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Phytochemical profiling of *Tagetes erecta* L. flowers at various blooming stages through optimized extraction of bioactive compounds for the development of functional juice

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Introduction: *Tagetes erecta* L. is a valuable medicinal plant and its edible flowers are a rich source of secondary metabolites that may vary at different blooming stages.

Objective: Current study aimed to evaluate the phytochemical profile of flowers at different blooming stages with varying treatments.

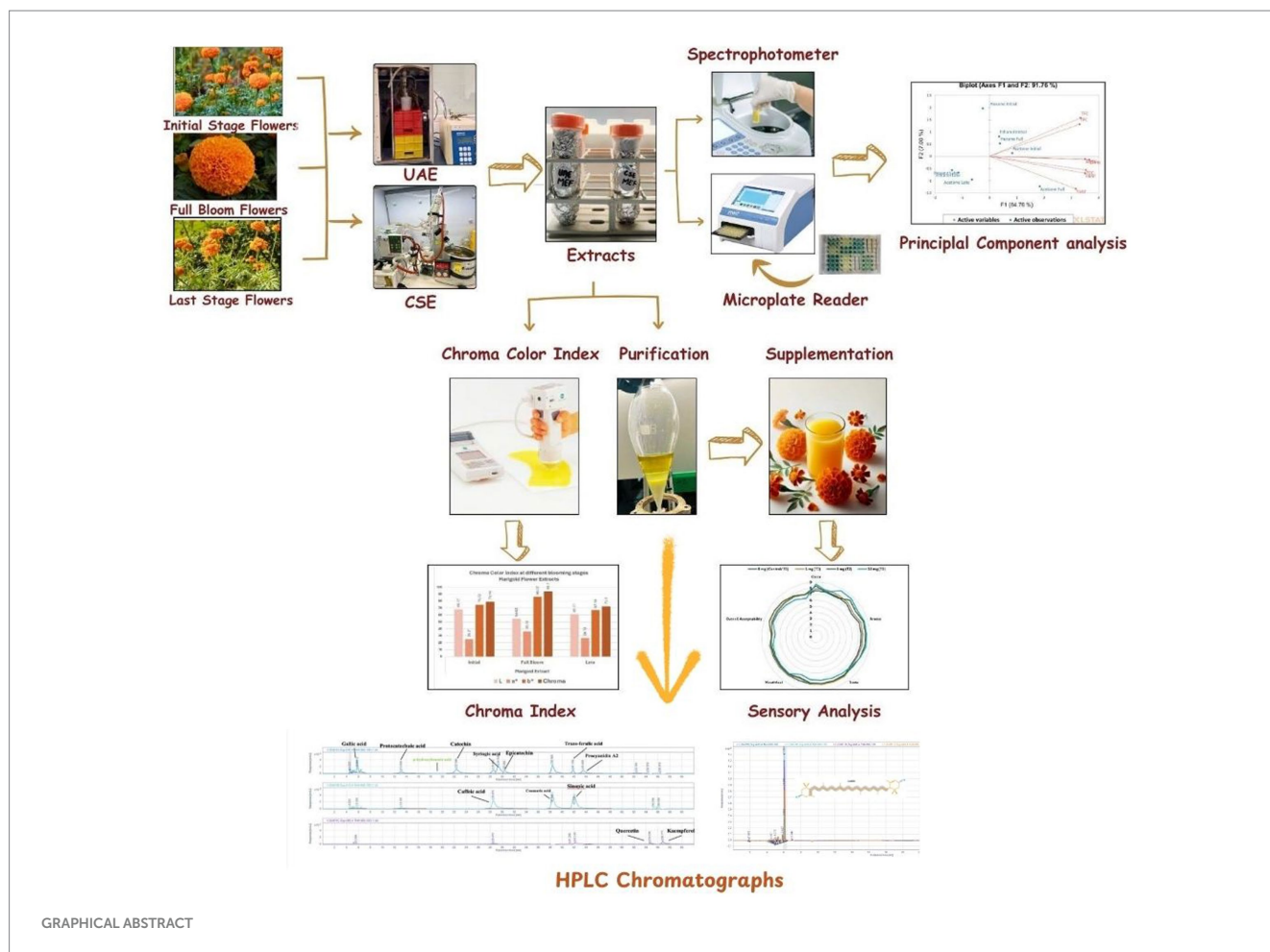
Methodology: Extracts were prepared by employing solvent extraction with ultra-sonification as pretreatment and by varying solvents to assess the phenolic and antioxidant activities. The high-performance liquid chromatography analysis was performed to quantify bioactive polyphenolics to validate effect of various stages and treatments on sample extracts.

Results: Study found that ultrasound-pretreated samples resulted higher yield and phenolic contents than solvent extraction (CSE) alone. The highest carotenoid content (427.39 mg/100 g) was observed for the pretreated full-bloom flower samples (MAF-1). Radical scavenging activities were observed highest (DPPH 96.19% and ABTS* 160.09 mmol TE/100 g) in *Tagetes* sample MAF-1 followed by MEF-1 and MAI-1. A highly significant and positive correlation was observed among all variables of phenolic and antioxidant assays. Various bioactive compounds including phenolic acids, flavonoids and carotenoids were quantified by HPLC-DAD. Results showed that the ultra-sonified extracts (MAF-1) resulted in higher gallic acid (109 mg/g), quercetin (11.74 mg/g), lutein (1.76 mg/g) and zeaxanthin (2.14 mg/g) followed by MEF-1 > MAI-1 > MEI-1.

Conclusion: The study results showed that the ultrasound-assisted extracts resulted in higher bioactive contents and antioxidant activities expected to deliver more value as additive to functional foods. It also reflects the potential of *Tagetes* flowers for the functional foods aiming to control early age-related disorders (ARDS).

KEYWORDS

Tagetes, ultrasonification, polyphenolic compounds, carotenoids, HPLC, functional juice



1 Introduction

The demand and use of natural bioactive components in food are growing with increasing consumer knowledge and industrial development (Barut et al., 2022). Carotenoids, mainly xanthophylls, are bioactive components that have gained increasing commercial and consumer attention. It can be used as a nutritional supplement for food fortification and in the pharmaceutical sector due to its potential therapeutic effect (Chitrakar et al., 2019). Extensive research has been conducted to evaluate various edible flowers and plants. It has been recognized that *Tagetes erecta* (Marigold) flowers are not only safe but also rich in phytochemicals of a therapeutic nature. It is one of the significant, economic and industrial plants that is now largely cultivated for commercial purposes (Chitrakar et al., 2019; Vural et al., 2023). According to an estimation, a large quantity of marigold flowers is grown worldwide (600,000 tons) (Singh et al., 2020) and has numerous commercial applications (Wu et al., 2023). The global market demand for marigold lutein is also continuously increasing, which may increase to USD 357 million by 2022 (Ma, 2017) and is expected to reach USD 405 million by 2027 (Saha et al., 2020). This increasing demand is a major challenge; therefore, to meet this challenge, local sources need to be investigated and improvements in extraction methods are required to obtain better yields, reduce

costs, and increase shelf stability for commercial applications. Among various species, *Tagetes erecta* L. is a commercially grown species and indigenous to Pakistan (Baig et al., 2021). Almost 40–50% of the flower consists of petals of various colors from light yellow to dark orange. Commonly identified bioactive compounds in marigolds are phenolics, flavonoids and carotenoids. Various databases reported different carotenoids in different marigold varieties includes; lutein, zeaxanthin, alpha-carotene, beta carotene, alpha cryptoxanthin, beta-cryptoxanthin (Rodrigues et al., 2019), and flavonoids including flavonols, flavanols, and flavones, mainly quercetin, quercetagetin, rutin, catechin, apigenin, acacetin, hesperidin, hesperetin, and patuletin etc. (Wu et al., 2023). These compounds give specific characteristics to marigold plants such as carotenoids, carotenes give color to marigold flowers, isothiocyanates contribute to the aroma while cyclotides are familiar as nutritious plant peptides.

Marigold flowers are a rich source of secondary metabolites that can be easily extracted and processed for commercial purposes. The significance of the proposed research is based on literature reporting that marigold extracts are rich in phytochemicals of therapeutic nature that play an important role as antioxidants (Kang et al., 2018), analgesic (Bashir and Gilani, 2008), anti-inflammatory (Chung et al., 2017), anti-viral (Siriamornpun et al., 2012), anti-clastogenic (Wang et al., 2006), anti-tumorigenic (Gansukh et al., 2019), cardioprotective

(Madaan et al., 2017). Recent studies have reported that it contributes to human cognitive functions being accumulated in the brain and macula, which helps to control age-related macular degenerative disorders (Jia et al., 2017; Yang et al., 2020) and cataracts (Padmanabha and Vallikannan, 2018). *Tagetes erecta* L. is commercially important because of its bioactive components. Commercially available lutein is derived from the petals of marigold flowers (Kaimainen et al., 2015) and has approved GRAS (generally recognized as safe) status, as well as an EU-approved commercial food colorant and food additive (E161b) for utilization in food products (Weigel et al., 2018). The European Food Safety Authority (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF), 2010) has recommended its daily allowance of 1 to 5 mg reported in EFSA (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF), 2010), because it is not naturally synthesized in the body and therefore; must be incorporated through diet (Jivan and Abbasi, 2019) to fulfill dietary requirements (Madaan et al., 2017). Therefore, diets rich in functional components are warranted against numerous age-related disorders (Nwachukwu et al., 2016). Functional beverages are now becoming more popular owing to additional health benefits to control age-related disorders especially age-related macular degenerative disorders (AMDs) (Corbo et al., 2014). Natural beverages such as juices, are rich sources of nutrients and act as a contemporary vehicle for numerous bioactive compounds and their fortification may improve sensory attributes (Rodríguez-Roque et al., 2016). Carotenoids are highly recognized bioactive components that are widely employed in the development of functional beverages, mainly fruit juices, drinks and milk (Rodríguez Roque, 2014). Marigold supplementation into various food products including dairy products (milk, yogurt, ghee) (Bhat et al., 2022; Xavier et al., 2014), beverages (Rodríguez-Roque et al., 2016) and tea (Ma et al., 2020) has been confirmed to not only improved the sensory attributes and bioavailability of bioactive components but also exert positive health effects.

