filter (Membrane solutions®).

Statistical analysis

The solubility assessments of flavonoids and mangiferin within the surfactant and cosurfactant groups was done using GraphPad Prism software version 10.0.1 (170). Graphs were also generated using the aforementioned software. Solubility datasets were subjected to one-way ANOVA to analyze the solubility of flavonoid and mangiferin among individual surfactants and cosolvents. The degree of significance was denoted by *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, and ****$p < 0.0001$. Conversely, if $p > 0.05$, the solubility test outcomes were considered statistically nonsignificant.

Result and Discussion

The identification of *Mangifera indica* L. plants was carried out at the Biology Learning Laboratory, Faculty of Applied Science and Technology, Universitas Ahmad Dahlan, with certificate number 461/Lab.Bio/B/XII/2023. The ethanolic extract of Arumanis mango leaves was prepared using 70% ethanol through the maceration method [18]. The advantage of the maceration method lies in its simple and easy process, capable of dissolving a significant portion of active compounds while avoiding damage to heat-sensitive constituents [21]. The water content in 70% ethanol serves as a swelling agent for plant materials, enhancing the contact surface with the extraction solvent. The use of ethanol can induce dehydration and damage plant cells due to the wall breakdown of the plant cells. Therefore, 70% ethanol exhibits a synergistic effect, enhancing the effectiveness of secondary plant metabolite extraction [21] including mangiferin, a molecule with intermediate polarity contained in mango leaves, also dissolves well in ethanol [22]. Moreover, the usage of 70% ethanol is recommended by the Indonesian Herbal Pharmacopoeia Edition II. One of the reasons is because it relatively volatile so it does not take long for the evaporation process [18]. To prevent antioxidant activity loss in flavonoids and degradation of mangiferin, the evaporation process using a rotary evaporator and water bath was conducted at a temperature not exceeding 50°C [17,21].

The properties of the ethanolic extract from Arumanis mango leaves can be seen in Table 2. The weight of the concentrated extract was determined to be 378.215 grams, following the utilization of 1500 grams of Arumanis mango leaf. This extraction process resulted in a yield percentage of 25.214%, highlighting the effectiveness of the extraction method.

Moisture content analysis is crucial due to the potential microbial and fungal growth associated with high water content in crude materials, ensuring the quality, safety, and efficacy of the product. In addition to compendial compliance, the toluene distillation method was chosen for its simplicity and speed. Toluene, with a

Table 2. Attributes of the ethanolic extract derived from arumanis mango

Parameter	Comments
Color	Dark brown
Taste	Bitter
Odor	Distinctive aroma of mango leaf
Yield	25.214%
Moisture content	8.59 ± 0.754% (requirement: 5 – 30%)

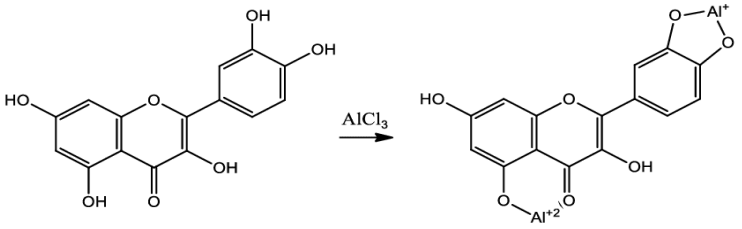

Figure 1. Formation of complexes through a reaction between hydroxyl groups present in flavonoids and aluminum chloride ($AlCl_3$)

lower density than water, facilitates the separation between toluene and water during distillation. Water was positioned below, while toluene remained above.

Prior to distillation, toluene saturation was conducted to purify it, as there might be residual water in the toluene. After adding the extract, the distillation process started until all the water content in the extract was drawn. Volume readings were taken when there were no more droplets on the collection contained. The moisture content in the concentrated extract of Arumanis mango leaves was determined to be 8.590 ± 0.754%, within the range of 5-30%, fulfil the specified requirements [23]. This result ensures the prevention of fungal and mold growth and signifies excellent storage stability, guaranteeing the quality of Arumanis mango leaf extract throughout its storage period [24].

