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The Effect of Various Extraction Methods and Solvents on the Phytochemical Contents and Antioxidant Capacities of Safflower Florets (*Carthamus Tinctorius* L.) from South Sulawesi

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Abstract

Safflower is a traditional medicine commonly applied for the treatment of varicella in South Sulawesi. The aim of this study is to study the effect of extraction methods and solvents on phytochemical content and antioxidant capacity in the extraction of safflower. The study began with sample extraction with various solvents, i.e., water, ethanol 70, methanol, ethyl acetate and hexane and various methods, i.e., reflux, Soxhlet, hot maceration, cold maceration and room temperature maceration. Each extract was standardized for its phytochemical content, i.e., phenolics, flavonoids and carotenoids and the antioxidant capacities were also determined by the radical scavenging 2,2-diphenyl-1-picrylhydrazyl, total antioxidant capacity, cupric ion reducing antioxidant capacity and Ferric reducing antioxidant power. The results indicated that the optimum total phenolic content was obtained when the florets were refluxed with methanol, flavonoids were refluxed with 70 % ethanol and carotenoids were Soxhleted with methanol. The research data also showed that 70 % ethanol and methanol have maximal antioxidant capacity. The results conclude that 70 % ethanol and methanol as solvents have the potential to extract the highest levels of phytochemical content and antioxidant substances.

Keywords: Safflower, *Carthamus tinctorius*, Kasumba turate, flavonoids, Phenolics, Carotenoids, Radical scavenging, Phosphomolybdenum assay, FRAP, CUPRAC

Introduction

Safflower is generally applied in South Sulawesi for the therapy of varicella [1,2]. Safflower or kasumba turate (the local name in South Sulawesi) is traditionally used in unconventional dosages or therapies. The Indonesian government is currently developing research on standardizing traditional medicine with regard to the efficacy, safety and quality of pharmaceuticals. Determining the chemical composition and pharmacological activity, as well as extracting with boiling water, are all part of standardization. Quinochalcones and flavonoids are the essential substances in safflower flowers [3]. Quinochalcones are red or yellow pigments contained in safflower florets, particularly carthamin, hydroxysafflor yellow A, safflor yellow A, safflamin C, safflamin A and safflomin A [4]. Hydroxysafflor yellow A is defined as a marker compound in standardization and is responsible for the activity produced by safflower flowers [3]. Mainly, the flavonoids in safflower flowers are flavanol glycosides, e.g., kaempfericides.

The chemical content and antioxidant capacity of the extract are influenced by the extraction techniques and solvents used. Selectivity, solubility, cost and safety are a few of the considerations that must be taken into account while choosing extraction solvents. According to the laws of similarity and intermiscibility, the solute will dissolve in solvents with close polarity (like dissolves like). Therefore, polarity values close to the polarity of the solute are necessary for solvents with high-performance extraction capacity. Nonpolar solutes can be extracted using n-hexan and ethyl acetate, whereas polar solutes can be extracted with methanol or ethanol. Diffusion and solute solubility both rise as the extraction temperature rises. On the other hand, overheating can result in solvent loss, which would remove unwanted

contaminants and break down thermolabile components. Conventional extraction techniques such as maceration, percolation and reflux extraction typically involve the use of organic solvents and necessitate a substantial volume of solvents as well as an extended extraction period [5]. This study aimed to study the effect of extraction methods and solvents on phytochemical content and antioxidant capacity in the extraction of safflower. The safflower extracts used in the therapy should be considered for optimal phytochemical content and efficacy.

