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Nutrients, bioactive compounds and antinutritional properties of marigold genotypes as promising functional food

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The rising demand for health promoting functional foods has sparked interest in diversifying diets by incorporating innovative items like edible flowers. Considering this, the nutritional composition, bioactive properties and antinutritional factors of the flowers of eight marigold genotypes (M_1 to M_8) were quantified and compared to elucidate their value and safety as functional food. The study outcomes highlighted significant differences among the genotypes for most of the attributes. Anthocyanin, carotenoid and lutein contents were observed within a range of 0.02 to1.90 mg/100 g, 5.02 to 11.08 mg/100 g and 0.19 to 9.78 µg/g, respectively. The content of sugars, β-carotene, vitamins (C and E) and minerals (sodium, potassium, calcium, magnesium and iron) were also found to be present in substantial amounts. The analysis of bioactive compounds revealed the richness in total phenolic (TPC) (428.58 to 592.71 mg gallic acid equivalent (GAE)/100 g) and flavonoid content (TFC) (135.06 to 233.39 mg quercetin equivalent (QE)/100 g). Among the assessed antinutrients, alkaloid, tannin and saponin exceeded permissible limits in the studied genotypes, while phytate remained within the safe range. However, the elevated levels of these antinutrients would not pose any problem if processed through methods such as soaking, boiling or cooking. Out of eight genotypes, M_1 had the highest content of anthocyanin (1.90 mg /100 g), reducing sugar (21.63 mg/100 g), and antioxidant activities. M_5 stood out with the highest levels of TSS (6.10 °Brix), β-carotene (0.50 mg/100 g), vitamin C (28.61 mg/100 g), Ca (225.33 mg/100 g), and TPC (592.71 mg GAE/100 g), while M_6 contained significant amounts of carotenoids (11.08 mg/100 g) and TFC (232.41 mg QE/100 g). Principal component analysis and cluster dendrogram findings further confirmed that among the eight studied genotypes, M_1, M_5 and M_6 genotypes were found as the most prominent with the remarkable contributions of the majority of the studied variables. Hence, these marigold genotypes could be considered as promising options to improve and diversify healthy diets, potentially serving as valuable sources of dietary supplements and functional food ingredients.

Keywords Antioxidant activity, Carotenoid, Edible flower, Lutein, Phytate, Secondary metabolites

Today's changing dietary habits and growing health awareness have brought about a significant shift in nutrition and food consumption scene that motivated consumers to look for functional foods as a key part of their nutrition plan[1]. Functional foods defined as food products that offer physiological benefits beyond basic nutrition, represent a proactive approach to wellness, harnessing the potential of bioactive compounds to promote health and prevent diseases[2]. Hence, there is an urgent demand to diversify dietary intake to enhance the nutritional quality of diets through the incorporation of functional foods.

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The quest for new sources of functional food offers the chance to include innovative items such as edible flowers, thereby diversifying our culinary experiences. Unlike various fruits and vegetables, edible flowers hold cultural significance as traditional food items in many regions globally, where they have been integrated into cuisines for centuries due to their aesthetic appeal and taste[3]. In general, the chemical composition of edible flowers resembles that of other plant-based foods, with high water content, low levels of fat and protein, and varying amounts of carbohydrates and minerals[4]. Several studies suggest that they possess numerous phytochemicals that play a role in their visual appearance, such as colorants like anthocyanins and carotenoids, as well as bioactive properties like phenolic compounds, flavonoids and other antioxidants[5]. The appeal of these compounds is directly linked to the wide range of health benefits they may offer when consumed, especially their antioxidant potential and various bioactivities, such as anti-inflammatory activity, cardioprotection and prevention against certain types of cancer[6]. Therefore, integrating edible flowers into newly designed recipes and food formulations could enrich the palette in human diet and broaden the dietary sources of nutrients and bioactive compounds on a daily basis.

