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Research article

Tablet formulation for ophthalmic disease prevention using a combination of lutein and naringin extracted from the flower of *Tagetes erecta* L. and fruit membrane of *Citrus maxima* (Burm.f.) Merr. extract

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ABSTRACT

This study aimed to develop a dietary supplement tablet for the prevention of ophthalmic diseases using extracts from *Tagetes erecta* L. flowers and *Citrus maxima* (Burm.f.) Merr. fruit membranes. To enhance the extraction of lutein from *T. erecta* flowers and total phenolic compounds and naringin from *C. maxima* fruit membranes, a novel technique, enriched fraction column chromatography, was employed. The optimized tablet formulation comprised 350 mg of *T. erecta* flower extract, 300 mg of *C. maxima* fruit membrane extract, 200 mg of corn starch, 1 % aerosil, and 1.2 % magnesium stearate. Each tablet contains 7.7 mg of lutein and 20.8 mg of total phenolic compounds, meeting daily dietary requirements. The developed tablet's physical properties, both before and after stability testing, adhered to USP standards. This supplement holds promise for preventing ophthalmic diseases and other health conditions associated with lutein and phenolic compounds, such as cardiovascular diseases and cancer.

1. Introduction

The World Health Organization reported that in 2019, at least 2.2 billion people had vision impairment globally. Of these, at least 1 billion people had vision impairment that could have been prevented [1]. Cataracts and age-related macular degeneration (AMD) are the most common causes of blindness in people aged >40 years in developing and developed countries, respectively [2]. With the rise of ageing societies, the increasing prevalence of age-related diseases presents significant challenges to healthcare systems worldwide.

Lutein, a carotenoid, is an effective functional compound with numerous biological properties that benefit human health [3]. It is highly concentrated in the macula, located in the central retina, and it is responsible for visual acuity and central vision because of the high concentration of photoreceptor cells [4]. It is also found in the human eye, protecting it against age-related eye diseases such as cataracts. Lutein has anti-inflammatory, antioxidant and blue light filtering properties. There is also evidence demonstrating other functions of lutein, including its ability to improve visual acuity and contrast sensitivity, as well as its neuroprotective effect in reducing cell loss and apoptosis after retinal ischaemia injury [4]. A meta-analysis in 2012 reported that increased intake of these carotenoids may offer protection against late AMD, with a statistically significant inverse association observed between lutein intake

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and neovascular AMD risk [5]. In addition, a 2015 systematic review that evaluated the relation between blood lutein concentrations and the risk of age-related cataracts reported that high concentrations were significantly associated with a decreased risk of nuclear cataracts [6]. Further research suggests that lutein is stored in fatty tissue, which may result in lower retinal lutein levels in individuals with obesity, potentially increasing their risk for certain eye diseases [4]. Since lutein can only be obtained through diet, supplementation is beneficial for maintaining adequate levels. A typical US diet contains 1–3 mg/day of lutein and zeaxanthin, whereas ~6 mg/day has been associated with a decreased risk of AMD. It has been suggested that plasma lutein concentration of 0.6–1.05 mmol/L is safe for humans while providing the expected beneficial effects [7,8]. Therefore, lutein supplementation is essential for supporting eye health and preventing ophthalmic diseases.

Phenolics represent the largest group of phytochemicals, responsible for a significant portion of antioxidant activity, with flavonoids being the most abundant type of naturally occurring phenolic compounds. Numerous studies have highlighted the activities of phenolic compounds and flavonoids in preventing eye disease through multiple mechanisms, including oxidative stress reduction and anti-inflammatory activity. Additional studies have shown that flavonoids protect various ocular cell types against oxidative stress induced cell death, improve endogenous antioxidant systems, inhibit inflammatory pathways, and enhance mitochondrial function [9,10]. There is also strong evidence that flavonoids positively affect vascular health by improving endothelial function [10]. Notably, oxidative stress and choroidal vascular dysfunction are the most crucial triggers of the pathogenesis of AMD. Oxidative stress is critical to ageing diseases, including AMD, mainly because of the much higher oxygen consumption by the retina than by any other tissue. Thus, the role of phenolic compounds, especially flavonoids, is promising for reversing oxidative stress and inflammation-associated damage and improving vascular function, potentially improving the clinical features of AMD [11]. This systematic review and meta-analysis demonstrated that flavonoids have a favourable effect on conditions associated with visual impairment. The relatively low cost, lack of serious side effects, and easy accessibility make flavonoid supplementation appealing [12].

The pomelo fruit membrane is an economical source of phenolic compounds, offering a sustainable use for Thailand's significant agricultural waste produced annually. The aqueous ethanolic extract of the pomelo fruit membrane is rich in flavonoids, vitamin C and carotenoids. The most abundant flavonoids in pomelo fruit are naringin, naringenin, neoeriocitrin and neohesperidine, naringin is the main constituent [13,14]. Various therapeutic effects of naringenin (aglycone portion of naringin) such as cardioprotective, cholesterol-lowering, anti-Alzheimer's, nephroprotective, anti-aging, antihyperglycemic, anti-osteoporotic and gastroprotective, anti-inflammatory, antioxidant, antiapoptotic, anti-carcinogenic, antiosteoporotic and anti-ulcer activities have been studied [13,14]. The total phenolic content in the pomelo fruit membrane ranges from 42.79 to 54.56 mg gallic acid equivalent/g and the total flavonoid content is within the range of 13.43–26.70 mg of rutin/g [15].

