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Antioxidant from marigold (*Tagetes erecta* L.) by ultrasonic assisted extraction and maceration in ethanol for preparation of essence product formulation

Pranudda Pimsee[1]* Nattharat Mahaisub[1], Nissara Phasee[1], Wannisa Keawbankrud[1], Patsorn Intasan[1]
Health Science and Aesthetic Program, Department of Science, Faculty of Science and Technology, Rajamangala University of Technology Krungthep, 2 Nang Linchi Road, Sathorn, Bangkok 10120, Thailand.
**E-mail: pranudda.p@mail.rmutk.ac.th*

Abstract:

This research aimed to study the optimum conditions for extracting active substances from marigolds (*Tagetes erecta* L.) by comparing two extraction methods, ultrasonic-assisted extraction, and maceration. These extractions were performed in 60% ethanol using marigold flower weight to solvent volume ratios of 1:15 and 2:15. It was found that the extraction by ultrasonic technique at a ratio of 2:15 was the ideal condition. The results showed that the total flavonoid and total phenolic contents were 505.94 ± 1.77 mg QE/g and 77.17 ± 1.10 mg GAE/g extract, respectively. The DPPH free-radical scavenging activity of this extract showed an IC_{50} value of 39.23 ± 2.40. The preparation of base essence products and the formulations containing 1.00%, 1.25% and 1.50% of marigold extracts showed that all the formulations had a pH in the range of 5.19 - 5.93 and had good stability

1. Introduction

A free radical is a substance occurs in human metabolism system, such as; respiratory system in cell level to provide energy to mitochondria, the injuries, or infection in body.[1] Moreover, there are some external factors as pollution, heavy metals, industrial solvents, pesticides, cigarette smoke, radiations, certain drugs or ultra violet light.[2] The free radicals are some atoms or molecules containing an unpaired electron in a valence shell or outer orbit.[3] The free radicals include superoxide anion, hydrogen peroxide, hydroxyl radical, organic hydroperoxide, etc.[4] If the body could not eliminate these free radicals, there will be the oxidative stress which leads to diseases as cancer, Alzheimer, allergies, diseases with eyesight and nerve system, wrinkles, or aging.[5]

Antioxidant is the substance to resist the oxidation reaction and decrease the free radical. There is the enzymatic antioxidant such as peroxidase, catalase and glutathione reductase. The non-enzymatic antioxidants include polyphenol, vitamin A, C, E and glutathione.[6] The antioxidants can be found in vegetable, fruit, juice and herb. There are several types of antioxidants mechanism such as free radicals scavenging, enzyme inhibition, metal chelation or singlet oxygen quenching.

Marigold flower (*Tagetes erecta* L.) is a biennial plant, age about 1 year, the compound leaves are in slender spear-shape. The marigold is a solitary flower. The color is bright yellow or orange-yellow. The petals are large and stacked together in several layers to be in circle shape. It originates in Mexico and countries in Latin America. There are many species. It is plantable in Europe, America, Asia including Thailand. The previous study found out that the substance extracted from the marigolds is able to prevent Gram's positive bacteria like *S. aureus* and *S. epidermidis*. The substance extracted from marigold flower has a capacity of antioxidant as it contains the phenolic and flavonoid compounds. Moreover, the marigold flowers substance extracted by using methanol can protect skin from getting aged by reduce the injury from the oxidation, deter the present of Matrix Metalloproteinase-2 and urge the collagen synthesis.[7]

There are many methods to extract the substance from herb which are maceration, infusion, decoction, percolation, digestion and soxhlet extraction, superficial extraction, ultrasound-assisted, and microwave-assisted extraction[8]. Maceration was a popular and inexpensive homemade technique for the preparation of tonic since a long time. Moreover, this technique was used for the extraction of essential oils and active compounds from plant materials. Ultrasonic assisted extraction, also called sound waves-assisted liquid extraction, is considered as one of the most efficient extract recovery techniques. In this method, acoustic vibrations are applied to the sample with frequencies above 20 kHz.[9] The advantage of extraction by this mean is that it requires less time of extraction than the maceration.