Marigold (*Tagetes* sp.), an important flower in the Asteraceae family, is extensively grown for ornamental, poultry and medicinal uses[7]. Originating from Mexico, it has been adapted in various regions across the tropics and subtropics, including India and Bangladesh[8]. Due to its vibrant bright yellow and orange color and its ability to thrive in diverse environmental conditions, it has become one of the major loose flower crops in Bangladesh. Different parts of this plant, including its flowers, are employed in folk medicine to tackle various health concerns and are utilized as natural insecticides, insect repellents, herbicides, bactericides and fungicides[9,10]. Furthermore, its flowers are added as an ingredient in salads, curries, tea, condiments and used as flavoring and coloring agents[11]. Phytochemical studies of its various parts have identified a range of chemical constituents, such as thiophenes, flavonoids, carotenoids and triterpenoids. The flower contains carotenoids including lutein, zeaxanthin, neoxanthin, violaxanthin, ß-carotene, lycopene, α-cryptoxanthin, phytoene and phytofluene[9]. In recent times, the significant lutein content in marigold has received increased attention, primarily due to growing physiological evidence supporting its role in maintaining human health and preventing diseases[12]. Furthermore, its flower extracts exhibited promising antioxidant properties, suggesting the potential utilization of this flower as functional food ingredients, thus opening up new avenues for exploration[7].

Besides the potential health benefits edible flowers offer, several safety concerns are associated with the presence of potential poisonous compounds[6]. Therefore, the varying composition of edible flowers, particularly new species not commonly or traditionally used in cooking, should be carefully considered due to the potential presence of unsafe compounds that could endanger human life. Even though marigold flowers can be considered as food sources, they have not been sufficiently exploited from the nutritional and phytochemical points of view in Bangladesh. Rather, large quantities of marigold flowers are usually discarded after use, disregarding their nutritional and functional value and contributing to environment pollution. Thus, the diversification of its utilization from aesthetic to functional food sources has the merits for reusing the waste as value-added products. Moreover, flowers of all the species may not be deemed edible due to the existence of antinutritional factors[4]. This fact warrants comprehensive research attention from the perspective of consumers who use flowers based on traditional knowledge without any medical or pharmaceutical supervision. Based on the aforementioned facts, it has been hypothesized that the marigold flowers may be effectively used as functional food with considerable amount of nutrients, bioactive compounds and less risk of antinutritional properties. Hence, the present research was undertaken to ascertain the nutrients, secondary metabolites and antinutritional properties of marigold genotypes and to screen out suitable marigold genotypes with better edible quality to be considered as functional food for improving and diversifying a healthy diet.

Materials and methods

Sample collection

Eight marigold genotypes with diversified flower size and color were chosen for the study (Supplementary Fig. 1) like M_1- deep maroon (small), M_2 - yellow petal with deep maroon tip (small), M_3 - reddish petal with orange edge (small), M_4 - deep orange (small), M_5 - bright yellow (small), M_6 - light yellow (large), M_7- light orange (large) and M_8 - bright orange (large). The seeds were collected from the department of Horticulture and sown in the research field of the same department of Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Gazipur, Bangladesh (24°09' N, 90°26' E) in the agro-ecological zone of Madhupur Tract (AEZ 28). The fully bloomed flowers were picked in the early morning and immediately transported to the laboratory. Upon arrival, their petals were manually separated, with the outer and innermost petals being removed leaving only the middle ones which were subsequently subjected to analysis for colorimetric properties, nutritional content and bioactive attributes. Besides, the collected petals were also shade-dried on wooden-framed trays with perforated netting in a well-ventilated area of the laboratory at room temperature (25 ± 2 °C) for 7 days. The dried petals were then ground and utilized to determine the lutein, mineral content and antinutritional composition.

Determination of color attributes

Marigold flowers were subjected to colorimetric analysis using a bench-top spectrophotometer (CR-5; Konica Minolta, Japan) as per[13] with 5 replicates. The CIE Lab* color space was applied to describe the color characteristics, employing the parameters L*, a* and b*. The L* value relates to a dark-bright scale representing the relative lightness in a range from 0 to 100 (0 = black, 100 = white). Color parameters a*and b* extend from −60 to 60. The negative a* value signifies green, while positive value indicates red. Similarly, the negative b* value represents blue, while positive value signifies yellow. The C* value designated as chroma indicates the purity or saturation of the color, while the hue angle (h*) is represented in degrees ranging from 0 to 360, with