Numerous products on the market claim to prevent eye disease with lutein, but none include both lutein and phenolic compounds. We aimed to develop a unique eye supplement that combines both lutein and phenolics, particularly flavonoids, to offer enhanced, synergistic health benefits. Marigold flowers were selected as a rich source of lutein, while pomelo fruit membranes, which is an agricultural byproduct were used for their high phenolic and flavonoid content. This study also examined the extraction methods for

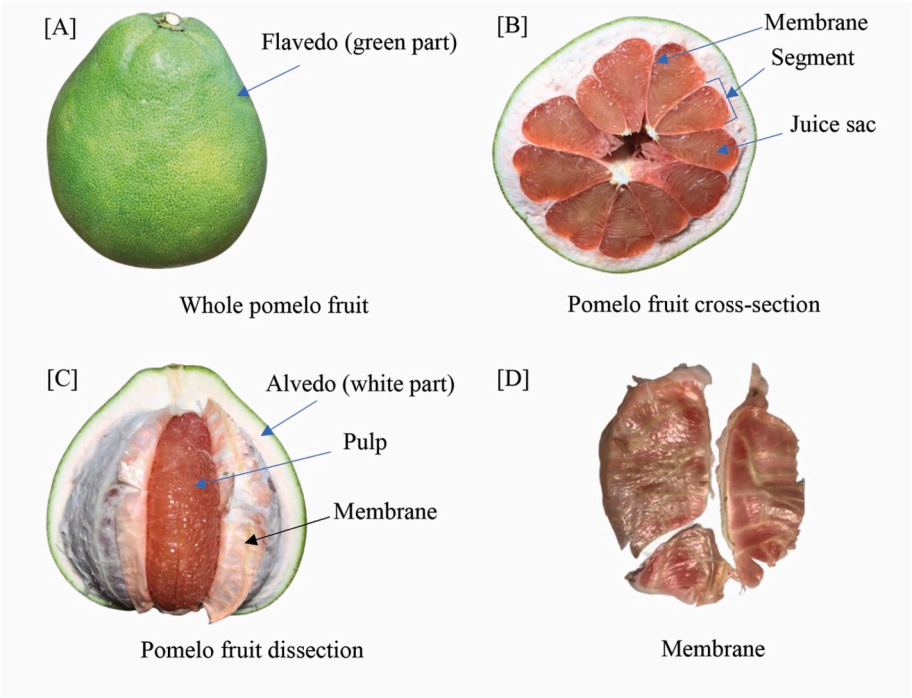

Fig. 1. Pomelo fruit: whole fruit [A], pomelo fruit cross-section [B], pomelo fruit dissection [C] and membrane [D].

marigold flowers and pomelo membranes, as well as the stability and quality control of the formulated tablet.

2. Materials and methods

2.1. Materials

2.1.1. Chemicals

Reference standards, lutein was purchased from Chengdu Biopurify (China), naringin from Sigma-Aldrich (St. Louis, MO, USA) and gallic acid from Carlo Erba (Italy). All other chemicals were of analytical grade, whereas high performance liquid chromatography (HPLC) grade solvents were used for HPLC analysis. Acetonitrile, acetone, ethanol, ethyl acetate, hexane, methanol, toluene, sodium sulfate, and sodium carbonate were purchased from Carlo Erba (Italy). Hydrochloric acid and potassium hydroxide were obtained from Ajax (New Zealand). Trifluoroacetic acid, silica gel and a TLC plate were purchased from Merk (Darmstadt, Germany). The Folin-Ciocaltue reagent was obtained from Loba Chemie (India).

2.1.2. Plant materials

The petals of the marigold flower (*Tagetes erecta* L.) were collected from Roi Et, northeast Thailand. The pomelo fruit membranes (*Citrus maxima* (Burm.f.) Merr.) (Fig. 1) were collected from Samut Songkhram, Central Thailand. Both authentic plants were identified by Wanida Caichompoo, a botanist at the Faculty of Pharmacy, Mahasarakham University. The voucher specimens of *T. erecta* (MSU. PH-AST-T1) and *C. maxima* (MSU.PH-RUT-C1) are deposited in the Faculty of Pharmacy, Mahasarakham University.

2.2. Methods

2.2.1. Marigold flower extraction

Marigold flowers were washed, dried in a hot air oven at 45 °C and ground to produce fine powder. The marigold powder was then macerated using four solvents (ethanol, acetone, ethyl acetate, and hexane) in a ratio of 100 g powder to 500 mL solvent for 7 days, with solvent replacement every 2 days. After combining the extracts, the mixture was evaporated to dryness under reduced pressure, and the yield of each extract was measured. The highest yield extract was further isolated by classical chromatography using silica gel as a stationary phase (250 g: 25 g of extract), gradient eluted with 2500 mL of hexane, hexane: ethyl acetate (10:1), and ethyl acetate at a flow rate of 4 ml/min. Fractions of 500 mL were collected and analysed by TLC with a mobile phase of hexane/ethyl acetate/methanol (10:3:1), detecting compounds at 446 nm using UV–visible spectrophotometry. Fractions with similar TLC patterns were combined and evaporated to obtain a concentrated extract. To achieve a dry powder form, various adsorbents such as corn starch, talcum, and aerosil were evaluated in different extract-to-adsorbent ratios, selecting the formulation with the least adsorbent content. The resulting powder was milled to an 80-mesh size to prepare it for tablet compression.