This study aims to extract the substantial substances from the marigold flower, to analyze the amount of phenolic and flavonoid by applying

the ethanol solvent by means of maceration and ultrasonic-assisted extraction, to analyze the antioxidant activity of the extracted substances, and to prepare the essence products which contain the marigold flowers extracted substances.

2. Materials and Methods

2.1 Plant materials

Marigold (*Tagetes erecta* L.) in full bloom, about 60 - 65 days old, from a garden in Surin province, Thailand. Washed with clean water 3 times. Let the marigold flowers drain. Selected only the flower petals and exposed to the sun for 5 days until the petals were completely dried. Pounded with a stone mortar until fine powder was obtained. Sift with a No. 10 sieve and store in a dry, clean, sealed glass bottle until further experiments.

2.2 Reagents

DPPH(2,2-diphenyl-1-picrylhydrazyl), gallic acid, ascorbic acid, quercetin, EDTA (ethylene diamine tetra – acetic acid), Folin-Ciocalteu reagent, sodium carbonate were purchased from Sigma – Aldrich. Sodium hydroxide and aluminium chloride were purchased from Kemaus. Dehydroxanthan gum, polyquaternium-51, panthenol, propylene glycol, polysorbate 20 and Galactomyces ferment filtrate were supplied from Myskinrecipes. All reagents were prepared using ultrapure water in all experiments.

2.3 Preparation of crude extracts

Marigold powder was extracted by maceration technique by weighing 10 and 20 g of ground marigold flower powder and soaking in 150 ml of 60 % ethanol, in a 250 mL conical flask. The ratio of marigold powder per solvent was obtained. Dissolved at 1:15 and 2:15 for 7 days at room temperature. The obtained marigold extract was filtered with filter paper No. 1 (Whatman No.1). Then the solvent was evaporated with a vacuum evaporator (Rotary evaporator, Buchi brand, model R-100), weighed the crude extract with a balance (Sartorius BSA224S-CW) and kept the marigold extract in the refrigerator at 4 °C in the dark.

For ultrasonic-assisted extraction, the ratio of marigold flowers to solvent was used as in maceration technique. The extraction time was 30 minutes with an ultrasonic machine (ULTRAsonic, model 104 H), filtered the extract with No. 1 filter paper, evaporated the solvent with a vacuum evaporator, weighed the extract, and stored in the refrigerator at 4 °C.

2.4 Determination of total flavonoid content

Determination of flavonoid content was modified from the method of Ramamoorthy et al.[10], 2 mL of marigold extract (1 g/L) were mixed with 1 mL of 2 % $AlCl_3$ solution and adjusting the volume with 1 mL of distilled water. Incubate the mixture at room temperature for 10 min. Absorption spectrophotometer (Jasco V-730 spectrophotometer) was used at 415 nm. Three replicates of the experiment were performed to determine the total flavonoid content based on quercetin standard curve in quercetin equivalent per gram of the extract (mg QE/g) unit.

2.5 Determination of total phenolic content

The total phenolic content was determined with Folin-Ciocalteu method according to the method of Zhou et al.[11] with some modifications. 0.1 mL of marigold flower extract was added to 1.15 mL of water, then added 0.25 mL of Folins-Ciocalteu reagent and 1.5 mL of 7.5% sodium carbonate. The substance was incubated at room temperature for 30 minutes. The absorbance was measured at a wavelength of 765 nm. (Jasco V-730 spectrophotometer). The experiment was repeated 3 times. The total phenolic content was calculated from the standard curve of gallic acid equivalent (GAE) as mg GAE/g extract.

2.6 Determination of antioxidant activity

Antioxidant activity of the extract was determined by using the scavenging of the stable 2,2-diphenyl-1-picryhydrazyl (DPPH) free radical according to the method of Mensor et al.[12] with some modifications. 1 mL of the sample was added in the test tube with 2 mL of DPPH in 0.2 mM of ethanol. The incubation was performed at room temperature for 30 min. The absorbance was measured at 517 nm by spectrophotometer Jasco V-730. The assay was repeated 3 times to determine the DPPH as a negative control by using ascorbic acid as the standard. Scavenging activity was calculated from the following equation.

$$\% \text{ scavenging activity} = [(A_c - A_s)/A_c] \times 100$$

When A_c is the absorbance of the control and A_s is the absorbance of the sample. Then, % scavenging concentration (IC_{50}) of the sample was determined, demonstrating the concentration that can trap 50% of free radical.