Materials and methods

Plants material, chemical and stock solution of sample

Plants material: Florets of safflower were collection of Department of Pharmacy, Faculty of Medicine and Health Sciences, Universitas Islam Negeri Alauddin Makassar, South Sulawesi, Indonesia. The florets are collected when the plants have reached the stage of full flowering, typically occurring approximately 120 - 150 days after the initial sowing. The samples were dried in a drying cabinet at 50 °C. Chemicals: acetone pure (Merck), aluminium chloride (Merck) solution (10 % w/w), ammonia (Merck) solution (1 %), ammonium acetate ($C_2H_7NO_2$) (Merck), ammonium molybdate ($H_{24}Mo_7N_6O_{24}.4H_2O$) (Merck), anhydride acetic acid (Merck), ascorbic acid (Merck), copper (II) chloride ($CuCl_2.2H_2O$) (Merck), deionized water (OneMed), chloroform (Merck), dichloromethane (Merck), dimethyl sulfoxide (Merck), DPPH (2,2-diphenyl-1-picrylhydrazyl) (Sigma Aldrich), Dragendorf reagent (potassium bismuth iodide solution), 70 % ethanol (Merck), ethyl acetate (Merck), Folin ciocalteau (Merck) reagent (7.5 % v/v), glacial acetic acid hexane (Merck), gallic acid (Sigma Aldrich), hydrochloric acid (37 %) (Merck), iron (III) chloride ($FeCl_3.6H_2O$) (Merck) reagent (5 %), Mayer's reagent (1.36 g mercuric chloride and 5 g potassium iodide in water (100 mL), methanol (Merck), neocuproine (Sigma Aldrich), quercetin (Sigma Aldrich), sodium acetate anhydrous (Merck), 7.5 % w/v sodium carbonate (Merck) solution, sodium dihydrogen phosphate ($NaH_2PO_4.H_2O$) (Merck), 98 % sulfuric acid (Merck), 2,4,6-Tris[2-Pyridyl]-S-Triazine (Sigma), Trolox (Sigma Aldrich).

Research design

The extraction optimization of safflower used 2 variabel, i.e., extraction methods and solvents. The same 70 % ethanol solvent was utilized in all of the methods used in this experiment: Soxhletation at 80 °C, reflux at 80 °C, hot maceration at 60 °C, cold maceration at 4 °C and maceration at room temperature at 25 °C. Concurrently, the following solvents were extracted using the same technique, maceration at room temperature: 70 % ethanol, water, hexane, ethyl acetate and methanol. The solvents and methods were selected because the variants are commonly used in herbal extraction, includes the solvents polarity, temperature and apparatus. Duncan statistics are used to calculate the significance of each variable.

Maceration method

The dried safflower (10 g) was macerated in 250 mL of 70 % ethanol. A rotary evaporator (Heidolph) was used to filter and concentrate the extract under vacuum after it had been left for 24 h. For the other solvents, methanol, water, ethyl acetate and n-hexane, the same process was carried out. The samples were kept at 4 °C for cold extraction, 60 °C for hot maceration in a water bath and 25 °C for maceration at room temperature [6].

Reflux and Soxhletion method

After transferring the 10 g of dried safflower into a round-bottom flask, 250 mL of 70 % ethanol was added and the mixture was refluxed for 2 h at 80 °C. The extraction's filtering and drying processes were comparable to maceration [7]. Similar reflux was used to extract safflower for the Soxhletation procedure, but the sample was transferred into the Soxhlet chamber. The soxletation procedure was applied at 80 °C in 3 cycles [8].

Extraction efficiency was calculated as extraction yield using the formula:

$$\text{Extraction yield} = \frac{\text{Dried extract}}{\text{Dried florets}} \times 100\ \%$$

Phytochemical screening

Screening for phytochemical content in the extract includes alkaloids, phenolics, triterpenoids/steroids, saponins and glycosides. Alkaloids were tested by Dragendorf and Mayer's test. To

prepare the alkaloid test solution, about five milligrams of the extracts were dissolved in 6 mL of 1 % hydrochloric acid, heated if necessary and filtered. Dragendorf test: The test solution was added to the Dragendorf reagent in a 1:1 ratio, alkaloid was present if an orange-red precipitate appeared. Mayer's test: The test solution was added to the Mayer's reagent in a 1:1 ratio, the presence of a cream-colored precipitate indicates an alkaloid [9].

Phenolics. A sample stock solution was added with 5 % $FeCl_3$ in a 1:1 ratio. If the extract produced a black or blue-green precipitate, it included tannins or phenolics [10].

Triterpenoids/Steroids were tested by Liebermann-Burchard and Salkowski test. The Liebermann-Burchard test involved shaking a dried extract (± 50 mg) in 1 mL of chloroform, adding a few drops of acetic anhydride and boiling the mixture in a water bath. 98 % H_2SO_4 was added to the test tube after the tubes had cooled. If a brown ring appeared at the intersection of 2 layers, making the upper layer green, the steroid was present, triterpenoids if deep red color. Salkowski test: Dried extract (± 50 mg) was shaken in chloroform (1 mL) and added 98 % H_2SO_4 alongside of the test tube. Triterpenoids were present if there was a reddish-brown coloration at the interface [11].

Saponins. After shaking dried extract (± 100 mg) in 2 mL of deionized water, saponin was detected if a froth formed and continued to remain heated after 5 min in a water bath [9].