2.2.2. Pomelo fruit membrane extraction

The pomelo fruit comprises four main parts: flavedo, albedo, endocarp, and membrane (Fig. 1). The flavedo is the outer peel (green layer), the albedo is the white layer between the peel and the pulp, the endocarp is the edible fruit and the membrane separates the fruit segments. This study utilizes the membrane, significant waste material collected annually from pomelo gardens. The membrane was dried in a hot air oven at 50 °C, then ground to a fine powder using an 80-mesh sieve. Membrane powder (100 g) was macerated in 300 mL of 95 % and 50 % aqueous ethanol for 7 days. Each extract was dried under reduced pressure to obtain a semi-solid extract. Various adsorbents, including corn starch, talcum, and aerosil, were tested to facilitate conversion from semi-solid to dry powder. The dry powder with the least amount of adsorbent required was chosen for formulation. Finally, the powder was milled to an 80-mesh particle size before tablet compression.

2.2.3. Determination of lutein in marigold flower extract

Sample preparation followed the method of Punphaew [16]. A mixture containing 100 mg of the marigold extract and a solvent mixture of hexane/acetone/ethanol/toluene (10: 7: 6: 7) was added into a 100 mL volumetric flask and gently shaken for 1 min. Then 2 mL of 40 % potassium hydroxide in methanol was added, and the flask was placed in a water bath at 56 °C for 20 min. The flask was cooled at room temperature in the dark for 1 h, followed by the addition of 30 mL of hexane. The final volume was adjusted to 100 mL using 10 % sodium sulfate, and the flask was left in the dark for another hour. Lutein content in the marigold extract was determined using high-performance thin-layer chromatography (HPTLC). HPTLC analysis was performed with a CAMAG HPTLC system (CAMAG, Switzerland) integrated with an automatic sample applicator (CAMAG, Linomat 5) and a TLC scanner (CAMAG, TLC scanner 4) using CATS software. Samples were applied on a 0.2 mm-thick plate pre-coated with silica gel, which was first activated by washing with methanol and drying at 110 °C for 10 min. Both samples and reference standards were then applied with a 20-μL syringe via the automatic applicator, maintaining a gap of 10 mm between the two bands under N_2 gas. The plate was developed in a twin trough chamber containing a mobile phase of hexane/ethyl acetate/methanol (10:3:1). Once the mobile phase reached a distance of 9 cm from the plate base, the plate was removed, dried for 15 min, and scanned at 446 nm by TLC scanner. Lutein contents were analysed by comparing retention time with the standard and quantified using a five-point calibration curve of different concentrations.

2.2.4. Determination of total phenolic content in pomelo fruit membrane extract

The total phenolic content of the extracts was evaluated using a colourimetric method with a Folin-Ciocalteu reagent, following the

method described in Ref. [17] with slight modifications. Results were compared against a calibration curve of the reference standard, gallic acid. The test sample (60 μL) was mixed with 4.74 mL of deionised water and 300 μL of the Folin-Ciocalteu reagent. After 5 min, 900 μL of sodium carbonate solution was added, and the mixture was left to react in the dark for 30 min. Absorbance was then measured at 765 nm against a blank reagent using a Jasco UV–Vis Spectrophotometer V-530 (Japan). Phenolic content was calculated as gallic acid equivalents (GAEs)/g of dry plant material based on a standard curve of gallic acid (50–500 mg/L, $Y = 0.0011X - 0.0012$, $R^2 = 0.9992$). All determinations were performed in triplicate.

2.2.5. Determination of total flavonoid content in pomelo fruit membrane extract

The aluminium chloride colourimetric method was used for the determination of the total flavonoid content of the sample. Quercetin served as the standard for the calibration curve. For each sample, 100 μL of diluted standard quercetin solution or extract was mixed with 20 μL of 5 % $NaNO_3$ and 35 μL of 10 % aluminum chloride. The mixture was incubated for 30 min at room temperature, and the absorbance was measured at 430 nm against a blank using a Jasco UV–Vis Spectrophotometer V-530 (Japan). The total flavonoid content of the samples was calculated based on the calibration curve. All measurements were performed in triplicate.

2.2.6. Determination of naringin in pomelo fruit membrane extract

The chemical profiles and naringin content of the pomelo fruit membrane extract were determined by HPLC. Both the pomelo membrane extract and naringin standard were dissolved in 95 % ethanol before qualitative and quantitative analyses. The HPLC system consisted of an Agilent 1260 Infinity II Analytical-Scale LC Purification System equipped with a diode array detector (Agilent 1260 Infinity II), an LC column (Phenomenex USA, Luna 5 μm (RP-C18) 250 × 4.6 nm and a guard column (Phenomenex 4.0). Data were integrated using Agilent HPLC LC1260 software (Agilent Technologies, California, USA). Separation was achieved via a linear gradient programme using H_2O containing 0.1 % trifluoroacetic acid (solvent A) and acetonitrile (solvent B) as the mobile phase. Elution was performed using a gradients system of A/B from 95/5 (5 min), 90/10 (5 min), 85/15 (10 min), 80/20 (10 min), 75/25 (10 min), 70/30 (10 min), 65/35 (5 min), 60/40 (5 min), 40/60 (5 min), 20/80 (5 min), 10/90 (5 min) and 100%B (5 min). The flow rate was set to 0.8 mL/min, and detection was carried out at 280 nm. Naringin content was quantified by comparing peak areas in the sample chromatograms with a calibration curve of the reference standards. Each experiment was performed in three replicates.