0° corresponding to red, 90° to yellow, 180° to green, and 270° to blue. The following equations were used to compute chroma (Eq. 1) and hue angle (Eq. 2):

$$C^* = \sqrt{\left(a^{*2} + b^{*2}\right)} \tag{1}$$

$$h^* = \arctan\left(\frac{b^*}{a^*}\right) \tag{2}$$

Estimation of coloring pigments

To estimate the anthocyanin content, 1 g marigold petals were ground and mixed with a 5 mL extraction solution of methanol, 6 M hydrochloric acid and water (70:7:23 v/v). After 24 h of incubation at 4 °C in the dark, the extract was centrifuged using a centrifuge machine (MPW-260R) at 5000 rpm for 15 min and the supernatant (3 mL) was measured for absorbance at 530 nm using a spectrophotometer (PD-303 UV Spectrophotometer; APEL Co.). The result was expressed as mg per 100 g fresh weight[14]. The anthocyanin content was measured using the Eq. (3):

$$Q_{AT} = A_{530} \times M^{-1} \tag{3}$$

Where, $Q_{At\,=}$ amount of anthocyanin, A_{530} = absorbance at 530 nm and M = fresh weight of sample used for extraction (g).

The carotenoid content of marigold was determined using the method outlined by[15]. Petals (100 mg) were extracted with 5 mL of 80% acetone overnight, stored in darkness at 4 °C for 24 h and then absorbance was measured at 663, 646 and 470 nm using a spectrophotometer, with 80% acetone as the blank. Finally, the carotenoid content was estimated applying the following Eq. (4):

$$\begin{aligned} &\text{Chl a } (\mu g/mL) = 12.21\ (A_{663}) - - 2.81\ (A_{646}) \\ &\text{Chl b } (\mu g/mL) = 20.13\ (A_{646}) - - 5.03\ (A_{663}) \\ &\text{Carotenoid } (mg/g) = \frac{1000\ (A470) - 3.27\ (\text{Chl a}) - 104\ (\text{Chl b})\ \times V}{229\ \times 1000\ \times W} \end{aligned} \tag{4}$$

where, V = Volume of acetone used (mL) and W = Weight of petal sample (g).

The lutein content was analyzed following the procedure of[16] with some modifications. Briefly, 10 g of dried flower sample was blended with 40 mL of acetone, filtered and the supernatant was centrifuged at 10,000 rpm for 10 min. Absorbance of the resulting supernatant was measured at 446 nm. Lutein concentration was calculated using the formula (5):

$$\text{Lutein content } (\mu g/g) = A \times V \times \text{dilutionfactor} \times € \times W \tag{5}$$

Where A is absorbance at 446 nm, V is volume of extract in mL, € is the absorption coefficient (2589) and W is dry weight of the sample.

Measurement of pH

To measure the pH value, 0.5 g of crushed petals were soaked in 5 mL of double distilled water following the procedure described by[13]. Then, the pH of the solution was determined with a digital pH meter (Digital Hanna pH Meter) after 2 h of occasional stirring.

Analysis of nutritional composition

Moisture content of marigold flower was assessed by drying a representative 1 g sample of petal in an oven at 100° C for 48 h until a constant weight was achieved, as described by[17] and calculated using the formula (6):

$$\text{Moisture Content } (\%) = (\text{Initial weight} - \text{Final weight})\,/\text{Initial weight} \times 100 \tag{6}$$

The total soluble solids (TSS) content was determined through the direct reading in a handheld refractometer (Model: Atago N1, Japan) by placing a drop of macerated petal sample on the prism, and the results were reported in °Brix[18].

The sugar content in the marigold samples was evaluated according to the protocol described by[19]. To accomplish this, extract solution was prepared by mixing 20 g of dried petals with distilled water. Then, the volume was adjusted to 100 mL by adding distilled water and centrifuged for 20 min at 4000 rpm. The supernatant was collected in a test tube and covered with foil paper.

For determining the total sugar content, 5 mL of the extract solution was mixed with 2 drops of 4 N HCl and heated on a sand bath for 30 min for hydrolysis. After cooling, 10 mL of Bertrand A and Bertrand B solution were added, followed by another 30 min heating on the sand bath and overnight cooling without disturbance. After that, Bertrand C solution was added. Finally, the solution was titrated with 0.4% potassium permanganate ($KMnO_4$) solution and the total sugar content (mg/100 g) was calculated by comparing with tabulated values.