Glycosides. Dried extract (± 100 mg) was added to 5 % H_2SO_4 and heated. After filtration, the filtrate was shaken with dichloromethane in a 1:1 ratio. The dichloromethane layer was shaken with 1 % ammonia. Glycoside is positive if a rose-pink to red ammonia layer develops [11].

Quantitative test of phytochemical contents

Quantitative phytochemical testing was carried out on the total content of phenolic, flavonoid and carotenoid compounds. The sample's stock solution was prepared by dissolving the dried extract in dimethyl sulfoxide to a final concentration of 2,000 mg/L. A stock solution of gallic acid was prepared at 250 mg/L and quercetin at 250 mg/L. Stock solution is diluted based on test specifications.

The total phenolic content was measured as gallic acid. The sample solution at 800 mg/L was taken at 0.5 mL and added 2.5 mL of 7.5 % Follin-Ciocalteu reagent. After incubation for 8 min, 2 mL of 7.5 % sodium carbonate was added. The mixture was incubated for 60 min and the absorbance was measured using a UV-Vis spectrophotometer (Genesys 20 Thermo Fisher Scientific) at 765 nm. The total phenolic content in the sample solution was calculated using the gallic acid standard curve (60 - 250 mg/L). Total phenolic content was calculated against the dried extract [12,13].

The total flavonoid content was measured as quercetin. The sample solution at 2,000 mg/L was taken 0.5 mL and added 0.2 mL of 10 % $AlCl_3$, 0.2 mL of 1 M sodium acetate and 6 mL of deionized water. The mixture was incubated for 30 min and the absorbance was measured using a UV-Vis spectrophotometer at 415 nm. The quercetin (20 - 100 mg/L) standard curve was utilized to calculate the total flavonoid content of the sample solution. The dried extract was used to calculate the total flavonoid content [12,14].

The total carotenoid content was determined using the Lichtenthaler [15] method and pure acetone as a solvent. To generate a 10 mL solution, precisely 10 mg of dried extract was dissolved in pure acetone. Using a UV-Vis spectrophotometer set to 661.6, 644.8 and 470 nm, the absorbance of the solution was measured [15,16]. Total carotenoid content in the sample solution was calculated using the formula:

$$C_a = 11.24(A_{661.6}) - 2.04(A_{644.8})$$

$$C_b = 20.13(A_{644.8}) - 4.19(A_{661.6})$$

$$Total\ carotenoid\ content = \frac{1000A_{470} - 1{,}90\ Ca - 63{,}14\ Cb}{214}$$

Antioxidant capacity assay

Antioxidant capacity was determined using the total antioxidant capacity (TAC), radical scavenging capacity (RSC), ferric reducing antioxidant power (FRAP) and cupric ion reducing antioxidant capacity (CUPRAC) methods TAC, RSC, FRAP and CUPRAC measured the capacity of redox reactions of the extract equivalent to ascorbic acid and Trolox. Each ascorbic acid and Trolox stock solution were prepared at a concentration of 200 mg/L in 96 % ethanol. The dried extract is dissolved in dimethyl sulfoxide to a final concentration of 2,000 mg/L to create the sample's stock solution. The stock solution is diluted in accordance with the test specifications.

TAC involves phosphomolybdate ion reduction in the presence of an antioxidant, resulting in a green phosphate/Mo(V) complex. TAC reagent was prepared by dissolving 3,864 mg sodium dihydrogen

phosphate, 4944 mg ammonium molybdate and 33.3 mL sulfuric acid (98 %) in deionized water to make a solution of 500 mL. The sample solution at 500 mg/L was taken at 0.5 and 5 mL of TAC reagent was added. After incubation for 90 min at 95 °C, the absorbance was measured using a UV-Vis spectrophotometer at 695 nm. The standard curve of ascorbic acid (50 - 250 mg/L) and Trolox (50 - 250 mg/L) was used to determine the antioxidant capacity in the sample solution. TAC was calculated against the dried extract [17,18].

RSC is based on antioxidants ability to reduce DPPH radicals. The reagent was 0.1 mM DPPH in methanol, freshly prepared. The sample solution at 500 mg/L was taken at 1 and 6 mL of DPPH reagent was added. After incubation for 60 min in a dark place, the absorbance was measured using a UV-Vis spectrophotometer at 515 nm. The standard curve of ascorbic acid (40 - 200 mg/L) and Trolox (8 - 40 mg/L) was used to determine the antioxidant capacity in the sample solution. The RSC of DPPH was calculated against the dried extract [17,18].