2.2.7. Tablet formulation

Three tablet formulations were developed by adjusting the ratios of inactive ingredients while keeping the marigold and pomelo extract powders fixed at 350 mg and 300 mg per tablet, respectively. The specified amounts of marigold and pomelo extract powders in the formulation were calculated based on the recommended daily intake of lutein and total phenolics to help prevent ocular diseases such as cataracts, glaucoma, and AMD [7]. The specific amounts of inactive ingredients and die diameters for each formulation are detailed in Table 1. Tablets were produced using direct compression with a hydraulic compression force of 4–6 N. The physical properties, including content uniformity, thickness, hardness, friability, disintegration time and dissolution of each tablet formulation were evaluated and compared.

2.2.8. Quality control of formulated tablets

Weight variation, content uniformity, thickness, hardness, friability, disintegration and dissolution were evaluated according to USP standard criteria for tablet formulations [18], as follows.

2.2.8.1. Weight variation. Thirty formulated tablets were randomly selected, and 10 tablets were weighed individually. Each tablet's weight was required to be within 85–115 % of the target tablet weight.

2.2.8.2. Content uniformity. The lutein and total phenolic content in each formulated tablet were determined using TLC densitometry and UV–Vis spectrophotometry, following established analytical methods.

2.2.8.3. Thickness. The thickness of 20 formulated tablets was measured using a vernier caliper, with a standard deviation not exceeding 5 %.

2.2.8.4. Hardness. Ten formulated tablets were sampled, and the hardness of each tablet was measured using a Stokes- Monsanto

Table 1
The ingredients in the developed tablets.

Ingredients	Formula		
	1	2	3
Marigold extract (mg)	350	350	350
Pomelo membrane extract (mg)	300	300	300
Corn starch (mg)	150	200	250
Aerosil® (%)	1	1	1
Magnesium stearate (%)	1.2	1.2	1.2
Diameter of die (mm)	12	13	13

hardness tester. The measured value should fall within the range of 4–7 kg/m^2.

2.2.8.5. Friability. The formulated tablets were randomly selected, and their friability was tested using a Roche friability machine. A total tablet weight of at least 6.5 g was used, with the machine set to rotate at 25 rpm for 4 min. The friability should not exceed 1 %.

2.2.8.6. Disintegration. Six formulated tablets were randomly selected, and one tablet was placed in each of the six tubes of the basket. The apparatus was operated using water as the immersion fluid, maintained at 37 ± 2 °C. Observations were made until the tablets had disintegrated completely. If one or two tablets failed to disintegrate completely, the test was repeated with 12 additional tablets. The requirement was met if at least 16 of the total 18 tablets tested had disintegrated completely.

2.2.8.7. Dissolution. In this study, three dissolution media were used to determine the optimal conditions: 900 mL of 0.05 M hydrochloric acid, 500 mL of phosphate buffer (pH 6.8) and 500 mL of 0.05 M hydrochloric acid with 0.8 % w/v sodium lauryl sulfate. One tablet was placed in the dissolution apparatus, and the dissolution medium (900 mL of 0.05 M hydrochloric acid) was equilibrated to 37 ± 0.5 °C. The apparatus was then operated at 100 rpm for 90 min. Samples (20 mL) from each container were withdrawn from a zone midway between the surface of the dissolution medium and the top of the rotating basket or blade, ensuring that the sample point was not less than 1 cm from the vessel wall at 10, 20, 30, 45, 60 and 90 min. The withdrawn aliquots were then replaced with equal volumes of fresh dissolution medium at 37 °C. Ten millilitres of each sample were extracted with ethyl acetate, and the amount of lutein was analysed. In addition, 10 mL of each sample was analysed for total phenolic content using a UV spectrophotometer. The lutein and total phenolic contents were calculated as percentages of the accumulated substance release at different time points.

2.2.8.8. Moisture content. The moisture contents of the pomelo fruit membrane extract powder, marigold flower extract powder, powder mixtures and formulated tablets were determined using a moisture analytical balance. Two grams of each sample were placed in an aluminium pan and heated at 105 °C for 30 min. The weights of the samples before and after heating were recorded, and the moisture content was calculated as a percentage.

$$\% \text{ Moisture content} = [(W1 - W2) \ / \ W2]x\ 100$$

where W1 = weight of the sample before drying.
W2 = weight of the sample after drying.

2.2.8.9. Microbial contamination. The obtained tablets were tested for microbial contamination, including *Escherichia coli*, *Clostridium perfringens* and *Salmonella* spp., to assess the quality of the raw materials and the manufacturing process. The samples were tested according to the AOAC official methods and the FDA Bacteriological Analytical Manual at the Center for Scientific and Technological Equipment, Suranaree University of Technology.

2.2.8.10. Heavy metal contamination. The tablets were tested for heavy metal contamination (e.g. arsenic, cadmium, mercury, and lead) to assess the quality of raw materials and agricultural processes. The samples were analysed using inductively coupled plasma mass spectrometry (ICP-MS) (Agilent, 7500ce) at the Center for Scientific and Technological Equipment, Suranaree University of Technology.

2.2.9. Stability

The formulated tablets were subjected to accelerated stability testing using the freeze-thaw cycle method. The samples were stored in an incubator at 45 °C for 12 h and in a refrigerator at 4 °C for 12 h, each for six cycles. Then, the physical and chemical properties of the developed tablets were analysed and compared before and after the testing.

2.3. Statistical analysis

The experimental results were expressed as mean ± standard error of the mean (SEM) from three replicates. General data were analysed using descriptive statistics, including percentages, means, or standard deviations, with Microsoft Excel. Differences between pre- and post-stability test measurements were assessed using the paired *t*-test. A p-values <0.05 were considered statistically significant.