The primary objective of this study is to quantify the phenolics, carotenoid content, and antioxidant activities of marigold flowers at various stages of blooming from the start of the season to the end of the flowering time. In addition, research aimed to evaluate the comparison of employing ultrasound as pre-treatment and conventional solvent extraction by using different organic solvents. Ultrasonication is one of the advanced extraction techniques in which ultrasound radiations of specific intensity are applied to extract bioactive compounds from cell materials. Ultrasound assisted extraction for bioactive compounds is based on two stages; washing stage, a relatively fast stage in which the extraction solvent easily penetrates the cell and causes breakage of cell walls to free inbound components while in the second stage components leach down through porous cells solid to the solvent matrix. In this study, the ultrasonication process is employed due to its ability to break cell wall of plant material and facilitate the extraction of phenolic compounds and may resulted higher antioxidant activities in pre-treated (UAE) extracts (He et al., 2024). Furthermore, the antioxidant assays and quantification (HPLC) of marigold flowers will justify the possibility of supplementing phenolics and carotenoids enriched extract to develop functional juice to accomplish the RDA of lutein and provide critical future insights into their potential to be utilized in nutraceuticals and pharmaceutical food products.

2 Methodology

2.1 Sample collection and preparation

Marigold flowers were collected at various blooming stages (Initial, full bloom, late) (Annex 1) from the local nursery of KFUEIT, Rahim Yar Khan, Pakistan. The marigold flower species was verified by a horticulture expert from the Department of Horticulture, Agriculture Engineering and Sciences, Khwaja Fareed University of Engineering and Information Technology. The samples were dried in an air-based food dehydrator (Model No. DI-2400) under specific conditions (Temperature, 40°C, Time 3–4 h). Dried flower petals were ground to a fine powder (60 mesh size), labeled, and kept separately in air-tight polyethylene bags and stored at –18°C.

2.2 Moisture analysis

Marigold flower sample moisture analysis was performed according to the standard gravimetric method AOAC 925.10 (Association of Official Analytical Chemists, 2023). Marigold flower petals of all different stages were weighed (5 g) for the estimation of moisture content. All samples were subjected to a hot-air oven (Qualtex Universal Series, 2000) at 105 ± 5°C for approximately 24 h. After the complete removal of moisture, samples were cooled in a desiccator, and moisture content was measured by the following formula;

$$M.C\% = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100$$

2.3 Extract preparation and purification

The extracts were prepared by mixing marigold flower powder samples of 5 g each into 50 mL of selected solvents; Ethanol (80%), Hexane (80%), and acetone (80%) (Surendranath et al., 2016), (Jalali-Jivan and Abbasi, 2020). In this method, samples were divided into six sets, three with pre-treatment and three without pre-treatment solvent extraction (Table 1). Extraction with pre-treatment as ultrasonication by using an Ultrasonifier (Branson, model Digital Sonifier 450) was performed by employing the optimal conditions (Amplitude 40%; Time: 5 min) reported by Shi et al. (2023). All the samples were carefully labeled and mixed with different solvents at a ratio of 1:10. After overnight incubation at room temperature (~25°C), the samples were stirred on a magnetic stirrer at 500 rpm in dim light. Samples were then filtered through Whatman filter paper No. 1 before being subjected to a rotary vacuum evaporator at 30°C for 10 min., and the concentrated extract was collected and pooled in Falcon tubes (50 mL).

2.3.1 Purification

Marigold oleoresin was purified to remove solvent residues by alkali treatment with KOH (50%) and ethanol (80%) by mixing at 250 rpm at a specific temperature of 50°C for 15 min (Jalali Jivan and Abbasi, 2019). Subsequent phase separation was performed to separate the oil phase, from the pure extract (Manzoor et al.,

TABLE 1 Sample treatment plan for extraction from marigold samples.

Solvents	Stages	Samples	
		With pre-treatment (UAE)	Without pre-treatment (CSE)
Acetone	Initial	MAI-1	MAI-2
	Full bloom	MAF-1	MAF-2
	Late	MAL-1	MAL-2
Hexane	Initial	MHI-1	MHI-2
	Full bloom	MHF-1	MHF-2
	Late	MHL-1	MHL-2
Ethanol	Initial	MEI-1	MEI-2
	Full bloom	MEF-1	MEF-2
	Late	MEL-1	MEL-2

Extraction Treatments are abbreviated as UAE: Ultrasound assisted extraction; CSE: Conventional solvent extraction. Marigold samples with different treatments were mentioned in abbreviations; Marigold Acetone Initial Sample with Pre-treatment "MAI-1," Marigold Acetone Initial Sample without Pre-treatment "MAI-2," Marigold Acetone Full-bloom Sample with Pre-treatment "MAF-1," Marigold Acetone Full-bloom Sample without Pre-treatment "MAF-2," Marigold Acetone Late Sample with Pre-treatment "MAL-1," Marigold Acetone Late Sample without Pre-treatment "MAL-2"; Marigold Hexane Initial Sample with Pre-treatment "MHI-1," Marigold Hexane Initial Sample without Pre-treatment "MHI-2," Marigold Hexane Full-bloom Sample with Pre-treatment "MHF-1," Marigold Hexane Full-bloom Sample without Pre-treatment "MHF-2," Marigold Hexane Late Sample with Pre-treatment "MHL-1," Marigold Hexane Late Sample without Pre-treatment "MHL-2"; Marigold Ethanol initial Sample with Pre-treatment "MEI-1," Marigold Ethanol Initial Sample without Pre-treatment "MEI-2," Marigold Ethanol Full-bloom Sample with Pre-treatment "MEF-1," Marigold Ethanol Full-bloom Sample without Pre-treatment "MEF-2," Marigold Ethanol Late Sample with Pre-treatment "MEL-1," Marigold Ethanol Late Sample without Pre-treatment "MEL-2".

2022). The samples were again centrifuged at 250 rpm for 5 min until two clear phases were obtained from which the orange colored liquid was collected and off-white solid coagulates were discarded.

2.4 Phytochemical analysis

2.4.1 Total carotenoids

The carotenoid content was measured using the method of Manivannan et al. (2021). For this purpose, marigold powder extracts were prepared by multiple re-extractions with a fresh solvent until the residues became colorless. After extraction, the oleoresin was subjected to rotary vacuum evaporation to separate the carotenoid pigments. Clear extracts were obtained and pooled to make up the volume and subjected to a spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) at 450 nm for total carotenoid assessment. Carotenoid contents were calculated (Equation 1) and expressed in mg/100 g marigold dry powder weight.

$$\text{Total carotenoids} = \left(A \times DF \times V \times C^{1\%} \right) / \left(A^{1\%} \times \text{Weight of sample} \right) \quad (1)$$

A → Absorbance, DF → Dilution Factor, V → Volume of extract, C^{1%} → Concentration of 1% solution, A^{1%} → Absorbance of 1% solution.

2.4.2 Total phenolic content

The total phenolic content of *T. erecta* was measured using the reducing Folin Ciocalteu Reagent following the method of Manivannan et al. (2021). For this purpose, the sample extract (25 μL) was mixed with 25 μL of FC reagent +200 μL of water and allowed to stand for reaction (5 min at 25°C). Sodium carbonate solution (10% w:w) was

prepared and 25 μL was added to the above reaction mixture, resulting in a blue color as the endpoint. Reaction samples were allowed to stand at 25°C for 60 min in the dark and then subjected to a UV-Vis spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) for absorbance measurement at a wavelength of 765 nm against gallic acid standard concentration ranging from 0–200 μg/mL. TPC was calculated (Equation 2) and expressed as mg GAE/g of dry sample weight;

$$C = c \times V / m \quad (2)$$

Where; C → Total content of Phenolic compounds, c → Concentration of Gallic acid (mg/L), V → Volume of extract, m → Weight of extract in gram.

2.4.3 Total flavonoid content

The total flavonoid content in marigold sample extracts was determined by employing the method adopted by Siriamornpun et al. (2012), marigold extract sample (80 μL) was mixed with an equal amount (80 μL) of sodium nitrite solution (50 g/L) and a 120 μL of 2% aluminum chloride. The sample extract was then mixed with 120 μL of sodium acetate and incubated for 2.5 h. The reaction mixture was vortexed for proper mixing, and the absorbance was measured at 440 nm with standard Quercetin (0–50 μg/mL). The results for TFC were calculated (Equation 3) and are expressed in mg QE/g of dry weight.

$$\text{TFC} = C (V / m) \quad (3)$$

Where; C → Concentration of Quercetin (mg/mL), V → Volume of Plant extract (mL), m → Weight of pure plant extract (g).

2.5 Antioxidant assays

2.5.1 DPPH (2,2-diphenyl-1-picrylhydrazyl) assay

Antioxidant assays of different marigold extracts were performed by measuring the DPPH radical scavenging activity, employed by Li et al. (2007), with slight modification in the method. Prepared extract samples at various concentrations from 50 to 100 μL aliquots were measured in labeled test tubes. The volume of aliquots was adjusted to 1,000 μL by using a methanol solution for the standard curve. 25 μL of sample extract was added into 275 μL of 0.1 mM DPPH (2 mg DPPH into 50 mL methanol) solution and was prepared and mixed well by shaking before incubation (dark cool place temp. 30°C for 30 min). As radical DPPH has the potential to capture hydrogen from the sample extract was indicated by its color transformation from purple to yellow. Samples were then subjected to UV-Vis spectrophotometer at a wavelength of 517 nm against the ascorbic acid standard curve (0–200 $\mu\text{g}/\text{mL}$). The absorbance was recorded to calculate (Equation 4) the free radical scavenging potential of the marigold extracts by using the following formula;

$$\text{RSA\%} = \left[\frac{A_c - A_s}{A_c} \right] \times 100 \quad (4)$$

Where; A_c is the blank standard and A_s is the sample extract.