The FRAP assay is based on an antioxidant's ability to reduce Fe^{3+} to Fe^{2+} in an acidic condition. The FRAP reagent is composed of 3 solutions: 0.02 M Fe(III) solution (540 mg $FeCl_3.6H_2O$ were and 2 mL hydrochloric acid [1 M] were dissolved in deionized water to make solution 100 mL), 0.01 M TPTZ solution (312 mg 2,4,6-Tris[2-Pyridyl]-S-Triazine was dissolved in 96 % to make solution 100 mL), Acetic buffer pH 3.6 (3.1 g sodium acetate and 16 mL glacial acetic acid were dissolved in deionized water to make solution 1,000 mL. FRAP reagent was prepared freshly by mixing 0.02 M Fe(III) solution, 0.01 M TPTZ solution and acetic buffer in 1:1:10 ratio. The sample solution at 500 mg/L was taken at 2 and 6 mL of FRAP reagent was added. After incubation for 30 min, the absorbance was measured using a UV-Vis spectrophotometer at 595 nm. The standard curves of ascorbic acid (4 - 20 mg/L) and Trolox (4 - 12 mg/L) were used to determine the antioxidant capacity of the sample solution. FRAP was calculated against the dried extract [19,20].

The CUPRAC assay is based on antioxidant's ability to reduce Cu^{2}+ to Cu+ in complex with neocuproine. The CUPRAC reagent is composed of 3 solutions: 0.01 M Cu(II) solution (426 mg $CuCl_2.2H_2O$ was dissolved in water to make solution 250 mL), 7.5 mM neocuproine solution (390 mg neocuproine was dissolved in 70 % ethanol to make solution 250 mL), 1 M pH 7 ammonium acetate buffer solution. CUPRAC reagent was prepared freshly by mixing 0.01 M Cu (II) solution, 7.5 mM neocuproine solution and 1 M pH 7 ammonium acetate buffer solution in a 1:1:10 ratio. The sample solution at 500 mg/L was taken at 2 and 6 mL of CUPRAC reagent was added. After incubation for 30 min, the absorbance was measured using a UV-Vis spectrophotometer at 595 nm. The standard curve of ascorbic acid (12 - 40 mg/L) and Trolox (4 - 12 mg/L) was used to determine the antioxidant capacity of the sample solution. CUPRAC was calculated against the dried extract [19,20].

Results and discussion

Effect of extraction methods

The effects of various extraction techniques on the chemical component and possible antioxidant effects have been studied. Temperature and technique effects were taken into consideration when designing the extraction procedures. The other factors stayed constant, including the sample, solvent ratio, particle size and source of the plant. Reflux (80 °C), Soxhlet extraction (80 °C) and maceration at 3 different temperatures-cold (4 °C), room temperature (25 °C) and hot (60 °C) are the experimental techniques examined in this study.

The extraction yield indicated that the hot maceration had the maximum extraction efficiency (**Tabel 1**). Higher temperatures can increase extraction yield because solvent access to the pores of the plant materials has been enhanced, thereby increasing the mass transfer rate [21]. The solubility and diffusivities of the compounds likewise increase as the extraction temperature rises [22]. Maceration was preferred over Soxhlet and reflux because it was more economical, cost- and time-efficient and convenient [23]. High temperatures are used for Soxhlet and reflux applications, however the extraction yield is generally lower than for high-temperature maceration. The difference may be caused by the fact that the maceration process takes 24 h but the Soxhlet and reflux extraction processes take only 3 and 2 h, respectively. The drawbacks of both Soxhlet and reflux are that they both need specialized equipment, use a lot of solvent, take a long time to process and even require heating during reflux [24,25].

Table 1 Efficiency of extraction in different methods using 70 % ethanol.

Methods	Extraction yield (%)
Soxhlet (80 °C)	19.9 ± 1.2^{c}
Reflux (80 °C)	25.8 ± 1.7^{b}
Hot maceration (60 °C)	31.1 ± 1.8^{a}
Cold maceration (4 °C)	18.9 ± 1.3^{c}
Maceration (25 °C)	25.6 ± 1.9^{b}

[a, b, c] the mean difference is significant at the 0.05 level using Duncan

Steroids/terpenoids, glycosides, alkaloids, phenolics and saponins are present in all extracts (**Table 2**). In light of the dyes present in safflower petals, the resultant extract exhibits a reddish-orange hue. Carthamine, safflor yellow A, B, safflomin C and precarthamine are examples of these natural dyes, which are red and yellow C-glucosylquinochalcone groups (phenolic compounds) [26]. Safflower florets contain alkaloids, e.g., safflospermidine A and safflospermidine B and coumaroylspermidine derivative; steroids, e.g., sitosterol, stigmasterol and daucosterol; and quinochalcone glycosides [27-29].