3. Results

3.1. Optimal method for the extraction of marigold

Four solvents (ethanol, acetone, ethyl acetate, and hexane) were used to macerate of marigold flowers. The results showed that the ethyl acetate extract yielded the highest extract content (18.48 %), followed by acetone (17.03 %), hexane (13.11 %) and ethanol (11.99 %). The ethyl acetate extract was further separated by column chromatography to obtain a lutein-rich fraction. Silica gel was used as a stationary phase, and a gradient of hexane/ethyl acetate (ranging from 10:0.5 to 0:100) was used as the mobile phase. Three fractions were collected, with fraction 3 providing the highest yield (36.52 %), followed by fractions 2 (22.48 %) and 1 (18.96 %).

Fraction 3 also contained the highest amount of lutein and was selected for tablet formulation. Aerosil (32.43 %) was used as an appropriate adsorbent to prepare the marigold extract powder for tablet formulation.

3.2. Optimal method for the extraction of pomelo fruit membranes

Two solvents (95 % and 50 % aqueous ethanol) were used to macerate the pomelo fruit membrane. The results showed that 95 % and 50 % aqueous ethanol yielded extract amounts of 19.51 % and 20.04 %, respectively, with no significant difference between them. Therefore, 95 % ethanol extract was selected for tablet formulation due to its superior drying efficiency. Aerosil (30.0 %) was used as a suitable adsorbent to prepare the pomelo extract powder.

3.3. Determination of lutein in marigold flower extract

The lutein content in the marigold extract powder was determined using a TLC densitometer and quantified through comparison with a calibration curve. Five concentrations of standard lutein, prepared from 0.024 mg/mL lutein solution and applied to TLC plates at 4–10 μL, were used to obtain the area under the curve (AUC). The lutein amounts and corresponding AUC values were plotted to generate the calibration curve, resulting in a linear equation of $Y = 5346.7X - 709.2$, with an $R^2 = 0.9982$. The lutein content in the marigold extract powder was found to be 2.19 %.

3.4. Determination of total phenolic content in pomelo fruit membrane extract

The phenolic content in the pomelo extract powder was determined using a colorimetric method with the Folin-Ciocalteu reagent and quantified against a calibration curve of gallic acid. Five concentrations of standard gallic acid (50–500 μg/mL) resulted in UV absorbance values ranging from 0.0531 to 0.5383. The concentration of gallic acid and UV absorbance values were plotted to generate a calibration curve with a linear equation of $Y = 0.0011X\text{-}0.0012$ and R^2 of 0.9992. The total phenolic content in the pomelo extract powder was 69.4 ± 0.26 mg GAE/g dry weight, corresponding to 6.94 % of the powder.

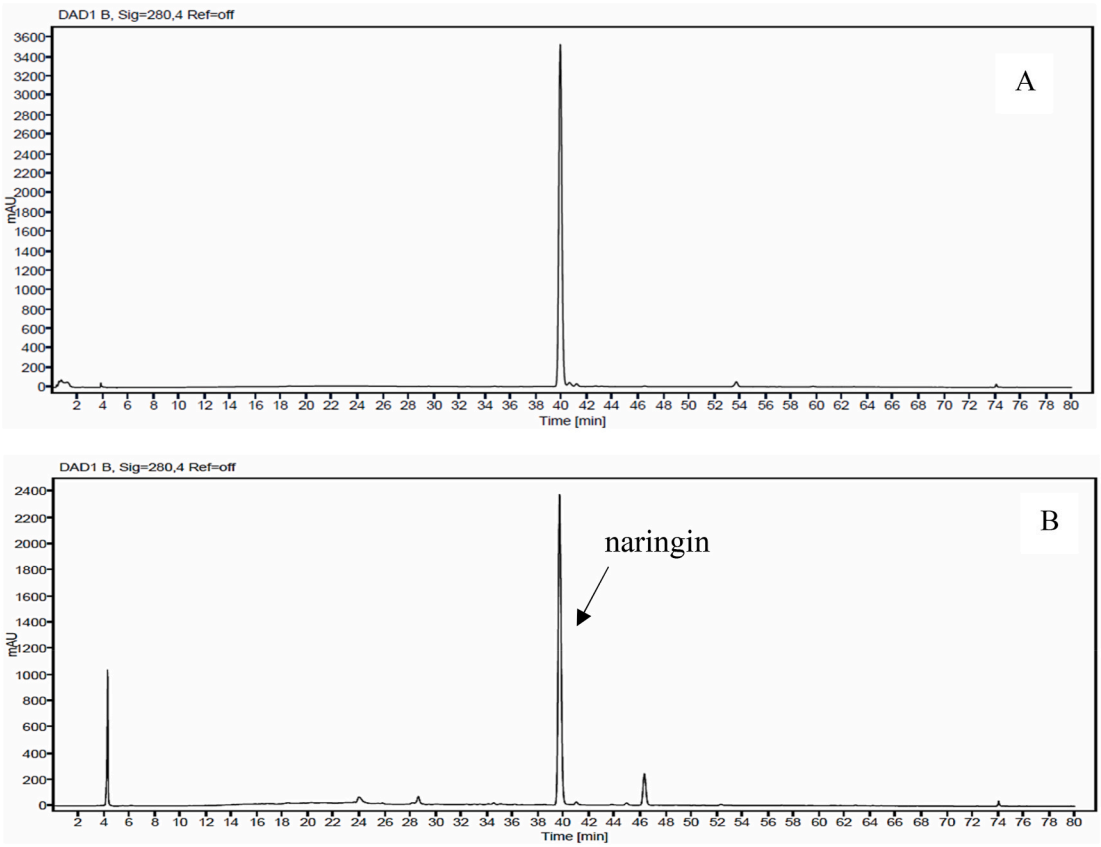

Fig. 2. HPLC chromatogram of naringin (A) and pomelo fruit membrane extract (B).