2.5.2 FRAP (ferric reducing antioxidant power) assay

The ferric ion⁻reducing antioxidant potential (FRAP) of *Tagetes erecta* was measured by the antioxidant activities measuring method employed by Duan et al. (2024). The FRAP reagent reaction mixture (dye) was prepared using 2,4,6 Tris(2-pyridyl)-s-triazine (10 mM), Ferric chloride (20 mM) and sodium acetate (300 mM) in 1:1:10. After reagent mix preparation, 20 μL marigold extract sample was mixed with 280 μL of FRAP dye solution and incubated in the dark for 10 min at 37°C. After incubation, absorbance was recorded at 593 nm using a UV-spectrophotometer. The standard curve for ferric ion reducing antioxidant potential was derived by varying the concentration of Trolox from 0 to 100 $\mu\text{g}/\text{mL}$ and the results were obtained by applying linear regression Eq. model (Equation 5) and expressed as mmol of Trolox/g DW.

$$\text{FRAP Potential} = \frac{\text{Change in sample absorbance } 0 - 4 \text{ minutes}}{\text{Change in standard from } 0 - 4 \text{ minutes} \times \text{Standard value}} \quad (5)$$

2.5.3 ABTS (2,2-azinobis-(3-ethylbenzthiazolin-6-sulfonic acid)) assay

The ABTS⁺ assay was performed to measure the radical scavenging potential of the marigold extract by following the method reported by Manzoor et al. (2022) with slight modifications reported by Ali et al. (2021). ABTS mix was prepared by mixing ABTS (7 mM) with potassium persulfate (140 mM) and the mixture was allowed to stand overnight at 22°C in the dark. The absorbance of ABTS⁺ stock solution was measured by subjecting dye at 734 nm (Abs 0.75 cm^{-1}). Sample aliquots were mixed with 10 μL of extract and 290 μL of ABTS⁺ in a well plate (incubated for 06 min) before recording its absorbance

against a standard curve of Trolox (0–500 $\mu\text{g}/\text{mL}$) at the same wavelength of 734 nm. The antioxidant activity of extracts was measured (Equation 6) in mmol of Trolox equivalent per gram (mmol TE/g) of dry powder using the following equation:

$$Y = -0.0005 X + 0.7094 \quad (6)$$

where; Y \rightarrow represents the absorbance and X \rightarrow mM of Trolox eq.

2.6 Chroma color analysis

The color of dried marigold flower samples at all three stages (full bloom, start and end season), and their extracts were analyzed by the colorimetric method (Gombač et al., 2021). Colorimetric values in CIELAB color coordinates; L*, a*, and b* were determined by using a chroma meter (Minolta Chroma meter CR-400). Chroma and Hue were calculated using the following Equation 7:

$$\begin{aligned} \text{Chroma } (C^*) &= \sqrt{a^{*2} + b^{*2}} \\ \text{Hue} &= \tan^{-1} \left(\frac{b^*}{a^*} \right) \times (\text{degrees}) \end{aligned} \quad (7)$$

where; a* and b* indicate color directions; +a* \rightarrow Red Direction, -a* \rightarrow Green direction, b* \rightarrow yellow direction. However, L* reflects the degree of lightness and darkness ranges from 0 to 100.

2.7 Quantification of marigold bioactive compounds through HPLC

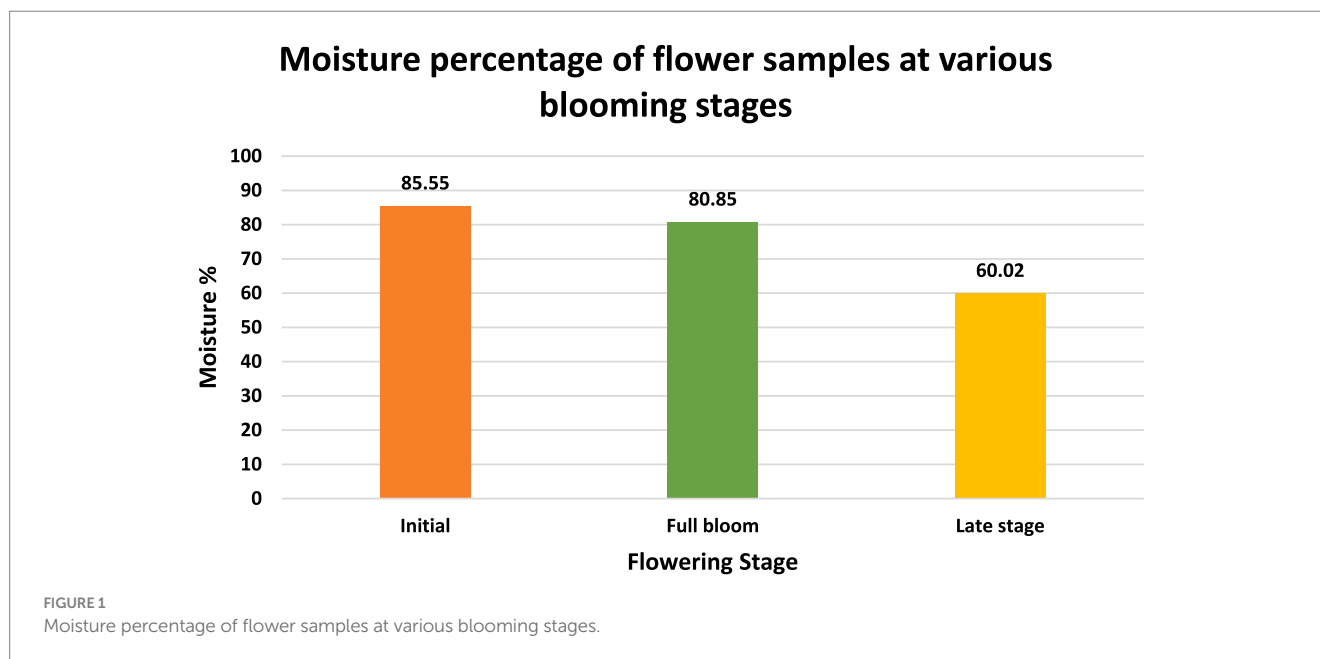
Prior to quantification of bioactive compounds, marigold sample extracts (1 mL) prepared with ultrasonication were filtered (0.45 syringe filters), diluted with extraction solvent (1:10) and filled in HPLC vials (Shi et al., 2023). Quantification of targeted bioactive compounds of extract samples was carried out by following the standard method of Suleria et al. (2020). High performance liquid chromatography (HPLC, Waters Alliance system, 2690) equipped with a reversed phase column (Synergi Hydro-RP) of dimensions 4.6 \times 250 mm with particle size 4 μm protected by guard column C18 ODS (Phenomenex, Torrance, CA, USA) and connected with a diode array detector (DAD Model 2,998, Waters). Binary mobile phase system consists of two mobile phases; Mobile Phase A [Milli-Q[®] H₂O/ acetonitrile (95:5)] and Mobile Phase B [Milli-Q[®] H₂O/acetonitrile (50/50)]. Flow rate was set to 0.8 mL/min and injection volume was set to 25 μL and column was operated at room temperature 25°C. Detector wavelengths was set at 280, 320, 370 and 450 for data acquisition. Various compounds identification was achieved by comparing retention time (RT) and the absorption spectra of the peak with standards (Sigma-Aldrich, Castle Hill, NSW, Australia).

2.8 Development of functional drink

Based on phytochemical profiling purified ultrasound pre-treated samples from different flowering stages were selected for further supplementation. To develop a functional drink, considering the

TABLE 2 Treatment plan for supplementation of purified extract in drink.

Sr#	Treatments	Concentration (mg)
1	T ₀	0
2	T ₁	1
3	T ₂	5
4	T ₃	10



recommended dietary allowance of lutein, the purified extract was added in various concentrations to drinks (Table 2). The drink was further evaluated for sensory characteristics, quality, and stability during the product storage study.

2.8.1 Sensory analysis

Supplementation of marigold powder extract was done with varying levels of supplementation. The treatment plan varies between 0 and 10 mg supplementation of purified extract in juice as follows; T₀ → 0 mg, T₁ → 1 mg, T₂ → 5 mg, T₃ → 10 mg. Sensory profile evaluation was performed using the 9-point hedonic scale (1–9) to assess sensory characteristics, including; color, taste, flavor, appearance, and total acceptability. Sensory evaluation was performed by trained panelists (10 food professionals) and consumers (40) of different age groups (18–50 years), selected randomly from the Institute of Food Science and Technology, KFUEIT, RYK, Pakistan. The samples were coded and randomly placed in sensory booths. Fifty milliliters of each treatment drink were presented to panel members and observations were recorded on Sensory Performa.

2.9 Statistical analysis

All experiments were performed in triplicate by using a randomized complete block design (RCBD). Solvents and marigold flowering stages were considered distinct factors, whereas the parameters evaluated were treated as dependent factors. Analysis

of variance (ANOVA) and mean comparison test (LSD) was performed using the SPSS program (Package version 28.0.1, 2021). Mean values are presented as standard deviation (\pm SD). Correlation analysis was carried out to determine the association among various variables, while principal component analysis (PCA) was performed to understand variability using the XLSTAT 2023 package.

3 Results and discussion

3.1 Moisture analysis

The moisture content of the marigold flower samples was measured at various blooming stages (Figure 1). Freshly procured flowers at each stage were subjected to moisture analysis to assess the moisture percentage. The moisture percentage in late-bloom samples was naturally lower (60.02%) than that of the highest (80.85%) for fresh full-bloom flowers followed by the initial stage (85.55%). Several factors can significantly affect the moisture content of edible flowers, such as growth or environmental conditions, maturity stage, harvest or post-harvest conditions, and drying methods (Bhave et al., 2020). Loss of moisture may be a result of environmental conditions such as temperature, which increases up to 40°C until the late season. The trend of higher moisture content in spring samples and lower moisture content in the off-season is supported by previous research conducted by Arefin et al. (2015).