To ascertain the reducing sugar content, 5 mL of extract solution was mixed with 10 mL of Bertrand A and Bertrand B solutions in a conical flask. After heating for 30 min and overnight cooling, the supernatant

was carefully decanted preserving the precipitate. The precipitate was washed thrice with distilled water. Then, Bertrand C solution was added to the precipitate, followed by titration with 0.4% $KMnO_4$ solution to determine the reducing sugar content (mg/100 g) by comparing with tabulated values.

According to[19], β-carotene analysis involved blending of 1 g of fresh sample with 10 mL of acetone: hexane (4:6) solution, followed by centrifugation and filtration by Whatman No. 1 filter paper. Subsequently, optical density readings were taken at 663, 645, 505 and 453 nm using a spectrophotometer to determine the β-carotene content using the formula (7):

$$\beta\,\text{carotene}\ \ (\text{mg}/100\ \text{g}) = 0.216\,(\text{OD}_{663}) + 0.452\,(\text{OD}_{453}) - 1.22\ \ (\text{OD}_{645}) - 0.304\ \ (\text{OD}_{505}) \tag{7}$$

Where, OD is the optical density at particular wave length; 0.216, 0.452, 1.22 and 0.304 are the absorption coefficient of the respective absorbance.

Vitamin C of the flower sample was estimated following the titration method of[19]. The same extract solution used for sugar determination was also employed for this analysis. In a conical flask, 2.5 mL of the prepared sample extract was combined with 2.5 mL of 5% KI along with 1 mL each of glacial acetic acid and 2% starch solution. After that, it was titrated with 0.001 N KIO_3 solution to determine the vitamin C content (mg/100 g) of the sample solution according to the Eq. (8):

$$\text{Vitamin C}\,(\text{mg}/100\ \text{g}) = (\text{T} \times \text{F} \times \text{V} \times 100)\,/\,(\text{v} \times \text{W}) \tag{8}$$

Where T is the titrated volume of 0.001 N KIO_3 (mL), F is 0.088 mg of ascorbic acid per mL of 0.001 N KIO_3, V is the total volume of sample extracted (mL), v is the volume of the extract (mL) titrated with 0.001 N KIO_3, W is the sample weight (g).

The procedure described by[20] was utilized with some modification to ascertain vitamin E content of marigold samples. Briefly, 1 g of sample was macerated in 20 mL of absolute ethanol for 3 h, filtered and then mixed with 1 mL each of 0.2% ferric chloride and 0.5% α-dipyridyl solution. After dilution to 5 mL with distilled water, absorbance at 520 nm was recorded. Various concentrations of alpha-tocopherol (0.2, 0.4, 0.6, 0.8 and 1.0 mg/mL) were employed to construct the standard curve to calculate vitamin E concentration using the following Eq. (9):

$$\begin{aligned} \text{y} &= 1.9283\text{x} - 6.2896 \\ \text{R}^2 &= 0.9661 \end{aligned} \tag{9}$$

The mineral content was assessed using an Atomic Absorption Spectrophotometer (AAS), following the procedures outlined by[19], with slight modifications. In this regard, 0.5 g of powdered sample was mixed with 5 mL of the mixture of HNO_3 and $HClO_4$ (5:1), then digested on sand bath for 3–4 h. After filtration, the filtrate was taken in a 100 mL conical flask and the volume was adjusted to 100 mL by adding distilled water up to the mark. Additionally, 10 mL of the extract was diluted to 50 mL with distilled water and analyzed using AAS (model-PinAAcle 900 H; PerkinElmer). The mineral concentration was quantified according to the Eq. (10):

$$\text{Mineral}\ (\%) = \text{Sample reading} \times \text{Final volume} \times \text{Dilution factor}/\text{Sample weight} \tag{10}$$

Determination of bioactive compounds

Bioactive compound analysis was conducted using methanolic extracts of marigold flowers following the methods outlined by[19]. Fresh marigold petals (1 g) were immersed in methanol (25 mL) and placed in a water bath at 30 °C for 2.5 h. After centrifugation at 6000 rpm for 15 min, the filtered supernatant was stored at 4 °C for further analysis.