3.5. Determination of total flavonoid content in pomelo fruit membrane extract

Five concentrations of quercetin (5–125 μg/mL) and UV absorbance were plotted to generate the calibration curve resulting in a linear equation of $Y = 0.0259X + 0.0122$ with an R^2 of 0.999. The total flavonoid content in pomelo extract was found to be 33.57 ± 0.14 mg quercetin equivalent/g extract.

3.6. Determination of naringin in pomelo fruit membrane extract

The HPLC chromatogram of the naringin and pomelo fruit membrane extract was shown in Fig. 2. The calibration curves of naringin showed a linear equation of $y = 333.54x + 757.58$. The naringin content in the pomelo fruit membrane extract was determined to be 11.19 ± 0.54 mg/g of crude extract.

3.7. Result of tablet formulation

We aim to develop a supplement tablet containing 6–20 mg of lutein and 20–50 mg of total phenolic compounds, aligned with the recommended daily dose [7]. The lutein content in the marigold extract powder and the total phenolic content in the pomelo extract powder were 2.19 % and 6.94 %, respectively. Based on these concentrations, each tablet would require 273.97–913.24 mg of marigold extract powder and 288.18–720.46 mg of pomelo extract powder. In this study, we included 350 mg of marigold extract powder and 300 mg of pomelo extract powder, providing 7.7 mg of lutein and 20.8 mg of phenolic compounds, respectively. The inactive ingredients used in tablet formulation comprised 1 % aerosil (as a glidant), 1.2 % magnesium stearate (as a lubricant), and varying amounts of corn starch (as a disintegrant and additive) at 150, 200 and 250 mg in three formulations. The total tablet weight was kept below 850 mg. Tablets were produced via direct compression, and each formula was tested for weight variation, hardness, thickness, and disintegration time, with results summarized in Table 2. Formula 1 included 150 mg of corn starch, a die diameter of 12 mm, and a disintegration time of 21.45 ± 0.61 min, meeting disintegration criteria. However, it was relatively thick, with high hardness and challenging powder flow that could impact weight consistency. Formula 2 used 200 mg of corn starch, a die diameter of 13 mm, a disintegration time of 13.51 ± 0.48 min, and a hardness of 6 N, passing all criteria. Formula 3, with 250 mg of corn starch and a die diameter of 13 mm, had a faster disintegration time of 10.03 ± 0.53 min, but the tablets were too thick and large. Based on these evaluations, formula 2 was selected for further studies. This formulation, comprising 350 mg of marigold extract powder, 300 mg of pomelo extract powder, 200 mg of corn starch, 1 % aerosil and 1.2 % magnesium stearate, with a die diameter of 13 mm and a compressive strength of 6 N, proved to be the most suitable for commercial production.

3.8. Quality control of the formulated tablets

3.8.1. Weight variation

The target tablet weight in this study was 850 mg, with an average measured weight of 850.24 ± 0.41 mg. The % RSD was 0.049 and all tablets fell within the acceptable range of 85–115 %.

3.8.2. Content uniformity

The required amounts of lutein and total phenolic compounds were not less than 6 and 20 mg/tablet, respectively. The analysed quantities were 6.24 ± 0.19 mg of lutein and 22.01 ± 0.65 mg of total phenolic compounds per tablet. The content uniformity of formulated tablets was in the range of 85–115 %, meeting the USP standard criteria.

3.8.3. Thickness

The average thickness of the formulated tablet was 4.90 ± 0.00 mm, with no tablet differing by more than 5 %.

3.8.4. Hardness

The average hardness of the developed tablets was 6 ± 0.00 kg/cm^2, with all tablets falling within the general range of 4–7 kg/cm^2.

3.8.5. Disintegration time

All sampled tablets disintegrated within 30 min, meeting the standard criteria, with an average disintegration time of 13.55 ± 0.31 min.

Table 2
The properties of developed tablets.

Formula	diameter (mm)	Weight (mg) (n = 10)	Thickness (mm) (n = 5)	Hardness (kg/cm^2) (n = 5)	Disintegration time (min) (n = 3)
1	12	808.79 ± 0.52^a	5.00 ± 0.00^a	7.50 ± 0.00^a	21.45 ± 0.61^a
2	13	858.68 ± 0.47^b	4.90 ± 0.00^a	6.00 ± 0.00^b	13.51 ± 0.48^b
3	13	909.63 ± 0.45^b	5.45 ± 0.00^a	6.00 ± 0.00^b	10.03 ± 0.53^b

The values represent the mean ± SEM within the same column followed by the different superscript letters are significantly different at $p < 0.05$.

3.8.6. Friability

The friability of the formulated tablet was 0.047 %, meeting the standard criteria.

3.8.7. Dissolution

The optimal dissolution conditions were evaluated using three dissolution mediums: 900 mL of 0.05 M hydrochloric acid, 500 mL of phosphate buffer (pH 6.8) and 500 mL of 0.05 M hydrochloric acid with 0.8 % w/v sodium lauryl sulfate. The result showed that the highest cumulative drug release percentage of total phenolic compounds was observed with 500 mL of 0.05 M hydrochloric acid containing 0.8 % w/v sodium lauryl sulfate (Fig. 3). However, none of the media was able to detect lutein, indicating that this condition was not suitable for dissolving lutein in the tablet.