Table 2 Phytochemical constituents of safflower extract in different methods.

Variants of methods	Phytochemical types						
	Alkaloid		Phenolics	Steroids/terpenoids		Saponin	Glycoside
	Dragendorf	Mayer		Lieberman Burchard	Salkowski		
Soxhlet	+	+	+	+	+	+	+
Reflux	+	+	+	+	+	+	+
Hot maceration (60 °C)	+	+	+	+	+	+	+
Cold maceration (4 °C)	+	+	+	+	+	+	+
Maceration	+	+	+	+	+	+	+

+ = presence, - = absence

When the carotenoids were extracted using a Soxhlet and the safflower florets were extracted using reflux, the maximum total amounts of phenolics and flavonoids were achieved (**Table 3**). Phenolics and flavonoids are the main ingredients in all methods; carotenoids constitute the supporting cast. Similar to the Soxhlet method for carotenoid extraction, the reflux approach demonstrated higher extraction efficiency for phenolic and flavonoid compounds when compared to other methods. Additionally, some researchers have discovered a number of samples that efficiently extract natural compounds using reflux techniques. According to the results published by Hong and Kim [30], reflux was demonstrated to be a more effective method than maceration for the extraction of phenolic compounds, flavonoids and rosmarinic acid. The study conducted by He *et al.* [31] found that the conventional reflux method showed similar effectiveness with the modern ultrasound-assisted extraction method according to extraction yields, total phenolic levels and total flavonoid levels of 4 Hosta species. In a different study, Kongkiatpaiboon and Gritsanapan [32] used reflux and sonication methods to successfully extract the alkaloid didehydrostemofoline from the root of Stemona collinsiae. These methods worked better than maceration, percolation and Soxhlet. Carotenoids have also been extracted from a variety of natural sources using Soxhlet. In a study conducted by Mezzomo *et al.* [33], the Soxhlet extraction method was employed to extract carotenoids from the leftovers of pink shrimp (*P. brasisiliensis and P. paulensis*). To produce extracts with a high concentration of carotenoids, particularly astaxanthin, hexane is the solvent utilized in the extraction procedure. In their study, Saldaña *et al.* [34] applied the Soxhlet with hexane to isolate carotenoids from freeze-dried carrots. Bashipour and Ghoreishi [35] conducted an extraction of β-carotene from freeze-dried aloe vera using Soxhlet with petroleum ether.

Table 3 Phytochemical content of Safflower extract in different methods.

Variants of methods	Total phenolics[a]	Total flavonoids[b]	Total carotenoids[c]
Soxhlet	146.4 ± 0.9[f]	43.5 ± 1.6[f]	30.8 ± 0[d]
Reflux	189.8 ± 3.6[d]	68.4 ± 2.9[d]	4.8 ± 1.1[g]
Hot maceration (60 °C)	172.0 ± 2.5[e]	45.3 ± 1.1[f]	25.2 ± 1.2[e]
Cold maceration (4 °C)	147.4 ± 3.4[f]	44.2 ± 0.5[f]	15.9 ± 0.1[f]
Maceration	169.1 ± 8[e]	54.5 ± 1[e]	6.1 ± 0[g]

[a] gallic acid equivalent (mg GAE/gram dry weight extract)
[b] quercetin equivalent (mg QE/gram dry weight extract)
[c] carotenoid equivalent (μg CE/gram dry weight extract)
[d, e, f, g] the mean difference is significant at the 0.05 level using Duncan

Antioxidant capacities were determined to be equivalent to those of ascorbic acid and Trolox using the DPPH, FRAP, CUPRAC and TAC (phosphomolybdenum assay) methods. Each antioxidant assay describes different biological activities. DPPH describes organic free radical scavenging [36]. FRAP, CUPRAC and TAC describe enzymatic reactions involving Fe^{3+}, Cu^{2+} and Mo^{6+} ions. Three ions act as cofactors that activate enzymes, e.g., hemoglobin, tyrosinase, nitrogenase, or oxidation-reduction enzymes [37].

Table 4 Antioxidant capacities of Safflower extract in different methods.