In accordance with the Indonesian Herbal Pharmacopoeia (2017), unless stated otherwise in the monograph, the maceration process is carried out utilizing a 70% ethanol [18]. This solvent selection aims to facilitate optimal extraction of all constituent components, both polar and nonpolar entities. The solubility of active extract components is essential for achieving uniform dispersion within the preparation. However, owing to the ethanol solvent extraction, certain non-polar constituents may exhibit poor solubility in water. Consequently, the incorporation of surfactants becomes imperative to enhance solubility and improve the homogeneity of active ingredients.

Surfactants can enhance the solubility of drug substances due to their amphiphilic structure. This characteristic enables surfactants to be frequently used for the dissolution of active ingredients with low water solubility by incorporating them into micelles. To evaluate the efficacy of surfactants in enhancing the solubility of active constituents present in the ethanolic extract of Arumanis mango leaves, a specific quantity of concentrated extract was solubilized in the surfactants. To separate the dissolved and undissolved parts, the sample underwent centrifugation, and the resultant supernatant was acquired for further analysis. The quantification of soluble compounds was conducted by detecting the total flavonoids and mangiferin present in the supernatant.

Flavonoids were identified through a reaction with $AlCl_3$, leading to the formation of complexes between aluminum ions and flavonoid compounds. $AlCl_3$ reacts with hydroxyl groups on flavonoid compounds, forming color complexes that are detectable using colorimetric assay (Figure 1). The flavonoid concentration was determined based on the calibration curve made using quercetin compound as standard [25]. The standard curve for quercetin can be seen in Figure 2A.

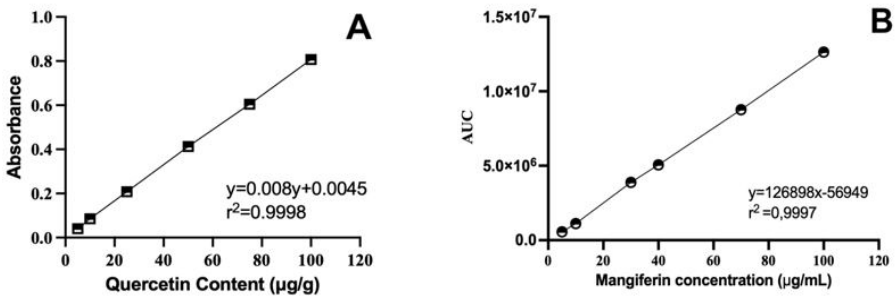

Figure 2. The standard curve for quercetin (A) and mangiferin (B)

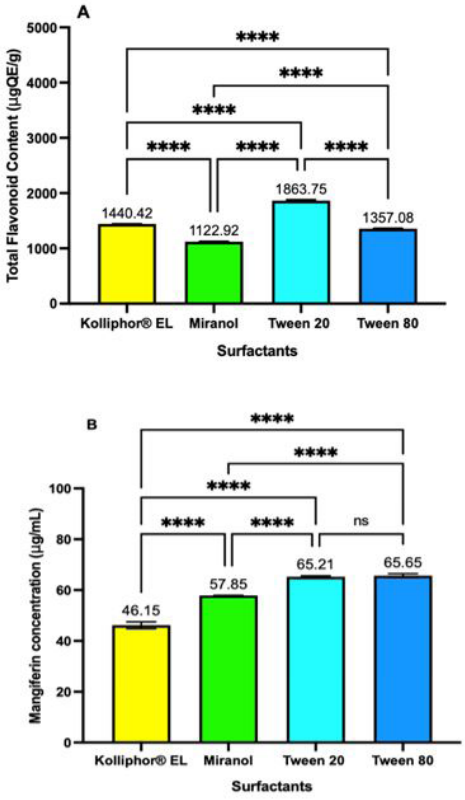

Figure 3. Quantitative analysis of Total Flavonoid Content (A) and Mangiferin Concentration (B) in different surfactant solutions

Figure 3A presents the outcomes of a comprehensive quantitative analysis aimed at evaluating the impact of various surfactants on the total flavonoid content and mangiferin concentration contained in the supernatant of the ethanolic extract of arumanis mango leaves. The figure depicts a clear comparison of total flavonoid content and mangiferin concentration across the different surfactant solutions employed. The optimal solubility of total flavonoids is observed with Tween 20, with a value of 1863.750 ± 0.838 µgQE/g, followed by Kolliphor EL (1440.417 ± 0.265 µgQE/g), Tween 80 (1357.083 ± 0,464 µgQE/g), and Miranol® C2M (1122.917 ± 0.340 µgQE/g). The statistical test showed that the solubility of flavonoids were significantly different in all surfactants (Figure 3A).