3.8.8. Moisture content

The moisture contents of the pomelo fruit membrane extract powder, marigold flower extract powder, mixture powder, and formulated tablets were 2.63 ± 0.37, 1.09 ± 0.11, 2.87 ± 1.42, and 4.52 ± 1.86 %, respectively. The results indicated that the moisture content of all samples did not exceed 8 %, meeting the standard criteria.

3.8.9. Microbial contamination

Microbial contamination of the developed tablets was assessed. The result showed that none of the samples were contaminated with *Escherichia coli*, *Clostridium perfringens* and *Salmonella* spp.

3.8.10. Heavy metal contamination

The developed tablets were tested for heavy metal contamination. The results showed that none of the samples contained arsenic, cadmium, mercury, and lead.

3.9. Stability

The stability of the formulated tablet was evaluated under accelerated conditions using the freeze-thaw cycle method. The amounts of lutein and total phenolic compounds in the formulated tablets before and after six freeze-thaw cycles are presented in Table 3. No significant differences were observed ($p > 0.05$). Additionally, the physical properties such as weight variation, content uniformity, thickness, hardness, disintegration time, friability, and dissolution were not significantly affected by the freeze-thaw cycles. The values for each property of the developed tablet before and after stability testing are summarized in Table 4.

4. Discussion

Although marigold flowers are a good source of lutein, the lutein content in a single tablet prepared using conventional extraction methods is insufficient to meet daily intake requirements. Therefore, a specialized extraction technique was employed to enhance lutein yield. The conventional extraction methods (simple maceration and soxhlet extraction) and novel methods (supercritical fluid extraction, enzyme-assisted extraction and microwave-assisted extraction) have been studied for the extraction of marigold flowers [19,20]. In this study, we developed an extraction method combining simple maceration with column chromatography to achieve high lutein yields. The results showed that the ethyl acetate extract yielded the highest extract content of dried powder (18.48 %), followed by acetone (17.03 %), hexane (13.11 %), and ethanol (11.99 %). The ethyl acetate extract was further separated by column chromatography using silica gel as a stationary phase and a gradient of hexane/ethyl acetate was used as a mobile phase, varying from a ratio of 10: 0.5 to 0: 100 to obtain three fractions. Fraction 3 gave the highest extract amount (36.52 %), followed by fractions 2 and 1 (at 22.48 % and 18.96 % of the crude extract, respectively). Fraction 3 also gave the highest amount of lutein (3.24 %). Compared to results from other studies, lutein content in different extracts typically ranged from 1.06 % to 2.10 % [20–22]. The developed

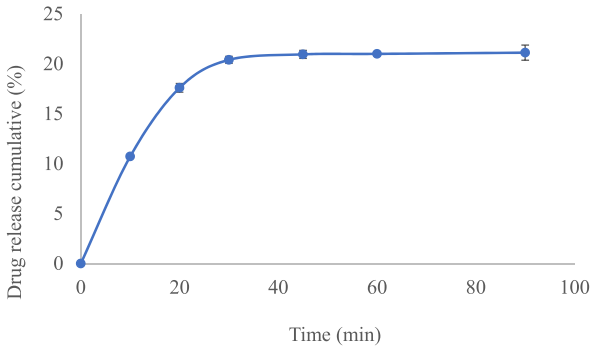

Fig. 3. Dissolution profile of total phenolic content in 0.05 M hydrochloric acid with sodium lauryl sulfate at 0.8 % W/V medium in the developed tablet before freeze-thaw cycle.

Table 3
The amounts of lutein and total phenolic compounds in developed tablets compared before and after the freeze-thaw cycle.

Lutein content (mg/tablet)		Total phenolic compound (mg/tablet)	
Before	After	Before	After
6.24 ± 0.19^{a}	6.16 ± 0.08^{a}	22.01 ± 0.65^{b}	22.30 ± 0.50^{b}

The values represent the mean ± SEM for the same compound before and after, with different superscript letters indicating significant differences at $p < 0.05$.

Table 4
The properties of developed tablets compared before and after the freeze-thaw cycle.

Weight (mg) (n = 10)		Thickness (mm) (n = 5)		Hardness (kg/cm^2) (n = 5)		Disintegration time (min) (n = 3)	
Before	After	Before	After	Before	After	Before	After
850.24 ± 0.41^{a}	850.02 ± 0.56^{a}	4.90 ± 0.00^{b}	4.90 ± 0.00^{b}	6.00 ± 0.00^{c}	7.00 ± 0.00^{c}	13.55 ± 0.31^{d}	13.66 ± 0.39^{d}

The values represent the mean ± SEM for the same property before and after, with different superscript letters indicating significant differences at $p < 0.05$.

technique produced lutein yields that were 1.5–3.0 times higher than those obtained from other common extraction methods. This method is a simple, low-cost, but highly effective method for extracting lutein from marigold flowers.

The extract obtained from the marigold flower was semisolid and not suitable for direct use in tablet formulation. Therefore, it needed to be made in powder form. Various adsorbents and their different concentrations were evaluated to determine the optimal ratio for transforming the marigold flower extract into powder. The ratio that produced a dry extract powder with the least amount of adsorbent was selected. The results showed that 32.43 % aerosil was suitable for making marigold extract powder. The resulting extract powder was fine, the humidity was less than 8 %, and contained 2.19 % lutein.