3.2 Extraction yield

Current research has focused on improving extraction from marigolds by employing various solvents at various blooming stages to obtain the maximum extract yield. The extraction methods were also varied by employing assisted ultrasound treatment. It has been observed that the solvent's nature, including solubility, dissolution strength, safety, cost effectiveness, and environmental safety, play important roles in extraction efficiency. The extraction results from marigold flowers showed highly significant differences in terms of marigold extract (oleoresin) at different flowering stages with different solvents employed for extraction, and extraction methods (Table 3). Furthermore, the two-way interactions between flowering stage \times solvents, flowering stage \times UAE, and solvent \times pre-treatment method also showed highly significant differences ($p < 0.05$). Similarly, highly significant differences were observed in the case of the three-way interaction among flowering times \times solvents \times pre-treatment techniques. The maximum extract yield was observed in pre-treated full bloom flower extracts (MAF-1: 24.26 mg/g) followed by ultrasound-assisted ethanol based extracts of full bloom sample (MEF-1: 17.66 mg/g) while the lowest yield was recorded for late season flower samples extracted with hexane with CSE (MHL-2: 5.22 mg/g). The solvent extraction efficiency is determined by the polarity of the extracted compounds, which is referred to as the extraction power of the solvent (Essien et al., 2020). Apart from the polarity concept of solvents, yield also depends on extraction conditions such as time, temperature and assisted treatments (Manzoor et al., 2022; Moomin et al., 2023). Higher yields of acetone are also supported by previous research, which reported that it is an efficient solvent that has substantial potential to extract carotenoids from marigolds (Surendranath et al., 2016). Thus, the presented results showed that the maximum extraction yield could be obtained using flowers acquired at full bloom and with the ultrasound-assisted extraction (UAE) method as compared to conventional solvent extraction. The results also showed that among the various solvents employed for extraction, acetone yields higher for various growth stages compared to other solvents due to its polarity and efficiency in extracting polyphenolic compounds (Subbiah et al., 2020).

In another research study, marigold lutein extract yield was observed higher with acetone, i.e., 15–20 mg/g per gram of dry

marigold petal powder (Jalali-Jivan and Abbasi, 2020). Evidence suggests that acetone is not only recognized for its higher yield, but is also considered a safe, green, and environmentally friendly solvent for use in the food and pharmaceutical industries (Jalali-Jivan et al., 2021). The obtained results of average yield are also supported by a recent research database that compared conventional solvent and ultrasound-assisted extraction and confirmed that the ultrasound-assisted extraction yield was higher (21.23 mg/g) than that of conventional solvent extraction (Manzoor et al., 2022). Current research has confirmed that pre-treatments like ultrasonication improve the extraction of bioactive components like phenolics, and carotenoids like lutein and their retention during storage (Sowbhagya et al., 2013).

3.3 Phytochemical screening of marigold extracts

Evidence confirms that the phytochemical profile of extracts can vary based on factors such as the part of the plant being used, its growth conditions, maturity stage at the time of harvest, extraction method, and the type of solvent used for extraction (Youssef et al., 2020). Based on the literature and extraction results, the pre-treated samples were further employed for the phytochemical analysis of marigold extracts.

3.3.1 Total carotenoids content

Total carotenoid content in various extracts ranged between 263.11 and 427.39 mg per 100 grams (Table 4). The extract results showed that the maximum carotenoid content was recorded for MAF-1 (427.39 mg/100 g) followed by MHF-1 (375.76 mg/100 g) in full bloom marigold flower samples of peak season. In contrast to full bloom, total carotenoids of initial stage samples were observed higher in MAI-1 (354.49 mg/100 g) followed by MEI-1 (338.11 mg/100 g). However, the lowest carotenoid content was observed in hexane extracts of marigold flower samples of the end season (MHL-1: 263.11 mg/100 g). Late-season flower samples resulted in lower carotenoid content and presented a lower chroma index representing lower chroma index, as flower color is considered an important indicator of carotenoids that may be affected by environmental conditions. Literature reported that carotenoid content may vary

TABLE 3 Extract yield at various blooming stages with varying solvents and methods.

Samples	With pre-treatment UAE (mg/g)	Samples	Without pre-treatment CSE (mg/g)
MAI-1	17.16 \pm 0.76 ^b	MAI-2	12.31 \pm 0.30 ^s
MAF-1	24.26 \pm 1.03 ^a	MAF-2	13.71 \pm 0.17 ^f
MAL-1	15.80 \pm 0.96 ^{cd}	MAL-2	7.25 \pm 0.38 ^l
MHI-1	15.58 \pm 0.06 ^{cd}	MHI-2	10.60 \pm 1.13 ⁱ
MHF-1	16.16 \pm 0.42 ^c	MHF-2	11.70 \pm 0.29 ^{gh}
MHL-1	14.36 \pm 0.20 ^{ef}	MHL-2	5.22 \pm 0.94 ^k
MEI-1	15.08 \pm 0.48 ^{de}	MEI-2	11.82 \pm 0.29 ^s
MEF-1	17.66 \pm 0.08 ^b	MEF-2	10.79 \pm 0.66 ^{hi}
MEL-1	13.66 \pm 0.15 ^f	MEL-2	5.92 \pm 0.15 ^t

UAE: Ultrasound assisted extraction, CSE: Conventional solvent extraction method; Samples abbreviations explained in Table 1. Values in columns for individual variables with different lettering (a, b, c, d, e, f, g, h, i) are significantly different at $p < 0.05$.

TABLE 4 Total carotenoids, total phenolics, and total flavonoid content in marigold extracts at different stages in pre-treated samples.

Samples	Total carotenoid content (mg/100 g)	Total phenolic content (mg GAE/g)	Total flavonoid content (mg QE/g)
MAI-1	354.49 ± 1.29 ^d	77.75 ± 0.39 ^c	120.47 ± 0.67 ^c
MAF-1	427.39 ± 0.56 ^c	85.02 ± 0.81 ^a	130.41 ± 0.96 ^c
MAL-1	285.37 ± 0.94 ^f	64.15 ± 0.92 ^e	84.92 ± 0.86 ^e
MHI-1	278.89 ± 1.02 ^e	80.23 ± 0.51 ^b	116.76 ± 0.78 ^f
MHF-1	375.76 ± 0.99 ^b	74.14 ± 0.16 ^c	126.14 ± 0.94 ^c
MHL-1	263.11 ± 2.02 ⁱ	61.41 ± 0.97 ^b	72.56 ± 0.77 ⁱ
MEI-1	338.11 ± 1.02 ^c	72.86 ± 0.32 ^f	122.88 ± 0.88 ^d
MEF-1	366.92 ± 0.41 ^c	76.29 ± 0.44 ^d	128.39 ± 0.30 ^b
MEL-1	271.54 ± 0.83 ^h	58.86 ± 0.66 ⁱ	78.27 ± 0.42 ^h

Samples abbreviations explained in Table 1; GAE (Gallic acid equivalent), QE (Quercetin equivalent).

Values in each column for individual variable with different lettering (a, b, c, d, e, f, g, h, i) are significantly different at $p < 0.05$.

depending on the genotype (Akshaya et al., 2017), variety, part of the plant, and level of maturity (Duan et al., 2024). It also depends on the plant source, processing, preservation, storage conditions (heat), and forms (powder, concentrates, extracts, etc.) (Šivel et al., 2014). Among the carotenoids, lutein, zeaxanthin and cryptoxanthin are reported as significant contributors to color pigmentation in marigold flower petals. Marigold flowers of dark color and more petals are found to have high carotenoid content (Akshaya et al., 2017) and are suitable for xanthophylls' extraction and exploitation for commercial food applications.

Various studies have reported that marigold has 18 different carotenoid compounds (Rodrigues et al., 2019) including, lutein (Kashyap et al., 2022), zeaxanthin, α -carotene (Tiwari et al., 2022), β -carotene (Elvira-Torales et al., 2019), α -cryptoxanthin, β -cryptoxanthin (Dias et al., 2018), phytoene, phytofluene, α -zeaxanthin, β -zeaxanthin, neoxanthin and antheraxanthin (Sowbhagya et al., 2004). These results are in line with previous research that reported that carotenoid content in various genotypes ranged from 19 to 525 mg per 100 g marigold flower. *Tagetes* genotypes of dark orange to yellow-orange colored flower petals were found higher in total carotenoids (Akshaya et al., 2017). The higher carotenoid content observed in the marigold acetone extract (15–20 mg per gram) is supported by previous research (Jalali-Jivan et al., 2019). It has also been reported that hexane extracts have a lower carotenoid content because of the specific solubility, stability and absorptivity of carotenoids (such as lutein and beta carotene) in organic solvents (Surendranath et al., 2016). Similarly, previous studies have supported the idea that extraction methods significantly affect the content of biologically active components. Carotenoid recovery can be improved to 97% by modifying extraction methods and conditions (Duan et al., 2024). In the present study, carotenoid content was slightly higher than in previous studies, which may be due to the application of ultrasound (40% amplitude for 5 min) that facilitates solvent penetration and leaching of the inbound components (polyphenolics) by breaching the plant cell wall (Manzoor et al., 2022; Ordóñez-Santos et al., 2021).

The findings of the current research are also supported by previous research results (Tungmunnithum et al., 2020), which reported that the combination of ultrasound technique with extraction resulted in enriched bioactive components in extracts from medicinal plants that

can be utilized in functional food development. These results are also supported by previous evidence that ultrasound radiation causes cavitation in cells to free bound components, making it available for improved extraction (Jalali-Jivan et al., 2019). Thus, the extraction of carotenoids has been improved by employing ultrasound pre-treatment, which not only provides a high extraction yield but also lowers the degradation rate.

3.3.2 Total phenolic contents

Total phenolic content was determined in pretreated sample extracts against standard gallic acid using a spectrophotometer and expressed as GAE on a dry weight basis (Table 4). The results of quantification for total phenolic content varied between 58.86 mg GAE/g to 85.02 mg GAE/g. TPC values for ultrasound assisted acetone based extracts of fully bloomed marigold flower sample (MAF-1) resulted in higher phenolic content (85.02 mg GAE/g) followed by MAI-1 (80.23 mg GAE/g) UAE extracts (hexane × initial stage) of marigold flower samples. In contrast to these results, end season sample extract of MEL-1 exhibited the lowest phenolic contents (58.86 mg GAE/g).