The high content of flavonoid solubilized in Tween 20 is unexpected since some research suggested that the solubility of flavonoids were higher upon solubilizing with Tween 80 than in Tween 20 [26]. Research by Vigneshwari (2023) demonstrated enhanced solubility of the flavonoid quercetin in Tween 80 over Tween 20. Similarly, Erawati suggested that Tween 80 can elevate quercetin solubility in citrate buffer pH 4.5 ± 0.2 to a greater extent than Tween 20. Notably, both Vigneshwari and Erawati focused on singular quercetin, while our current investigation utilized an extract containing various flavonoids, including cicerin-7-malonylglucoside, apigenin, apigenin 7-O-glucuronide, reynoutrin, and eupatorin [27]. However, a study on the total flavonoid content in Chrysanthemum morifolium extracts indicated higher values when flowers were extracted using Tween 20 compared to extraction using Tween 80 [28].

Furthermore, the extraction of mango leaf constituents was carried out using 70% ethanol, a solvent known for its efficacy in extracting both polar and nonpolar components. However, the polar nature of 70% ethanol suggests a preference for the extraction of flavonoids with higher polarity. There is also a distinction in polarity between Tween 20 and Tween 80, characterized by their hydrophilic-lipophilic balance (HLB) values of 16.67 and 13.82, respectively [29]. This implies that Tween 20 exhibits higher polarity than Tween 80 [28,30]. The hydrophilic characteristics of substances extracted with ethanol 70% make it more easy to be solubilized in Tween 20, thus, a high total flavonoid content in extract solubilized with Tween 20.

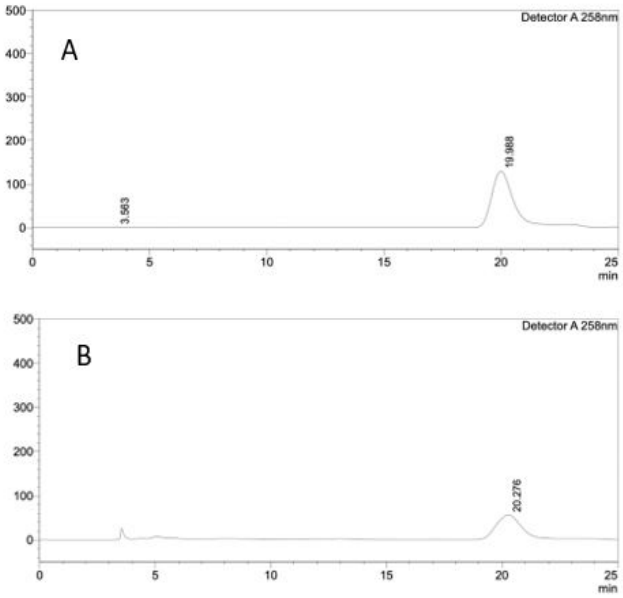

Figure 4. The chromatographic profile of the standard mangiferin (A) and sample (B) exhibits the retention time of mangiferin

The solubility evaluation of mangiferin was determined using the reverse phase – high performance liquid chromatography (RP-HPLC) method with a standard curve, as illustrated in Figure 2B. The chromatographic profile of the standard exhibits the mangiferin chromatogram response at minute 19.988, as depicted in Figure 4A. The chromatographic profile of mangiferin in the sample emerges at a time close to the standard, at minute 20.276 (Figure 4B). The obtained retention time results are closely aligned with the study conducted by Retnaningtyas et al (2019), indicating a time of minute 18.939 [20]. This confirms the presence of similarity in the components of the tested compound with the mangiferin standard used. Analysis results reveal that the highest solubility of mangiferin is achieved with Tween 80 (65.645 ± 1.207) µg/ml among surfactants, followed by Tween 20, Miranol C2M, and Kolliphor EL (Figure 3).