The total phenolic content (TPC) was measured spectrophotometrically using the Folin-Ciocalteu method. In brief, 0.5 mL of the methanolic extract was combined with 2.5 mL of the Folin-Ciocalteu reagent and 2 mL of 7.5% sodium carbonate. After incubating this mixture at 30 °C for 1 h in the dark, its absorbance was measured at 760 nm against a methanol blank as standard. Various concentrations of gallic acid (10, 20, 40, 60, 80, 100 and 200 µl) were used to establish a standard curve (Supplementary Fig. 2). Finally, TPC was extrapolated from the standard curve using the following Eq. (11):

$$\begin{aligned} \text{y} &= 0.0176\text{x} + 0.1016 \\ \text{R}^2 &= 0.9917 \end{aligned} \tag{11}$$

The findings were expressed as mg gallic acid equivalent (GAE) per 100 g of fresh weight.

Determination of the total flavonoid content (TFC) was carried out following the aluminum chloride colorimertic method. During analyses, 100 µl of methanolic extract, appropriately diluted, was mixed with 100 µl of 10% (w/v) $AlCl_3$ and 100 µl of 1 M potassium acetate. Afterward, the mixture was incubated in the dark at room temperature for 40 min, followed by measuring absorbance in a spectrophotometer at 420 nm against the methanol blank. Different concentrations of quercetin (10–100 µg/ mL) were utilized to establish the standard calibration curve (Supplementary Fig. 3), from which TFC was quantified using the Eq. (12):

$$\begin{aligned} \text{y} &= 0.0268\text{x} - 0.1864 \\ \text{R}^2 &= 0.9796 \end{aligned} \tag{12}$$

The results for TFC were expressed as mg quercetin equivalent (QE) per 100 g of fresh weight.

The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay was employed to assess the antioxidant activity of marigold samples, based on the scavenging ability of antioxidants towards the stable DPPH radical. The sample extracts and ascorbic acid were prepared at concentrations of 20, 40, 80, and 100 µg/ mL and methanol was added up to a volume of 3 mL. Subsequently, methanolic DPPH solution (prepared by adding 0.004 mg of DPPH to 100 mL of methanol) was added. After incubating the reaction mixture in dark for 30 min, the absorbance was measured at 517 nm against a blank (methanol) using a spectrophotometer. The radical scavenging activity was determined as following Eq. (13):

$$\%\ Radical\ scavenging\ activity = \frac{A0 - A1}{A0} \times 100 \tag{13}$$

where, A_0 = Absorbance of control and A_1 = Absorbance of sample.

The inhibition concentration (IC_{50}) derived from the graph plotting the percent radical scavenging activity against the concentration of extract for both standard and test samples was used to assess the antioxidant capacity. A lower IC_{50} value indicates higher antioxidant activity. The IC_{50} estimation was performed using the following formula (14):

$$IC_{50} = \frac{(y - b)}{a} \tag{14}$$

where, y is substituted with 50; a and b are determined by plotting regression lines separately for each sample.

Assessment of antinutritional factors

The phytate content in the samples was quantified using the methodology outlined by[21]. 2 g of petal sample was macerated with 100 mL of 2% HCl and left to stand for 3 h. Subsequently, the mixture was centrifuged for 10 min at 13,000 rpm and filtered. Afterward, 25 mL of the supernatant was transferred to a separate conical flask containing 5 mL of 0.3% ammonium thiocyanate solution, followed by the addition of 53.5 mL of distilled water. The resulting mixture was titrated against a standard ferric chloride solution (0.00195 g of iron per mL) until a reddish-brown color persisted for 5 min. The phytate content was then calculated using the Eq. (15):

$$\text{Phytate}\ (\%) = \text{Titre value} \times 0.00195 \times 1.19 \times 100 \tag{15}$$

The alkaloid content of marigold flower was analyzed as per the methods described by[21]. To accomplish this analysis, 5 g of pulverized sample was macerated with 200 mL of 10% acetic acid in ethanol, covered with aluminum foil and left for 4 h. After filtration, the solution was concentrated to one-fourth of its original volume in a water bath. Concentrated ammonium hydroxide was then added drop wise to the concentrated solution until complete precipitation (cloudy fume) occurred. The solution was allowed for settlement and the resulting precipitate was washed with diluted ammonium hydroxide and subsequently filtered. The residue was dried, weighed and the alkaloid content was determined accordingly (16):

$$\text{Alkaloid}\ (\%) = \frac{\text{Weight of precipitate}}{\text{Weight of original sample}} \times 100 \tag{16}$$