The total phenolic content was observed higher with acetone, followed by ethanol and hexane for full bloom and initial stage samples, whereas for end stage samples TPC values were much lower even with acetone. This fact is supported by Subbiah et al. (2020) who reported that environmental conditions (high temperature/heat) cause a substantial reduction in phenolics and flavonoids in marigold flowers, which in turn causes a significant reduction in the antioxidant potential of extracts. Evidence reported that *Tagetes erecta* contains 18 phenolic acids and their derivatives, including gallic acid, quercetin, di-gallic acid, chromotropic acid, caffeic acid, syringic acid, *p*-coumaric acid, α -coumaric acid, ellagic acid, ferulic acid, iso-ferulic acid, sinapic acid, chlorogenic acid, protocatechuic acid, chlorogenic acid, *p*-OH benzoic acid, vanillic acid, and 3,4,5 tri-methoxy cinnamic acid (Manivannan et al., 2021; Moliner et al., 2018). Results for total phenolic content were correlated with previous research (Siriamornpun et al., 2012) that evaluated TPC content in marigold extracts 55–60 mg GAE per g DW in fresh and dried marigold flower samples, respectively. Another study reported total phenolic content in methanolic extracts of marigold 74.8 mg TAE per gram dry powder (Kang et al., 2018). Evidence reported that the highest TPC content

was recorded with polar solvents (ethanol) compared to non-polar solvents (hexane), 223 mg GAE and 3.1 mg GAE per gram of extract, respectively (Li et al., 2007). Literature also confirmed that extraction is a complex process that is not only influenced by the solvent or its polarity but also by the chemical and physical properties of components and their concentration in extracts (Zhu et al., 2020). Thus, the evaluated results with acetone as an efficient solvent for total phenolic content are supported by the fact that polar solvents have more potential to solubilize and extract phenolic compounds (Li et al., 2007).

3.3.3 Total flavonoid content

The total flavonoid content ranged between 72.56 and 130.41 mg QE/g in various marigold extracts (Table 4). The quantification results of total flavonoid content revealed that extract prepared with ultrasound assisted method showed a higher TFC value (130.41 mg QE/g) for MAF-1 followed by MEF-1 (128.39 mg QE/g) for full bloomed sample extracts. The total flavonoid content of the end-season sample extracts was the lowest for MHL-1 (TFC 72.56 mg QE/g). This variation might be based on the type of flavonoids that have different solubilities in different solvents. Evidence has reported that marigold has numerous important flavonoids mainly in glycosylated forms including flavanols; quercetin, quercetagenin, quercitrin, rutin (Wu et al., 2023), flavanols; catechin, kaempferol, flavones; apig-6-arbinose-8-galactose, apig-6-rhamnose-8-glucose, apig-7-neohesperoside, apigenin, acacetin neo-rutinoside, flavanones; naringin, naringenin, hesperidin, hesperetin, and patuletin (Rhama and Madhavan, 2011), 6-hydroxykaempferol-3-O-hexoside, etc. The extraction method is also important for obtaining potential flavonoids in the extract, which may result in higher antioxidant activities in *Tagetes* plant species (Ayub et al., 2017). It has been observed that extracts contain various flavonoids, mainly flavanols thereby, resulting in different antioxidant activities with different solvents based on the type of component, its solubility based on polarity (Youssef et al., 2020). Previous research confirmed that total flavonoid content varies by modifying the method of extraction thereby, giving a TFC value of 110 mg QE/g with the conventional solvent extraction method that increased to 140 mg QE/g with the advanced extraction method (SCFA). The obtained results are supported by another study that reported a higher TFC value (133 mg RTE/g) in dried marigold extract than in fresh flowers (TFC 107 mg RTE/g) (Siriamornpun

et al., 2012). The results also showed that the total flavonoid content was higher with acetone followed by ethanol in full-bloom sample extracts than in late-stage samples. This fact is supported by previous research by Li et al. (2007), who evaluated various cultivars of Chinese marigold flowers and reported that the total flavonoid content was higher in polar solvents like ethanol compared to hexane, varying from 28 to 93 mg RE per gram. The current study found a higher flavonoid content than that reported in previous studies due to ultrasound pre-treatment. These results are supported by recent evidence which confirmed that ultrasound radiation caused cavitation. The ultrasonic waves generate tiny bubbles that swiftly implode, releasing energy, shear strains, and micro-jets that enhance organic solvent infiltration and resulted cell wall disruption. The approach enhances mass transfer, extraction yield, and preservation of heat-sensitive bioactive components, hence resulting in higher bioactive content as compared to conventional extraction methods (Manzoor et al., 2022).

3.4 Antioxidant assays (DPPH, FRAP, ABTS)

The antioxidant potential is the free radical scavenging activity of MFP extracts against oxidants that cause oxidative damage to cells. The total antioxidant activity of the extracts was determined by the most suitable, reliable and easy-to-perform methods including DPPH, ABTS, and FRAP assays. The antioxidant potential of various marigold flower powder extracts, treated with ultrasound was evaluated in various solvents (Table 5). The highest DPPH radical scavenging activity was observed in acetone based extracts of full bloom flower samples (MAF-1: 96.19%) followed by MEF-1 (85.56%) of same stage samples. However, antioxidant activities in end-season/late flowering stage samples showed the least radical scavenging activities (MAL-1 > MEL-1 > MHL-1). Extracts of marigold flowers at full blooming showed significant scavenging activities owing to their potential to scavenge free radical ABTS^{•+}. The highest RSA was observed in the extract sample of MAF-1 followed by the MAI-1 (160.09 mmol TE/100 g and 141.23 mmol TE/100 g, respectively). However, the MHL-1 extract samples of MFP showed minimal free radical scavenging activity at 42.27 mmol TE/100 g (Table 5).

A ferric-reducing antioxidant power (FRAP) assay was performed to investigate the free radical scavenging potential of the MFP extract

TABLE 5 Antioxidant activities of marigold extracts at different blooming stages in pre-treated samples.

Samples	DPPH (RSA %)	ABTS (mmol TE/100 g)	FRAP (mmol TE/100 g)
MAI-1	82.88 ± 1.30 ^b	141.23 ± 2.71 ^b	110.32 ± 2.15 ^c
MAF-1	96.19 ± 1.95 ^a	160.09 ± 3.94 ^a	120.47 ± 2.45 ^a
MAL-1	57.31 ± 1.85 ^f	66.12 ± 2.62 ^g	83.01 ± 2.02 ^f
MHI-1	61.54 ± 1.64 ^e	72.76 ± 1.50 ^f	64.7 ± 1.51 ^g
MHF-1	68.49 ± 0.91 ^d	86.54 ± 1.53 ^e	98.29 ± 2.16 ^d
MHL-1	40.11 ± 1.32 ^h	42.27 ± 0.89 ^j	53.2 ± 2.26 ⁱ
MEI-1	73.65 ± 1.45 ^c	132.19 ± 2.03 ^d	89.76 ± 2.03 ^e
MEF-1	85.56 ± 2.16 ^b	138.82 ± 1.5 ^c	112.19 ± 2.34 ^b
MEL-1	53.72 ± 1.93 ^g	57.33 ± 1.99 ^h	61.55 ± 1.47 ^h

Samples abbreviations explained at Table 1; RSA % (Radical scavenging activity percentage), TE (Trolox equivalent).

Values in each column for individual variable with different lettering (a, b, c, d, e, f, g, h, i) are significantly different at $p < 0.05$.

by estimating the conversion rate of ferric ions (Fe^{+3}) concentration to ferrous ions (Fe^{+2}). The results showed that marigold flower samples of full bloom had higher reducing power (FRAP) for the extracts compared to other stage samples. Ferric radical scavenging activities were highest in MAF-1 (120.47 mmol TE/100 g) followed by MEF-1 (112.19 mmol TE/100 g) marigold full-bloom flower extract samples. In contrast to this MHL-1 showed the lowest reducing power (53.20 mmol/TE/100 g). Among the extracts the highest radical scavenging activities (DPPH and ABTS) and reducing power assay (FRAP) were observed highest in acetone based extracts followed by ethanol and hexane extracted samples at various stages. This fact was supported by research evidence which reported that phenolic and flavonoid compounds are hydrophilic in nature and have better solubility in polar solvents (like acetone, and ethanol) (Shi et al., 2024; Subbiah et al., 2020). Thus, the increase in phenolic and flavonoid contents may improve the antioxidant activities of MFP extracts (Gong et al., 2012).

Furthermore, it was seen that various *Tagetes* species exhibit percent inhibition of DPPH in ethanol up to 89% (Ingkasupart et al., 2015) and 92% DPPH in methanolic extract (Četković et al., 2004). Another research also investigated the antioxidant activities of marigold extracts and reported that antioxidant potential is correlated with the content of total phenolics and flavonoids in extracts (Li et al., 2007). The observed DPPH radical scavenging potential was also supported by previous research studies in which marigold genotypes were investigated, and it was observed that DPPH % ranged between 68 and 82% (Akshaya et al., 2017). Literature reported that orange cultivars of *Tagetes* represent the higher DPPH % recorded between 76.16 and 89.90% (Ingkasupart et al., 2015). The higher inhibition percentage of MP acetone extract is supported by Surendranath et al. (2016) who reported that it has more potential to extract lutein from samples and other phenolics that reduce oxidative stress by donating electrons to scavenge free radicals and turn it into a stable form. Results for FRAP and ABTS⁺ were also in agreement with previous research, which reported that radical scavenging potential is positively correlated with the bioactive contents of extracts. The highest scavenging activity (ABTS⁺ 172 mmol TE) was observed in the genotype with the highest flavonoid content, while the highest reducing power (FRAP 141 mmol TE) was observed in the genotype with the highest phenolic content (Manivannan et al., 2021). Lower antioxidant activities in late season samples may also result from stressful environmental conditions such as higher temperature and genetic factors may affect the flavonoid content (Aryal et al., 2019; Shi et al., 2024), which in turn cause significant reduction in antioxidant potential (Gong et al., 2012) that depends on the number and position of free hydroxyl groups of flavonoids.