The addition of co-solvents is an effective strategy to enhance the solubility of nonpolar active ingredients by reducing the polarity of a considerable amount of solvents closer to the nonpolar solute [9]. The results of the solubility assessments involving various co-solvents are illustrated in Figure 5. In this phase of the investigation, a selection of co-solvent solutions was strategically employed. The Figure 5 presents a systematic comparison, offering a visual representation of the influence of these co-solvents on the solubility of flavonoids (A) and mangiferin (B). The study evaluated the co-solvents' capacity to enhance the extraction efficiency of total flavonoids and mangiferin. As indicated in Figure 5, the solubility of flavonoids is optimized when employing PEG 400 of 1309.583 ± 0.292 µgQE/g extract, followed by glycerin (841.458± 0.374 µgQE/g) and transcutol (701.042 ± 0.449 µgQE/g). On the other hand, transcutol (61.761 ± 1.314) µg/ml emerges as the co-solvent with optimal mangiferin solubility, followed by PEG 400 (58.981 ± 0.945 µg/ml) and glycerin (50.108± 0.572 µg/ml), with all exhibiting statistically significant differences.

PEG 400 demonstrates superior ability in dissolving flavonoids (1309.583 µg/ml), surpassing glycerin (841.458 µg/ml) by 1.56 times and transcutol (701.042 µg/ml) by 1.87 times. Glycerin has an HLB value of 20, while PEG 400 and Transcutol have HLB values of 13.1 and 4.2, respectively [29,31,32]. PEG 400, which is more hydrophobic than glycerin, shows higher solubility for quercetin owing to increased hydrophobic interactions with the active compounds (Sermkaew and Plyduang, 2020).

Co-solvents typically reduce the chemical potential of a solution by decreasing the hydrogen-bond density of water, thereby creating a less polar environment that allows more drug molecules to dissolve. Accordingly, the less polar nature of PEG 400 enhances its solubilization capacity (HLB 13.1) compared to that of glycerin (HLB 20). This suggests that flavonoid molecules preferentially dissolve in non-polar environments [33].

Mangiferin is slightly soluble in ethanol and water, and

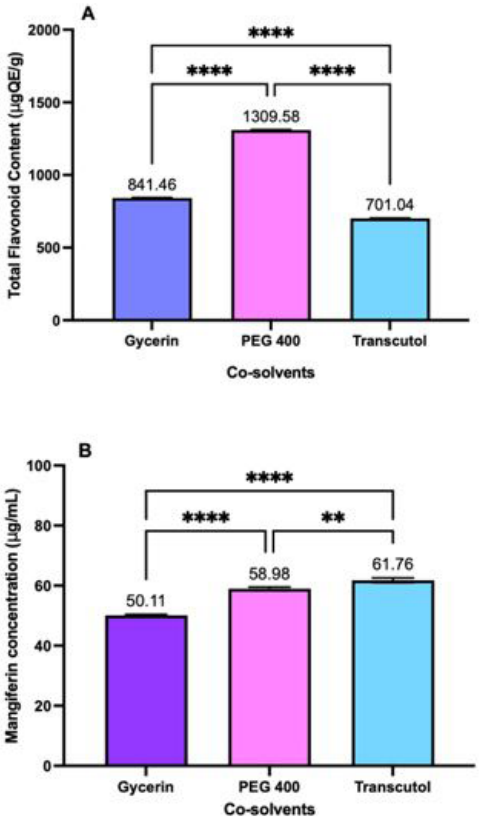

Figure 5. Quantitative analysis of (A) Total Flavonoid Content and (B) Mangiferin Concentration in different co-solvent solutions

insoluble in some non-polar solvents [22,34]. Transcutol has calculated HLB 4.2, but reported HLB 8.61 so it can be assumed as a water-soluble cosolvent [29]. This is similar to the nature of mangiferin, which is a molecule with intermediate polarity. Therefore, figure 5B shows that Transcutol can dissolve mangiferin the most compared to the more hydrophilic PEG 400 and Gycerin.

Regarding mangiferin solubility, PEG 400 displays a relatively lower value (58.981 µg/ml) than transcutol (61.761 µg/ml). However, the quantity of dissolved mangiferin in PEG 400 is only 0.95 times less than that dissolved in Transcutol. From this analysis, it can be concluded that PEG 400 stands out as the most optimal co-solvent for dissolving both flavonoids and mangiferin.

Conclusion

An investigation into the effects of surfactants and cosolvents has been conducted on the solubility of total flavonoids and mangiferin in Arumanis mango leaf extract (*Mangifera indica* Linn). Tween 20, as a surfactant, and PEG 400, as a cosolvent, emerge as promising candidates for enhancing the solubility of active compounds in Arumanis mango leaf extract. These components hold the potential for utilization in the formulation of topical thermosensitive in situ gel.

Conflict of Interest

The authors have no conflicts of interest regarding this investigation.

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