The flower extract was analyzed for the tannin content as per[22]. The powdered sample (0.5 g) was mixed with 75 mL of distilled water, gently heated and boiled for 30 min. After centrifugation at 2000 rpm for 20 min, the supernatant was collected and adjusted to 100 mL with distilled water. The extracted sample (1 mL) was further diluted by adding 75 mL of distilled water. Afterward, Folin–Denis reagent (5 mL) and sodium carbonate (10 mL) were added to the diluted sample, followed by topping up the volume to 100 mL with distilled water. The mixture was then incubated for 30 min, and the absorbance was measured at 700 nm. The tannin content in the sample was extrapolated with a regression graph for the tannic acid solution using the Eq. (17):

$$\begin{aligned} y &= 0.0051x + 0.0789 \\ R^2 &= 0.9638 \end{aligned} \tag{17}$$

For saponin determination, 0.5 g of powdered sample was mixed with 50 mL of 20% ethanol and heated in a hot water bath at 55 °C for 4 h. After filtration, the residue was re-extracted with another 50 mL of 20% ethanol. The filtrates were combined, concentrated to 20 mL on a hot water bath at 90 °C. The concentrate was transferred into a separating funnel and 20 mL of diethyl ether was added and shaken briskly. The mixture was allowed for settlement until two distinct layers (ether and aqueous) were formed. The lower fraction was collected and reintroduced into the separating funnel. Then, 20 mL of n-butanol was added, followed by three washes with 10 mL of 5% sodium chloride. The upper fraction was collected and evaporated to a constant weight in an oven at 40 °C[23]. The saponin content in the sample was calculated using the following Eq. (18):

$$\text{Saponin } (\%) = \frac{\text{Weight of residue}}{\text{Weight of original sample}} \times 100 \quad (18)$$

Antinutrient to mineral molar ratio

The molar ratio of antinutrient (phytate) to minerals (K, Ca, Mg and Fe) was determined by the following Eq. (19):

$$\text{Molar ratio} = \frac{\text{Concentration of antrinutrient } (\mu\,g/g)/\text{Molar mass of antrinutrient } (\mu\,g/mol)}{\text{Concentration of mineral } (\mu\,g/g)/\text{Molar mass of mineral } (\mu\,g/mol)} \quad (19)$$

where, molar mass for phytate – 660 g/ mol, K- 39.0983 g/mol, Ca – 40.08 g/ mol, Mg – 24.31 g/ mol, Fe – 55.85 g/ mol.

Statistical analysis

All analyses were performed using R software (version 4.1.2) and the results were presented as mean ± standard deviation (SD) of three replicates. The significance level of the data was tested by analysis of variance (ANOVA) and Duncan's multiple range test (DMRT) was used to compare the means at 5% level of probability. Additionally, correlation matrix, cluster analysis, principal component analysis (PCA) and biplot analysis were carried out using R packages including agricolae, factominer, factoextra, ggplot2 and corrplot.

Results

Color attributes

From the analyzed colorimetric parameters of different marigold flowers (Table 1), it is evident that significant variations ($p < 0.05$) in terms of petal lightness (L*), redness/greenness (a*), yellowness/blueness (b*), hue angle (h*) and chroma (C*) values were prominent among the eight tested genotypes. The lightness or luminosity of the light yellow flower of M_6 genotype was the highest with an average value of 72.13 which signifies a bright nature of its flowers. Though the deep red flowers of M_1 genotype had the lowest L* value (26.4) indicating darker color, it showed the highest a* value (33.73) giving a justification of red color tendency followed by M_4, M_8 and M_3 genotype. Meanwhile, the lowest value regarding redness (0.52) was noticed in M_6 genotype. In terms of yellowness, M_8 genotype producing bright orange flower expressed the highest mean (73.71) having statistical unity with M_2 followed by M_5 genotype. The hue angle, which defines the basic color of a sample by its position on the color spectrum, was maximum (1.56) in the M_6 genotype, placing it closer to the greenish-yellow region. This was significantly different from the other genotypes, whose colors leaned more toward red or orange, associated with lower values. In addition, chroma or color intensity of the marigold flowers ranged from 51.57 to 80.39 where M_8 genotype was found with maximum intensity (80.39) having statistical similarity with that of M_5, M_2 and M_4 genotype. On the contrary, flowers of M_1 genotype possessed minimum value regarding yellowness (39.0), hue angle (0.86) and color intensity (51.57).