Our results showed a large variation in radical scavenging activities with various solvents at different marigold flowering stages. This variation is supported by previous studies that compared total antioxidant activities (DPPH, FRAP, ABTS⁺) in MF extracts prepared using conventional solvent extraction (CSE) and ultrasound-assisted extraction (UAE) (García-Castello et al., 2015). Literature has reported that the UAE method resulted in higher radical scavenging activities (DPPH 92.87%; ABTS⁺ 0.74 mM TE) and reducing power (FRAP 0.95 mM TE) per gram dry weight of marigold flower petals compared to conventional solvent extraction (Manzoor et al., 2022). In conclusion, the study results support the fact that UAE has no adverse effect on the antioxidant potential of the matrix indicating no

degradation of bioactive components such as phenols and flavanols that are the major contributors to scavenging activities. Plant growth, flowering stages, season, and maturity may cause considerable variation in the radical scavenging and antioxidant activities of the extract. Also, the choice of solvent depends on the target compounds, which have a specific nature to be extracted based on their polarity and solubility. The antioxidant potential of marigold flowers and extracts has been exploited in commercial food and pharmaceutical industries owing to its potential to treat various chronic disorders, cognitive, ophthalmological, NAFLD and age-related disorders such as AMDs.

3.5 Quantification of bioactive compounds in marigold extracts

Quantification of bioactive compounds including phenolics, flavonoids and carotenoids in various marigold extracts was performed through HPLC-DAD. Presented results showed the distribution of various bioactive compounds at various wavelengths (λ) and retention time (RT) including phenolic acids [gallic acid (RT 5.76 min; 280 λ), coumaric acid (RT 38.36 min; 313 λ), syringic acid (RT 29.33 min; 280 λ), sinapic acid (RT 42.02 min; 313 λ)], flavonoids [quercetin (RT 54.63 min, 350 λ), catechin (RT 22.34 min; 280 λ) and kaempferol (RT 56.77 min; 350 λ)] and carotenoids [lutein (RT 6.03 min; 450 λ), zeaxanthin (RT 7.24 min, 450 λ) and fucoxanthin (RT 4.79 min; 450 λ)] in different solvent extracts of marigold (Table 6).

The highest total concentration of bioactive compounds was observed in ultrasound treated acetone extract (MAF-1) followed by ethanol extract. Among phenolic acids, gallic acid found in all extract samples while coumaric acid and syringic acid were not detected in hexane extracts. Other important class of polyphenolics detected was flavonoids including Quercetin, catechin and kaempferol. Highest concentration of Quercetin was found in MAF-1 (11.74 mg/g) followed by MEF-1 (10.90 mg/g) while the lowest concentration was observed in MHF-1 (0.92 mg/g). Among carotenoids; lutein was detected (0.41–1.76 mg/g) in all extract samples except late stage extracts of marigold flower. This may be due to the loss of bioactive compounds that are thermolabile and can be easily degraded at higher temperature (Jalali-Jivan et al., 2021). Lutein and zeaxanthin detected at specific wavelengths (430–575 nm) and retention time is also in line with previous studies of Kashyap et al. (2022) and Liu et al. (2011).

Current research findings supported by previous literature reported that efficient extraction of carotenoids is highly dependent on appropriate solvent selection (Becerra et al., 2020). Results showed that marigold hexane extracts resulted least content of lutein and other carotenoids not detected in all stage sample extracts (Table 6). This may also be resulted due to higher polarity of xanthophylls than the extracting solvent (hexane), making it more suitable for non-polar carotenoids (like carotenes) than polar xanthophylls. Presented results are also supported by previous study reported that acetone resulted highest lutein recovery due to its strong ability to dissolve polar carotenoids (Low et al., 2020). Evidence also reported that the polar functional groups of lutein and zeaxanthin make them more soluble and extractable in polar solvents like acetone, ethanol and tetrahydrofuran than hexane (Saini and Keum, 2018; Kopec et al., 2012). Morón-Ortiz et al. (2024) reported that organic solvents pose

TABLE 6 Quantitative comparative analysis of bioactive compounds in various ultrasound extracts of marigold flowers.

Samples	Phenolic acid			Flavonoids			Carotenoids			Sum of bioactive compounds	
	Gallic acid (mg/g)	Coumaric acid (mg/g)	Syringic acid (mg/g)	Sinapic acid (mg/g)	Quercetin (mg/g)	Catechin (mg/g)	Kaempferol (mg/g)	Lutein (mg/g)	Zeaxanthin (mg/g)		Fucoxanthin (mg/g)
MEI-1	32.72 ± 0.08 ^d	2.43 ± 0.49 ^a	-	2.76 ± 0.22 ^a	6.96 ± 0.49 ^a	-	-	1.40 ± 0.12 ^{bc}	-	-	46.26
MEF-1	37.76 ± 0.13 ^c	1.59 ± 0.01 ^b	1.84 ± 0.20 ^a	2.19 ± 0.01 ^a	10.90 ± 0.38 ^b	-	-	1.66 ± 0.17 ^{ab}	2.03 ± 0.06 ^c	0.27 ± 0.05 ^b	58.0.23
MEL-1	9.51 ± 0.15 ^e	1.66 ± 0.30 ^b	-	-	1.14 ± 0.04 ^c	-	1.77 ± 0.18 ^a	-	1.04 ± 0.04 ^c	-	15.11
MAI-1	42.94 ± 0.23 ^b	1.56 ± 0.16 ^b	-	2.35 ± 0.33 ^a	1.45 ± 0.07 ^c	1.60 ± 0.07 ^a	-	1.28 ± 0.09 ^{bc}	-	-	51.17
MAF-1	109.67 ± 0.77 ^a	2.39 ± 0.08 ^a	1.62 ± 0.08 ^a	2.58 ± 0.57 ^a	11.74 ± 0.35 ^b	-	-	1.76 ± 0.30 ^a	2.14 ± 0.09 ^a	1.02 ± 0.01 ^a	132.92
MAI-1	9.52 ± 0.11 ^e	1.71 ± 0.12 ^b	-	-	2.80 ± 0.10 ^d	-	1.76 ± 0.01 ^a	-	-	-	15.79
MHI-1	1.40 ± 0.10 ^g	-	-	-	-	-	-	1.15 ± 0.16 ^c	-	-	2.55
MHF-1	6.00 ± 0.18 ^f	-	-	-	0.92 ± 0.06 ^c	-	1.80 ± 0.13 ^a	1.52 ± 0.11 ^{abc}	1.51 ± 0.14 ^b	-	11.74
MHL-1	1.45 ± 0.07 ^g	-	-	-	-	-	-	0.41 ± 0.05 ^d	-	-	1.86

Samples abbreviations explained at Table 1; Values in each column for individual variable with different lettering (a, b, c, d, e, f, g, h, i) are significantly different at $p < 0.05$.

high risk (health and environmental) and resulted relatively low carotenoid yield upon extraction.

Presence of phytochemicals like phenolic acids in marigold extract samples also reflects that extract has good pharmaceutical and nutritional properties. Gallic acid ($C_7H_6O_5$) is a natural phenolic compound mostly present in medicinal plants and has known for its pharmacological activities like anti-inflammatory, cardiovascular, gastrointestinal, neuropsychological and metabolic functions (Kahkeshani et al., 2019). Quercetin ($C_{15}H_{10}O_7$) is an important member of flavonoid which is highly recognized for its antioxidant, anti-cancerous and antiaging properties (Shi et al., 2023). Lutein ($C_{40}H_{56}O_2$) consists of hydroxyl group and has ability to cross the blood ocular barrier (BOB) and helps to control age related macular disorders (Duan et al., 2024).

3.6 Chroma color analysis

Color is a significant aspect of the flower as it reveals the presence of valuable components such as carotenoids. Color values play a significant role in determining the qualities and nature of pigments in flower petals. The chroma color of the marigold flower samples was measured at various blooming stages and then its extract was prepared to compare the color index for each stage (Table 7). A highly significant effect of flowering stages on the chroma values of marigold extracts was observed in our study. CIELAB values showed the highest mean chroma index in full bloom stage marigold extract and petals C^* 93.70 and 70.84, respectively. L^* was the highest (68.17) in the initial stage sample extract whereas b^* was the highest (86.37) in the full bloom marigold extract. While the lowest chroma index was recorded in the petals of marigolds during late stages/end season. These results are supported by literature that investigated diverse species of *Tagetes* and reported higher chroma values (C^* 79) of orange cultivars as compared to light yellow to off-white petals (C^* 42). The CIE b^* value reflects the yellow direction of color and is observed higher in extracts than in powder (Manivannan et al., 2021). This fact is supported by the evidence that the marigold coloring component in flower petals is in the bound form (oleoresin). The extract was prepared and purified from oleoresin to free lutein resulting in a higher chroma color index (Jalali Jivan and Abbasi, 2019). The opposite trend was observed for the hue compared to the chroma color index when the results for both petals and extracts were compared. Hue is the angular measurement ranging between 0° and 90° representing the red-to-yellow color index. The presented results showed that hue degrees were higher for flower samples compared to extracts. In contrast to the chroma index, the maximum hue value (88.08) was recorded for the marigold flower petals. These chroma color values indicate the saturation and intensity of the coloring pigment in the samples. The flowers of *Tagetes erecta* L. are orange to yellow in color; therefore, its coloring component saturation may be higher at the initial and full bloom flowering stages than in the late season. At the full bloom stage, when the flower blooms, its petals increase in number and size, and the content of carotenoid compounds increases (Youssef et al., 2020).

The presented results were also supported by previous literature reporting that the extraction method and solvent polarity (interaction with free lutein from oleoresin) resulted in a carotenoid-rich extract that showed a higher chroma index (Kurniawan et al., 2019). This

TABLE 7 Mean chroma color index at different blooming stages of marigold flowers and its extracts.

Samples	Stages	L	a*	b*	Chroma index	Hue
Marigold flower petals color	Initial	59.93 ± 0.94 ^a	10.13 ± 0.56 ^b	60.79 ± 0.92 ^b	61.63 ± 0.98 ^b	80.54 ± 0.40 ^b
	Full bloom	56.69 ± 1.14 ^b	13.98 ± 0.03 ^a	69.44 ± 2.00 ^a	70.84 ± 1.96 ^a	78.61 ± 0.33 ^a
	Late	44.27 ± 0.55 ^c	1.18 ± 0.09 ^c	35.35 ± 0.82 ^c	35.37 ± 0.82 ^c	88.08 ± 0.16 ^c
Marigold extract color	Initial	68.17 ± 0.78 ^a	25.21 ± 1.48 ^b	75.02 ± 1.37 ^b	79.14 ± 1.76 ^b	71.43 ± 0.73 ^a
	Full bloom	54.63 ± 0.71 ^c	36.34 ± 0.99 ^a	86.37 ± 0.76 ^a	93.70 ± 1.08 ^a	67.19 ± 0.38 ^c
	Late	61.11 ± 0.98 ^b	26.72 ± 0.60 ^b	67.19 ± 0.70 ^c	72.30 ± 0.86 ^c	68.32 ± 0.25 ^b

Values in each column for individual variable with different lettering (a, b, c) are significantly different at $p < 0.05$.