Table 1. The composition and function of each substance of essence product

Ingredients	Functions	Base formula (%w/w)	Formula 1 (%w/w)	Formula 2 (%w/w)	Formula 3 (%w/w)
Dehydroxanthan gum	Thickener	0.30	0.30	0.30	0.30
Triethanolamine	Adjust pH	0.20	0.20	0.20	0.20
Polysorbate 20	Stabilizer	0.50	0.50	0.50	0.50
Propylene glycol	Humectant	2.00	2.00	2.00	2.00
Glycerin	Humectant	3.00	3.00	3.00	3.00
Galactomyces ferment filtrate	Humectant	2.00	2.00	2.00	2.00
Panthenol	Humectant	0.10	0.10	0.10	0.10
Polyquaternium-51	Humectant	0.20	0.20	0.20	0.20
Ethylene diamine tetra-acetic acid	Preservative	0.10	0.10	0.10	0.10
Dimethylol dimethyl hydantoin	Preservative	0.50	0.50	0.50	0.50
DI water	Solvent	91.10	90.10	89.85	89.60
Marigold extract	Antioxidant	-	1.00	1.25	1.50

2.7 Preparation of essence product

The composition and function of each substance of essence produce formulation are shown in Table 1. The base formulation was prepared by adding dehydroxanthan gum to the beaker which contained DI water as the specific amount. The dehydroxanthan gum was dissolved and heated until homogeneous, then added triethanolamine, glycerin, propylene glycol, galactomyces ferment filtrate, polysorbate 20, panthenol, polyquaternium-51, ethylene diamine tetra-acetic acid and dimethylol dimethyl hydantoin, respectively, until a homogeneous product was obtained. The products according to formula 1, 2 and 3 were added marigold flower extract at 1.00%, 1.25% and 1.50%, respectively.

Product Stability was determined by observing the cream texture, color, smell, separation and pH. The pH of finished product, after keeping at room temperature for 14 days, was measured by using the pH meter (SI Analytics, Lab885).

3. Results & Discussion

3.1 Crude extracts

The extraction of the substantial substances from marigold flowers by maceration and ultrasonic-assisted extraction with the ratio of marigold powder to solvent (60% ethanol) as 1:15 and 2: 15 w/v provided %yield of the extraction to marigold powder weight (w/w) as shown in Table 2. It was found that the extraction by using ultrasonic-assisted extraction with the marigold powder weight to solvent ratio of 2:15 w/v shown the highest %yield (4.14 ± 0.34 %) followed by the maceration at the ratio of 2 : 15 (3.12 ± 0.28 %) which was close to the ratio of 1 : 15 by ultrasonic-assisted extraction technique (3.09 ± 0.25 %) and the lowest %yield was obtained by the maceration at the ratio of 1:15 (2.57 ± 0.17 %).

Table 2. The percentage (%) yield of crude extracts obtained from different methods.

Method	Ratios (w/v)	Percent yield
Maceration	1 : 15	2.57 ± 0.17
	2 : 15	3.12 ± 0.28
Ultrasonic extraction	1 : 15	3.09 ± 0.25
	2 : 15	4.14 ± 0.34

3.2 Total flavonoid and phenolic contents

Table 3 illustrated the total flavonoid contents that were analyzed by the reaction with $AlCl_3$ and phenolic contents of marigold flower extract. The results showed that the extraction by using ultrasonic-assisted extraction method with 60 % ethanol at the ratio of 2:15 w/v provided the highest flavonoid content at 505.94 ± 1.77 mgQE/g and at the ratio of 1:15 w/v obtained the flavonoid content as 396.95 ± 2.59 mgQE/g. The maceration gave 339.52 ± 1.03 and 364.53 ± 2.56 mgQE/g for 2:15 and 1:15 ratios, respectively.