Coloring pigments

Significant difference ($p < 0.01$) concerning coloring pigment content was also observed in diverse colored marigold petals (Table 2). The total anthocyanin content of the different genotypes investigated varied between 0.02 and 1.90 mg/100 g with the maximum amount observed in genotype M_1 (1.90 mg /100 g) which was statistically different from the others. On the contrary, M_4 genotype had the minimum value of anthocyanin content (0.02 mg/100 g) having statistical unity with M_8, M_5, M_6 and M_7 genotype of marigold. As for total carotenoid content, significantly the higher amount (11.08 mg/100 g) was noticed in M_6 genotype while flowers of M_2 possessed the lowest content of total carotenoid (5.02 mg/100 g). While focusing on the lutein content, the estimated value was recorded to be the highest in M_4 genotype (9.78 µg/g) followed by M_3 and the lowest value (0.19 µg/g) was noted in the flower of M_6 genotype.

Genotype	Flower color	L*	a*	b*	h*	C*
M_1	Deep maroon	26.40 ± 0.14 f [x]	33.73 ± 0.57 a	39.00 ± 0.53 f	0.86 ± 0.02 e	51.57 ± 0.03 d
M_2	Yellow with maroon tip	51.14 ± 0.59 d	28.50 ± 0.58 cd	73.26 ± 0.77 a	1.20 ± 0.01 b	78.61 ± 0.84 a
M_3	Reddish with orange edge	46.27 ± 1.44 e	29.58 ± 3.90 a-d	57.62 ± 1.14 e	1.10 ± 0.04 d	64.82 ± 2.80 c
M_4	Deep orange	51.86 ± 0.13 d	33.11 ± 0.82 ab	70.77 ± 0.63 b	1.10 ± 0.01 cd	78.13 ± 0.80 a
M_5	Bright yellow	57.39 ± 1.04 c	29.12 ± 0.78 bcd	73.15 ± 1.34 ab	1.19 ± 0.01 b	78.73 ± 1.37 a
M_6	Light yellow	72.13 ± 0.12 a	0.52 ± 0.01 e	61.93 ± 0.60 d	1.56 ± 0.00 a	61.93 ± 0.60 c
M_7	Light orange	59.94 ± 0.17 b	26.95 ± 0.33 d	66.82 ± 0.49 c	1.19 ± 0.01 b	72.05 ± 0.40 b
M_8	Bright orange	56.35 ± 0.64 c	32.07 ± 0.24 abc	73.71 ± 0.44 a	1.16 ± 0.01 bc	80.39 ± 0.31 a

Table 1. Chromaticity parameters of fresh marigold flowers of eight genotypes. L*—lightness; a*—green–red components; b*—blue–yellow components; C*—chroma, h*—hue. [x]Data presented as means ± standard deviation in each column followed by the same letter(s) are not significantly different at $p < 0.05$ as determined by Duncan Multiple Range Test (DMRT) using the R software.

Genotype	Anthocyanin (mg/100 g)	Carotenoids (mg/100 g)	Lutein (µg/g)
M_1	1.90 ± 0.11 a[x]	10.00 ± 0.01 b	8.28 ± 0.30 bc
M_2	1.25 ± 0.16 b	5.02 ± 0.00 e	7.88 ± 0.30 c
M_3	0.46 ± 0.07 c	6.67 ± 0.58 d	9.16 ± 0.20 ab
M_4	0.02 ± 0.01 d	8.33 ± 0.58 c	9.78 ± 0.24 a
M_5	0.04 ± 0.01 d	6.67 ± 0.58 d	3.99 ± 0.48 e
M_6	0.04 ± 0.01 d	11.08 ± 1.00 a	0.19 ± 0.01 f
M_7	0.06 ± 0.01 d	9.13 ± 0.00 c	3.30 ± 0.41 e
M_8	0.03 ± 0.01 d	6.66 ± 0.57 d	6.40 ± 0.72 d

Table 2. Coloring pigment content of various marigold flower genotypes. [x]Data presented as means ± standard deviation in each column followed by the same letter(s) are not significantly different at $p < 0.01$ as determined by Duncan Multiple Range Test (DMRT) using the R software.