TABLE 8 Effect of different levels of supplementation on sensory characteristics of the supplemented drink.

Treatments	Color	Aroma	Taste	Mouth feel	Overall acceptability
T ₀	7.56 ± 0.057 ^d	7.50 ± 0.042 ^c	7.95 ± 0.042 ^d	7.81 ± 0.06 ^a	7.43 ± 0.042 ^d
T ₁	7.87 ± 0.06 ^c	7.75 ± 0.04 ^b	8.05 ± 0.014 ^a	7.78 ± 0.015 ^b	7.74 ± 0.014 ^b
T ₂	8.35 ± 0.057 ^b	8.22 ± 0.014 ^a	7.83 ± 0.013 ^b	7.67 ± 0.014 ^c	8.12 ± 0.014 ^a
T ₃	8.65 ± 0.035 ^a	8.35 ± 0.071 ^a	7.82 ± 0.028 ^c	7.65 ± 0.01 ^d	7.63 ± 0.028 ^c

Values in each column for individual variable with different lettering (a, b, c, d) are significantly different at $p < 0.05$.

Marigold pure extract supplemented as; T₀ Control (0 mg), T₁ (1 mg), T₂ (5mg), and T₃ (10mg).

indicates that at the bloom stage flower color intensity increases which reflects the concentration of coloring compounds, and carotenoid content, resulting in higher antioxidant potential (Manivannan et al., 2021). The coloring characteristic of the marigold flower is imparted by these coloring agents that are majorly saturated in its petals. Conclusively, dark-colored *Tagetes* species and their extracts are rich repositories of carotenoid coloring compounds (like lutein) that have the potential to be utilized as natural colorant (E 161b) in food and also have a significant therapeutic effect in pharmaceuticals (Nwachukwu et al., 2016).

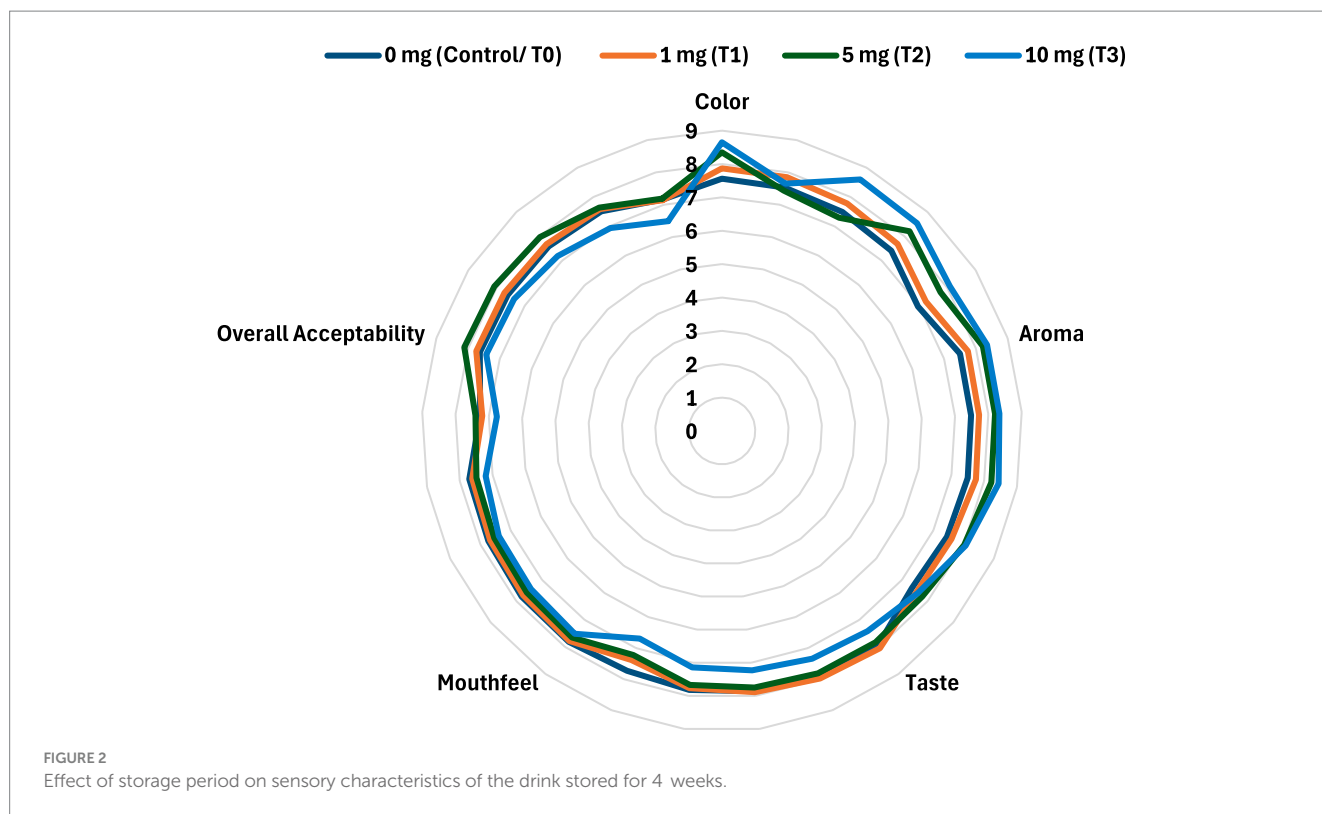
3.7 Product sensory attributes

Organoleptic evaluation of the supplemented drink confirmed that marigold extract can improve the sensory profile. The results of sensory attributes of the supplemented drink showed a significant effect of supplementation of the marigold extract on its sensory characteristics. It was observed that T₃ has a maximum color score (8.65) followed by T₂ (8.35), whereas the lowest score was recorded 7.56 for T₀. Overall sensory results for T₀ and T₁ were similar and observed closely due to the levels of supplementation in T₁.

Taste and aroma are important parameters for the sensory evaluation of products. The results for aroma and taste varied between 7.50–8.35 and 7.95–8.05, respectively. The highest scores for aroma and taste were recorded for T₃ (8.35) and T₁ (8.05), followed by T₂ (8.22) and T₀ (7.95), while the lowest scores were recorded for T₀ (7.50) and T₃ (7.42), respectively. The mouth feel and overall acceptability ranged between 7.81–7.65 and 8.12–7.63, respectively. The overall acceptability was recorded highest for T₂ (8.12), followed by T₁ (7.74) > T₃ (7.63) (Table 8). Taste and aroma are crucial parameters in the sensory evaluation and storage study of drinks. The results showed that supplementation imparts a pleasant aroma and color to drink which was recorded highest for T₃ followed by T₂. Evidence reported that the aroma of the product was improved with the supplementation of marigold because of its aromatic flavoring compounds (pipertone, piperitone, α -terpineol, terpinolene,

etc.) (Moghaddam et al., 2021). The results for flavor improvement were also supported by Chitrakar et al. (2019) who reported that essential oil components such as isothiocyanates may impart flavor to supplemented food products made with edible flower extracts. The obtained results are also supported by the literature that supplementation with marigolds has good coloring potential that significantly improves the product's sensory attributes and overall acceptability compared to the control. The sensory profile for color is in line with the previous studies that reported that supplementation with marigold lutein significantly improved the sensory characteristics of the product, including aroma, taste, color, and overall acceptability (Kim et al., 2023; Xavier et al., 2018).

The storage study of the supplemented drink was also performed for about 1 month (Figure 2). The results showed that the color of T₀ (control) was observed lowest (6.96) compared to other treatments at the end of the storage period. The highest color value was observed for T₃ (8.09) followed by T₂ on the 27th day of storage. At the end of the storage, T₂ scored the highest (7.79) followed by T₃ (7.63) for aroma, whereas T₀ scored the least (7.39). The highest score for taste was observed for T₀ (7.73) followed by T₁ (7.38) and T₂ (7.22) even on the 27th day of storage. The color of T₃ was stable but the sensory score for taste and mouthfeel of T₃ samples decreased to 6.69 and 6.76, respectively at the end of the storage period. The overall acceptability of the supplemented drink (T₃) was also scored lowest (6.49) while the highest (7.19) was the case for T₂ (on the 27th day) with the progression of the storage period. In contrast to T₃ other treatments' taste and overall acceptability were not significantly affected by storage time and conditions, this may be a result of higher supplementation levels that make juice a little more viscous and darker colored as marigold extract was purified to concentrate bioactive lutein. Also, carotenoids separated in food sources into the mouth while ingestion do not have much impact on food characteristics however release rate may be affected by the increased concentration or content of carotenoids in supplemented foods (Geng et al., 2023). Results are also supported by previous research reported that carotenoids are susceptible to degradation during storage by certain factors mainly oxygen, light, and heat (increased in temperature) (Weigel et al., 2018). The higher the content



the greater the reactivity of bioactive compounds. The overall sensory characteristics of functional food products are affected by storage conditions and duration.

Marigold has been widely investigated as an additive and supplement to food products to replace artificial additives and to add product nutritional value (Corbo et al., 2014). Literature reported that marigold supplementation in various food products not only improved the sensory characteristics (especially color) of the developed products but is also expected to deliver various health benefits that may result from its bioactive components (Becerra et al., 2020). In conclusion, organoleptic evaluation of the product showed that treatment with 5 mg had similar and/or greater acceptance than the control treatment. The recommended dietary allowance for lutein may be accomplished by supplementation into the most popular foods in all age groups. Therefore, developing a functional drink with marigold supplementation is a feasible strategy. Literature reports that marigold-supplemented products play an important role as nutrient carriers, accomplish lutein daily intake, and improve its bioavailability and accessibility which may result in delaying various age-related disorders (Bhat et al., 2022).