The suitable solvents for extracting the phenolic compound from the pomelo fruit membrane were assessed using 95 % and 50 % aqueous ethanol. The percent yields from both extracts were not significantly different; however, 95 % ethanol was selected for extraction of the pomelo fruit membrane due to its superior drying efficiency. The obtained extract was semisolid, making it unsuitable for direct use in tablet formulation. Various adsorbents and their different concentrations were evaluated to determine the optimal ratio for preparing a pomelo extract into a powder. The result indicated that 30 % aerosil exhibited a suitable adsorbent for transforming a pomelo fruit membrane extract into powder. The phenolic content in the pomelo fruit membrane was relatively high (69.4 mg GAE/g dry weight) compared to other plant sources [23]. The HPLC profile of the pomelo extract revealed that the main component was naringin (11.19 mg/g dry weight). These findings suggest that the most abundant total phenolic compounds of pomelo fruit membrane are flavonoids, particularly naringin. The phenolic content in the pomelo extract powder was 6.94 %, which is sufficient for daily intake to provide protection against optic diseases [7,8]. Moreover, the pomelo extract was obtained from agricultural waste material, making it a low-cost option. Another advantage of the pomelo extract is that it does not have the green colour of chlorophyll, which often affects the appearance of general natural products. Therefore, pomelo extract is an excellent source of phenolic compounds and flavonoids for use in dietary supplements.

This study successfully developed a dietary supplement tablet for preventing ophthalmic disease. The optimal tablet formulation consists of *T.erecta* flower extract (350 mg), *C. maxima* fruit membrane extract (300 mg), corn starch (200 mg), aerosil (1 %), and magnesium stearate (1.2 %). Each tablet provides 7.7 mg of lutein and 20.8 mg of total phenolic compounds, meeting the body's daily requirements. We formulated a supplement tablet containing 6–20 mg of lutein and 20–50 mg of total phenolic compounds, which are sufficient for daily intake to prevent ocular diseases such as cataracts, glaucoma and AMD [7,24]. In this study, we determined lutein only in free form, however, bound form lutein (esterified with long-chain fatty acids) is also found in marigold flowers [2]. Both free and esterified lutein compounds are absorbed from foods and dietary supplements, but the ester form requires prior de-esterification by intestinal enzymes [25]. Thus, the amount of lutein absorbed may exceed 7.7 mg per tablet, which is sufficient for daily intake to help prevent ocular diseases.

The quality control of formulated tablets was assessed using various methods, including weight variation, content uniformity, thickness, hardness, disintegration, friability, dissolution, moisture content, microbial contamination, heavy metal contamination, and stability. All tests complied with the standard criteria outlined in both the US Pharmacopeia and the Thai Herbal Pharmacopeia.

Dissolution conditions for formulated tablets were evaluated using three different media: 900 mL of 0.05 M hydrochloric acid, 500 mL of phosphate buffer (pH 6.8), and 500 mL of 0.05 M hydrochloric acid with 0.8 % w/v sodium lauryl sulfate (SLS). The highest cumulative drug release percentage for total phenolic compounds was observed with 0.05 M hydrochloric acid containing 0.8 % w/v SLS, but lutein was undetectable across all media. These conditions were, therefore, unsuitable for lutein dissolution in the formulated tablets. As is already known, lutein contains two cyclic end groups (beta- and alpha-ionone rings). It has a C40 isoprenoid structure that is insoluble in water but soluble in fats and lipophilic solvents [26]. The medium with sodium lauryl sulfate is often applied for dissolution testing of insoluble compounds. In this study, however, although the medium with SLS was used, lutein remained undetected. Anselmo et al. developed a dissolution method for lutein using sodium lauryl sulfate at concentrations of 1 % and 2 % along with polysorbate 80, which is higher than those used in this study. The samples could quantified by HPLC coupled to a diode-array detector [27]. For further development of a dissolution method that can simultaneously assess lutein and total phenolic compounds

in developed tablets, it may be essential to consider higher SLS concentrations, the addition of lipophilic solvents, and appropriate analytical instruments.

5. Conclusion

A tablet formula containing marigold flower and pomelo fruit membrane extracts for preventing eye diseases was successfully developed. The active compounds provide several protective functions, including shielding the macula from blue light, improving visual acuity and scavenging harmful reactive oxygen species**.** The amounts of lutein and total phenolic compounds in each tablet meet the body's daily requirements. The physical properties of the developed tablet, both before and after the stability test, complied with the USP standard criteria. This tablet is suitable for preventing ophthalmic diseases and may also be beneficial for other conditions related to the activity of lutein and phenolic compounds, such as cardiovascular diseases [28] and cancer [29].

CRediT authorship contribution statement

Pornpun Laovachirasuwan: Writing – review & editing, Writing – original draft, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Natthanan Chainom:** Visualization, Methodology, Investigation, Formal analysis, Data curation. **Sirinthra Kaengin:** Methodology, Investigation, Formal analysis, Data curation. **Somsak Nualkaew:** Writing – review & editing, Writing – original draft, Resources, Project administration, Methodology, Investigation, Formal analysis, Conceptualization.

Consent to participate

Not applicable.

Availability of data and materials

All data are available in the manuscript.

Ethical approval

Not applicable.

Consent for publication

Not applicable.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this article.

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List of abbreviation

AUC	Area under the curve
AMD	Age-related macular degeneration
HPLC	High-performance liquid chromatography
HPTLC	High-performance thin-layer chromatography
TLC	Thin layer chromatography
USP	The United States Pharmacopeia
UV	Ultraviolet
Vis	Visible

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