Variants of methods	TAC		RSC DPPH		FRAP		CUPRAC	
	A	B	A	B	A	B	A	B
Soxhlet	124.2 ± 0.9[c]	85.5 ± 0.3[c]	89.1 ± 0.2[d]	32.8 ± 0[e]	20.0 ± 0.2[d]	25.9 ± 0.2[d]	61.4 ± 1[e]	47,9 ± 1.5[d]
Reflux	173.1 ± 4.9[a]	103.6 ± 1.9[a]	117.4 ± 8.9[b]	39.9 ± 1.6[c]	21.9 ± 0.3[c]	28.9 ± 0.4[c]	86.6 ± 1.1[c]	66.3 ± 1.5[c]
Hot maceration (60 °C)	148.7 ± 12.1[b]	94.6 ± 4.6[b]	147.8 ± 1.2 [a]	51 ± 0.2[b]	23.3 ± 0.2[b]	30.7 ± 0.3[b]	102.3 ± 0.1[b]	77.7 ± 0.2[b]
Cold maceration (4 °C)	140.3 ± 8.5[b]	91.6 ± 3.2[b]	154.8 ± 5.1[a]	53.5 ± 0.9 [a]	25.8 ± 0.7[a]	33.8 ± 0.9[a]	110.9 ± 1.1[a]	84 ± 1.6[a]
Maceration	141.6 ± 4.9[b]	81.8 ± 1.9[c]	104.7 ± 7.1[c]	35.1 ± 1.3[d]	16.6 ± 0.6[e]	24 ± 0.7[e]	63.6 ± 1.1[d]	49.5 ± 1.7[d]

A = ascorbic acid equivalent (mg AAE/g dry weight extract). IC_{50} DPPH of ascorbic acid is 111.8 mg/L.
B = Trolox equivalent (mg TE/g dry weight extract). IC_{50} DPPH of Trolox is 18.9 mg/L.
[a, b, c, d, e] the mean difference is significant at the 0.05 level using Duncan

The antioxidant capability of the safflower extract is also impacted by variations in the extraction process. The highest antioxidant capacity was often found during low-temperature maceration, with the exception of TAC during reflux (**Table 4**). It is true that extracting at high temperatures can make phytochemicals more soluble and diffusible, which will shorten the extraction process and increase the amount of phytochemicals extracted. However, extracts may contain chemicals that are easily destroyed at high temperatures, such as those seen in hot maceration, reflux and Soxhlet [5]. As a result, their antioxidant capacity decreases. Certain substances exhibit a specific reactivity towards the reduction of Mo^{6+} ions (Keggin ions) and increase the antioxidant capacity as measured by TAC. Phosphomolybdenum can react with a wide range of compounds, from polar (phenolic, ascorbic acid, etc.) to nonpolar (tocopherol or carotenoids) [38].

Effect of solvent

Polarity is one of the most critical extraction parameters that influences extraction efficiency. The effective solvent must dissolve the compounds and extract them from the matrix. The most successful solvent for extracting the chemical compounds in safflower was 70 % ethanol, while nonpolar solvents were less effective (**Table 5**). The polarity of 70 % ethanol, a mixture of water and ethanol (3:7), is between water and ethanol. Water, the most polar solvent, can attract molecules that are approximately 70 % ethanol. According to the statistics, the bulk of the chemical compounds in safflower are polar or polar intermediates. 70 % ethanol has been shown to be effective in dissolving polar and polar intermediate

molecules containing COO or OH groups, such as organic acids, phenolics and flavonoids. According to Thouri *et al.* [39], this particular group has the ability to engage in interactions with ionics or hydrogen bonds, specifically with polar sites or ions originating from the solvent. Similar findings were reported by Junlatat and Sripanidkulchai [40], who discovered that macerating safflower florets with 74 % ethanol produced an extraction yield of 28.3 %.

Tabel 5 Efficiency of extraction in different solvents using maceration at room temperature.

Solvents	Extraction yield %
70 % ethanol	25.6 ± 1.9[a]
Water	23.7 ± 1.5[a]
Hexan	4.8 ± 0.5[c]
Ethyl acetate	4.1 ± 0.7[c]
Methanol	12.2 ± 0.9[b]

[a, b, c] the mean difference is significant at the 0.05 level using Duncan

In comparison to water, hexane and ethyl acetate, alcoholic solvents, such as 70 % ethanol and methanol, have better capabilities for extracting chemical compounds. Both 70 % ethanol and methanol have the ability to extract polar molecules like phenolics/tannins as well as non-polar chemicals like steroids and terpenoids (**Table 6**). Water dissolves polar molecules selectively, whereas hexane or ethyl acetate selectively solubilizes non-polar chemicals. Safflower florets contain a wide range of chemical components, including phenolics, flavonoids, alkaloids, steroids/terpenoids, saponins, glycosides and others [27-29].