3.8 Correlation and principal component analysis of extract with phytochemicals and antioxidant assays

Correlation analysis was performed, and a highly significant and positive correlation was observed among all parameters. The relationship between the extract yield, TCC, TFC, and TPC, and antioxidant activities of the plant was investigated. Some studies have reported that antioxidant activities have a non-significant association

with phytochemical compounds in plants, whereas other studies have presented a positive and highly significant correlation between bioactive compounds and antioxidant assays.

Yield showed a highly significant and positive correlation with all parameters under consideration (TPC, TFC, TCC, and all antioxidant assays) (Table 9). Total phenolic contents are also positively and significantly associated with all other attributes (TFC, TCC, DPPH, and ABTS). TPC was highly correlated with TFC (0.915), whereas it was the least correlated with FRAP (0.778). A highly significant correlation with all antioxidant assays (DPPH, ABTS, and FRAP) was recorded with total phenolics and carotenoids. DPPH showed a significant and positive association with both ABTS (0.963) and FRAP (0.936) while the ABTS was strongly associated with the FRAP (0.896).

The PCA outcomes showed that the first principal component (PC-I which only has Eigenvalue >1) accounted for 84.76% of overall variation, while PC-II and PC-III have Eigenvalues <1 and contributed approximately 7 and 4.78% in overall variance, respectively. It was observed that the first three PCs cumulatively described approximately 96.54% of the total variability. The partial weight of each parameter for each of the PCs was determined, and the findings revealed that the carotenoid pigmentation and radical scavenging activity assessed using DPPH, ABTS, and FRAP accounted for approximately 61.15% of the overall variation in PC1, with DPPH (16.24%) contributing the most, followed by TCC (15.14%) together with ABTS (14.89%), and FRAP contributed the least (14.88%).

In PC-I total flavonoid content (13.52%), total phenolics (13.19%), and extract yield (12.13%) imparted the least variability. Variation assessed by flavonoids, phenolic content, and extract yield contributed approximately 87.67% in PC-II, with an individual proportion of 36.14%, 26.15, and 25.38%, respectively. Moreover, in PC-II, variables

TABLE 9 Linear correlation between yield, total phenolic content, total flavonoid content, total carotenoids and antioxidant assays.

Variables	Yield	TPC	TFC	TCC	DPPH	ABTS
TPC	0.736*					
TFC	0.587	0.915**				
TCC	0.826*	0.746**	0.821*			
DPPH	0.818*	0.835**	0.854**	0.909**		
ABTS	0.736	0.772**	0.812*	0.859**	0.963*	
FRAP	0.778*	0.712*	0.778	0.936**	0.936*	0.896*

*Significant correlation with $p \leq 0.05$; ** Significant correlation with $p \leq 0.01$.

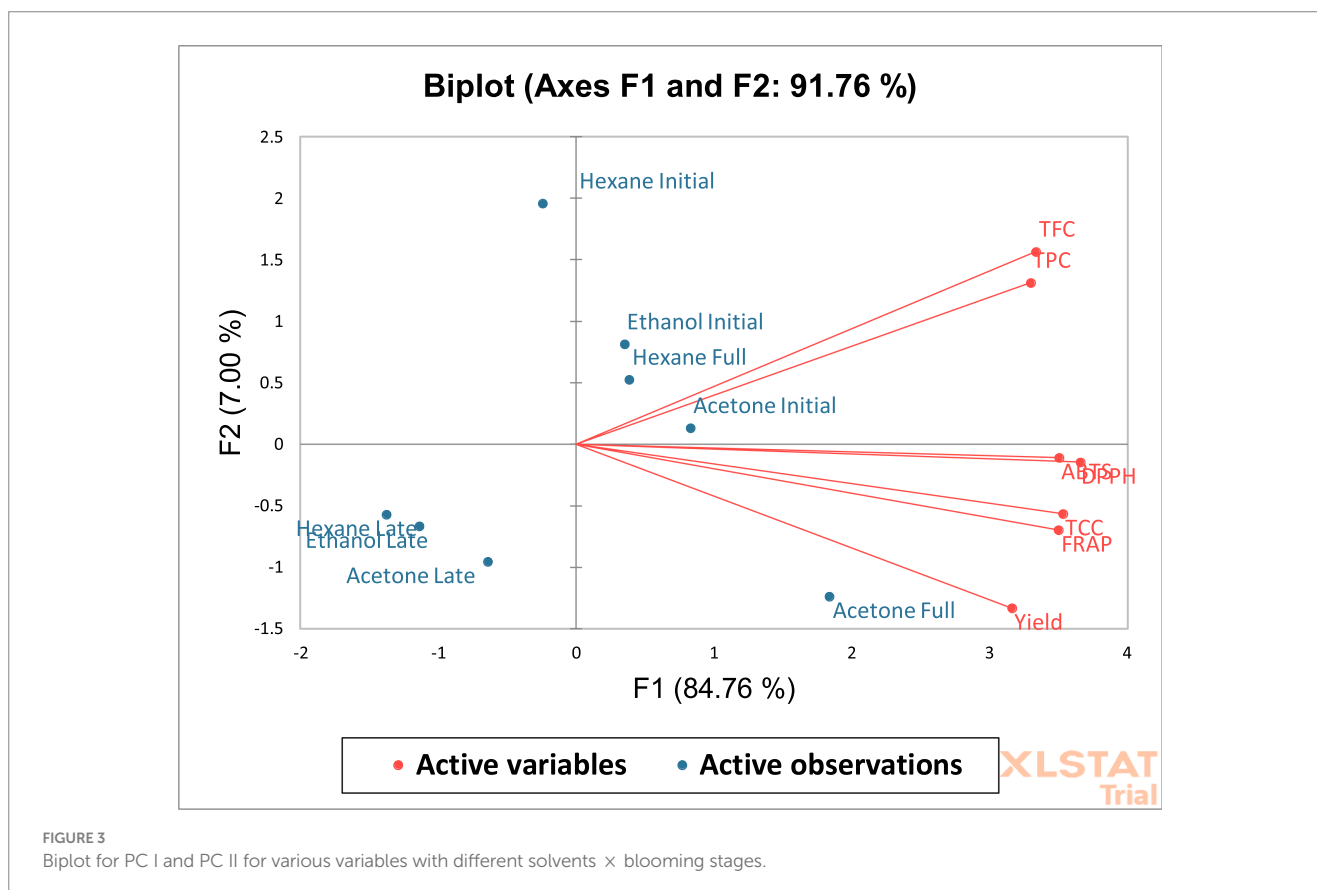


FIGURE 3 Biplot for PC I and PC II for various variables with different solvents × blooming stages.

like TCC and all antioxidants' assays cumulatively accounted for less than 15% of the overall variance, contrasting opposite findings were recorded for these factors in PC-I. Individually extract yield accounted for 45.05% of the total variation, while extract yield together with TPC accounted for approximately 70.43% variance in PC-III.

A PCA biplot (union of vectors for parameters and mapping of solvent × flowering) was developed and the vector stretch for individual parameters was drafted from the origin in order to unveil the association among the variables by the cosine of the angles among various vector stretches. The PCA biplot revealed that the strong association among variables acquired the position in the same quadrant as presented in Figure 3. DPPH with ABTS and TCC with FRAP, showed positive and strong correlations with each other as represented by the angular proximity of the vectors, whereas yield showed a positive and moderate association with all described factors in the 1st quadrant (+, -). Similarly, in the 2nd (+, +) quadrant, angular proximity is quite evident among

TFC and TPC which corresponds to a positive and strong association between both variables. Moreover, the cosine of the angles presents a positive correlation among all parameters under consideration (TFC, TPC, ABTS, DPPH, TCC, FRAP, and extract yield). PCA biplots for specific solvent × flowering stage such as acetone × full bloom which has higher extract yield, carotenoid content, TFC, TPC and antioxidant activity (DPPH, ABTS, and FRAP) were plotted quite adjacent in the 1st quadrant (+, -). Solvent × flowering stages which are rich in TFC and TPC, took the position in the 2nd quadrant (+, +), while solvent × flowering stage which have shown quite low values for these factors were quite far away from each other (factors from solvent × flowering stage) and positioned in the 4th quadrant (-, -).

A significant correlation between carotenoids, phenolics, flavonoids, and antioxidant assays showed that the phytochemical compounds of marigold extracts could be the principal contributors to antioxidant activities. Previous research has supported the positive

and highly significant correlation between the total phenolics, total flavonoids, and antioxidant activities of flower extracts (Youssef et al., 2020). Some other databases also found a positive correlation of total phenolic contents, carotenoid contents, and radical scavenging activities of plant extracts (Akshaya et al., 2017; Kaisoon et al., 2011).

4 Conclusion

The study concluded that ultrasound-treated marigold flower samples not only resulted in better yield but also contained more bioactive compounds including total carotenoids, total phenolic compounds, and flavonoids. Full bloom sample extracts (MAF-1) exhibited higher phenolic contents and antioxidant activities through ultrasound assisted extraction. Further, HPLC quantification also confirmed phenolics and carotenoids bioactive compounds (gallic acid, coumaric acid, quercetin, catechin, lutein, zeaxanthin) in higher concentration in polar organic solvent extracts of marigold full bloom flower samples. Study findings highlight the significant potential of *Tagetes* extracts and its suitability as food processing additive or fortificant in nutritional supplements or functional products. However, there is gap in existing literature regarding relationship between bioactive polyphenolic compounds, their blooming stages and bioavailability. Future study planned to fully evaluate *Tagetes* flowers (MFP) for its polyphenolic compounds with focus on exploring these aspects via LCMS-QToF MS/MS and in-vitro bioaccessibility through stimulated digestive model to confirm bioactive compounds recovery and bioaccessibility at various stages of digestion.

Data availability statement

The data that support the findings of this study are available on request from the corresponding author.

Author contributions

AyS: Conceptualization, Methodology, Writing – original draft, Formal analysis, Investigation, Data curation. AK: Project administration, Conceptualization, Writing – review & editing. TM: Conceptualization, Investigation, Methodology, Writing – review & editing. MC: Conceptualization, Funding acquisition, Data curation, Writing – review & editing. AS-M: Data curation, Investigation, Writing – review & editing. SA: Conceptualization, Data curation,

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fsufs.2025.1474848/full#supplementary-material>

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