Moreover, the total phenolic contents showed the same trend with the total flavonoid content. Ultrasonic-assisted extraction ratios of 2:15 and 1:15 presented the total phenolic contents of 77.17 ± 1.10 and 34.11 ± 0.74 mgGAE/g, respectively. The maceration extraction showed the lower total phenolic content than the ultrasonic-assisted extraction. The phenolic contents were obtained

by maceration with the ratio of a 1:15 and 2:15 were 19.93 ± 3.10 mgGAE/g and 28.95 ± 2.16 mgGAE/g, respectively.

Table 3. Total flavonoid and phenolic contents of the extracts.

Method	Ratios (w/v)	Total flavonoids (mgQE/g extract)	Total phenolic (mgGAE/g extract)
Maceration	1 : 15	364.53 ± 2.56	19.93 ± 3.10
	2 : 15	339.52 ± 1.03	28.95 ± 2.16
Ultrasonic extraction	1 : 15	396.95 ± 2.59	34.11 ± 0.74
	2 : 15	505.94 ± 1.77	77.17 ± 1.10

3.2 Antioxidant activity

Antioxidant activity of marigold flower extract was analyzed by DPPH method. The DPPH is stable radical in alcohol solvent. Light can be absorbed at wavelengths of 515-517 nm. After DPPH solution receives electrons from antioxidants, the solution will be converted from purple to yellow. The % scavenging activity was calculated by using ascorbic acid as the standard solution. The %scavenging concentrations (IC_{50}) of the extract were shown in Table 4.

The results showed that the extraction by using ultrasonic-assisted extraction with 60% ethanol at the marigold powder to solvent ratio of 2 : 15 w/v had the highest antioxidant activity with an IC_{50} of 39.23 ± 2.40 mg/L, at the ratio of 1: 15 w/v for the same method was 44.31 ± 3.51 mg/L, while the maceration provided 41.67 ± 2.46 and 45.84 ± 3.12 mg/L for the ratio 2: 15 and 1: 15, respectively. The free radicals of the extract were also lower than the antioxidant activity of ascorbic acid that was used as a standard solution.

Table 4. DPPH radical scavenging ability of the extracts and ascorbic acid.

Method	Ratios (w/v)	IC_{50} (mg/L)
Maceration	1 : 15	45.84 ± 3.12
	2 : 15	41.67 ± 2.46
Ultrasonic extraction	1 : 15	44.31 ± 3.51
	2 : 15	39.23 ± 2.40
Ascorbic acid	-	36.12 ± 0.65

3.3 Physical and chemical properties of the essence product

Table 5 showed the physical characteristics including texture, color, separation and pH of the essence products with 3 difference formulas compared with the original formula were observed at the end of preparation and after 14 days at room temperature.

From the experiment result, after 14 days, the base formula had a smooth, clear, colorless texture, without segregation of product layers and had a pH of 5.19. Formula 1 and 2, which contained 1.00% and 1.25% of marigold flower extract showed dark yellow and pH of 5.45 and 5.67, respectively. The third product formula containing 1.50% extract was smooth, darker yellow than other formulas and pH of 5.93. There was non-separable of layers of all three product formulas indicates that the products are stable.

Table 5. Physicals characteristics and pH of essence product

Formula	Color	Separation	pH
Base formula	clear, colorless	non-separable	5.19
Formula 1	bright yellow	non-separable	5.45
Formula 2	bright yellow	non-separable	5.67
Formula 3	dark yellow	non-separable	5.93

4. Conclusion

This study showed the result of the extraction method for extracting substantial substances of flavonoid and phenolic compounds from marigold flowers. The maceration and ultrasonic-assisted
extraction methods were used with various ratio of marigold powder weight to solvent (60 % ethanol). The result showed that the optimum ratio for two those extraction methods are 1:15 and 2:15. Moreover, the extracts from marigold flowers have antioxidant effects. It can be applied as an important substance for making skin care, cosmetic products or other health products. The obtained essence product had a smooth texture, light yellow, good stability and pH in the range of 5.19 – 5.93, which was the suitable pH for human use. In addition, marigold extract can be applied in other cosmetic products as well.

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