Table 6 Phytochemical constituents of Safflower extract in different solvents.

Variants of solvents	Phytochemical types						
	Alkaloid		Tannins	Steroids/terpenoids		Saponin	Glycoside
	Dragendorf	Mayer		Lieberman Burchard	Salkowski		
70 % ethanol	+	+	+	+	+	+	+
Water	+	+	+	−	−	+	+
Hexan	+	+	+	+	+	−	−
Ethyl acetate	+	+	+	+	+	−	−
Methanol	+	+	+	+	+	+	+

+ = presence, − = absence

The largest amounts of phytochemical compounds are recovered from safflower florets using 70 % ethanol and methanol solvents. When safflower florets were extracted in methanol, the total phenolic and carotene concentrations were at their maximum. When 70 % ethanol was used to extract the flavonoids, the total flavonoid content was highest. Phenolics and flavonoids are the primary ingredients detected in all safflower extracts, with carotenoids present in trace amounts. Carthamin, a flavonoid glycoside derivate, has been examined as the main component in safflower flowers [4]. Similar results were also reported by Ebadia *et al.* [41] that was the highest total phenolic content found in the methanol extract (245 mg GAE/g dry weight extract), followed by the acetonic extract (202 mg GAE/g dry weight extract) and the ethanolic extract (141 mg GAE/g dry weight extract). In another study, Salem *et al.* [42] found that extraction using 80 % acetone (15.09 mg GAE/g dry weight extract and 15.09 mg QE/g dry weight extract) and 80 % methanol (14.94 mg GAE/g dry weight extract and 14.94 mg QE/g dry weight extract) produced the highest concentration of phenolic and flavonoid compounds. Safflower florets have been shown to contain carotenoids, such as xanthophyll and carotene. In a study conducted by Salem *et al*. [43] that was reported an increase in carotenoid levels of 35 %, in safflower with 50 % water deficit treatment (594 µg xanthopylls equivalent and 79 µg carotene equivalent in 100 g dry weight flower var Jawhara). Meanwhile, in research by Salem *et al.* [44] reported an increase in carotenoid levels of 19 % in safflower with 10 g/L NaCl treatment (over 60 mg/100 g dry flowers var Jawhara). The optimal extraction of flavonoid glycosides and polar aglycones is achieved by using a mixed solvent with water, in this case 70 % ethanol [45]. The prevalent solvent for extracting phenolic and flavonoid compounds is hydroalcoholic [45,42].

Table 7 Phytochemical content of Safflower extract in different solvents.

Variants of solvents	Total phenolics[a]	Total flavonoids[b]	Total carotenoids[c]
Ethanol 70 %	169.1 ± 8[d]	54.5 ± 1[d]	6.1 ± 0[f]
Water	129.4 ± 10.1[c]	41.5 ± 1.8[c]	0.1 ± 0[h]
Hexan	54.8 ± 10.7[e]	7.7 ± 0.1[f]	4 ± 0.3[g]
Ethyl acetate	74.6 ± 3.3[e]	13.3 ± 0.6[e]	23.2 ± 0.1[e]
Methanol	180.7 ± 24.2[d]	52.6 ± 1.1[d]	35.8 ± 1.2[d]

[a] gallic acid equivalent (mg GAE/gram dry weight extract)
[b] quercetin equivalent (mg QE/gram dry weight extract)
[c] carotenoid equivalent (µg CE/gram dry weight extract)
[c, d, e, f, g] the mean difference is significant at the 0.05 level using Duncan

The antioxidant capacity may be increased by using the appropriate solvent. Methanol and ethanol extracts have a substantial antioxidant capacity of 70 %, as evaluated by RSC DPPH, FRAP and CUPRAC. TAC results show that the hexane and ethylacetate extracts have a notable antioxidant ability (**Table 8**). This observation implies that the extract's strong antioxidant capacity is mostly due to the significant contribution of polar to non-polar components. Numerous investigations have also looked into the safflower florets' and blossoms' potential as. In a study conducted by Karimkhani *et al.* [45], the antioxidant activity of 4 safflower cultivars was examined. The researchers stated that the IC50 of the flower methanol extract ranged from 233 to 299 mg/L. According to a study conducted by Salem *et al.* [26], the flowers at the fructification stage exhibited the highest antioxidant capacity as measured by FRAP and DPPH. During this particular phase, the carthamine isolate exhibited the highest content, measuring 1641 µg/g of dry weight. Additionally, the FRAP antioxidant capacity was determined to be 589 trolox equivalent mM/L, while the IC50 DPPH value was determined to be 0.86 mg/L. In their study, Erbaş and Mutlucan [46] reported the CUPRAC antioxidant capacity and DPPH scavenging effect (%) of various safflower genotypes. Notably, the safflower genotype US10 exhibited the highest antioxidant capacity, CUPRAC (95.1 TE mg/g dry flower) and DPPH scavenging effect (14 %). Erbaş and Mutlucan [46] further elucidated that a positive correlation exists between elevated antioxidant activity and increased concentrations of total phenolics. According to Soumia *et al.* [47], a 70 % ethanol extract of flowers had a TAC antioxidant capacity of 25.69 AAE mg/g dry weight. The present study's conclusions differ from those of earlier investigations due to variations in sample preparation, extraction methods and sample origins; as a result, the chemical composition is different. Phytochemicals high in antioxidants, such as flavonoids and phenolics, easily dissolve in polar or intermediate-polar solvents like hydroethanolic or methanolic. Safflower contains more than 200 distinct phytochemicals, such as coumarins, fatty acids, polysaccharides, phenylethanoid glycosides, flavonoids and steroids. Precarthamin, ch, safflomin A, hydroxysafflor yellow A, safflor yellow A, safflor yellow B and anhydrosafflor yellow B are the principal phenolic chemicals present in safflower flowers. These compounds are classified as quinochalcone derivatives with the typical structure of C-glycosylated cyclohexanonedienol. In addition, the flavonoid compounds existing include kaempferol, quercetin, apigenin, scutellarein and acacetin. These flavonoids are mainly bound to saccharides to form flavonol glycosides [3]. The safflower also contains various carotenoid compounds, including lutein, zeaxanthin, lycopene, neoxanthin, violaxanthin and β-carotene [43].

Table 8 Antioxidant capacities of Safflower extract in different solvents.

Variants of solvent	TAC		RSC DPPH		FRAP		CUPRAC	
	A	B	A	B	A	B	A	B
Ethanol 70 %	141.6 ± 4.9[d]	81.8 ± 1.9[d]	104.7 ± 7.1[a]	35.1 ± 1.3[a]	16.6 ± 0.6[b]	24 ± 0.7[b]	63.6 ± 1.1[a]	49.5 ± 1.7[a]
Water	87.6 ± 9.5[e]	72.9 ± 2.4[e]	29.7 ± 1[b]	7.8 ± 0.2[b]	6.7 ± 0.2[c]	9.6 ± 0.2[c]	42.4 ± 0.9[b]	34 ± 1.4[b]
Hexan	181.7 ± 7.1 [a]	137.7 ± 3[a]	22.7 ± 2.7[b,c]	1 ± 0.5[d]	6.4 ± 0.2[c]	1.6 ± 0.3[e]	24.9 ± 1.8[c]	22.8 ± 2.8[c]
Ethyl acetate	167.3 ± 5.5[b]	101.4 ± 2.1[b]	15.6 ± 3[c]	2.6 ± 0.5[c]	2.6 ± 0[d]	4.4 ± 0[d]	12.4 ± 1.2[d]	12.1 ± 1.8[d]
Methanol	154.7 ± 4.9[c]	97.1 ± 1.9[c]	106.2 ± 5.5[a]	35.1 ± 0.6[a]	20.3 ± 0.1[a]	25.9 ± 0.1[a]	62.1 ± 0.5[a]	48.4 ± 0.7[a]

A = ascorbic acid equivalent (mg AAE/g dry weight extract). IC_{50} DPPH of ascorbic acid is 111.8 mg/L.
B = Trolox equivalent (mg TE/g dry weight extract). IC_{50} DPPH of Trolox is 18.9 mg/L.
[a, b, c, d, e] the mean difference is significant at the 0.05 level using Duncan

Based on this research, the extraction method was efficient at high temperatures but could not maintain its antioxidant capacity. In comparison, the solvent that is dominant in extracting the antioxidant substances is methanol or 70 % ethanol.

Conclusions

The phytochemical content and antioxidant capacity of safflower floret extract are affected by the solvent and extraction process. Polar solvents have the potential to extract more phytochemicals and antioxidants. The hot approach extracts more phytochemical content than the cold method, although it has the opposite antioxidant capacity. Cold maceration with methanol and 70 % ethanol as solvents was discovered to be a potentially useful method for extracting phytochemical components and antioxidant chemicals.

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