Genotype	pH	MC (%)	TSS (°Brix)	Total sugar (mg/100 g)	Reducing sugar (mg/100 g)
M_1	5.67 ± 0.59[NS]	88.32 ± 0.58 a[x]	6.07 ± 0.12 a	23.48 ± 0.93 c	21.63 ± 0.50 a
M_2	5.70 ± 0.30	87.89 ± 0.91 a	5.00 ± 0.20 b	23.01 ± 2.22 c	17.01 ± 0.20 bc
M_3	5.70 ± 0.30	85.76 ± 0.83 bc	5.97 ± 0.15 a	31.53 ± 1.76 b	15.84 ± 2.67 cd
M_4	5.80 ± 0.10	88.40 ± 0.49 a	4.13 ± 0.15 c	43.43 ± 3.42 a	19.23 ± 0.85 ab
M_5	5.70 ± 0.20	86.83 ± 0.88 abc	6.10 ± 0.10 a	26.54 ± 2.54 bc	10.90 ± 0.95 ef
M_6	5.30 ± 0.10	87.77 ± 0.81 ab	5.00 ± 0.26 b	13.66 ± 0.71 d	11.54 ± 0.50 e
M_7	5.50 ± 0.20	85.68 ± 0.43 c	3.90 ± 0.10 c	13.81 ± 0.90 d	8.01 ± 0.22 f
M_8	5.40 ± 0.20	88.52 ± 0.46 a	5.07 ± 0.12 b	15.98 ± 1.12 d	13.45 ± d0.56 e

Table 3. pH and nutritional compositions of different marigold genotypes. NS = Non significant. [x]Data presented as means ± standard deviation in each column followed by the same letter(s) are not significantly different at $p < 0.05$ as determined by Duncan Multiple Range Test (DMRT) using the R software.

pH and nutritional composition

The pH value of marigold flowers did not show significant differences across the selected genotypes, with all genotypes having statistically similar pH values (5.3 to 5.8), indicating to be acidic (Table 3).

The nutritional composition of the marigold flowers presented in Table 3 depicted significant differences ($p < 0.05$) in each of the recorded parameters among the selected genotypes. The values obtained for the percentage moisture contents of the flowers was found within the range of 85.68 to 88.52%, with M_8 genotype showing the highest content (88.52%) having statistical similarity with that of M_4, M_1 and M_2 whereas flowers of M_7 had the lowest content (85.68%) followed by M_6 and M_5 genotype. In terms of TSS, M_5 genotype performed better with the highest value (6.10 °Brix) having statistical similarity with M_1 and M_3 genotype while the lowest value (3.90 °Brix) was recorded in M_7 genotype showing statistical unity with M_4 genotype. The values found for the total sugar content fluctuated between 13.66 and 43.43 mg/100 g, where the highest accumulation occurred in the flowers of M_4 (43.43 mg/100 g) being significantly different from the others. The lowest value was recorded in M_6 (13.66 mg/100 g) which was statistically at par with M_7 and M_8 genotypes. Meanwhile, reducing sugar was calculated maximum (21.63 mg/100 g) in case of genotype M_1 followed by M_4 while M_7 genotype showed minimum content (8.01 mg/100 g) of reducing sugar followed by M_5 genotype.

Genotypic differences regarding β-carotene and vitamin C content in marigold flowers were noticed significant ($p < 0.05$) whereas no statistical variability ($p > 0.05$) was observed in terms of floral vitamin E content (Table 4). Maximum level of β-carotene and vitamin C was estimated 0.50 and 28.61 mg/100 g, respectively in M_5 genotype being statistically distinct from the others. Conversely, minimum amount of β-carotene (0.22 mg/100 g) was observed in M_4 which was statistically similar to that of M_6 genotype whereas M_7 genotype was found with the minimum content of vitamin C (7.48 mg/100 g). However, non-significant variation was recorded among the genotypes in terms of vitamin E content which varied from 3.96 to 4.03 mg RE/g.

With respect to the mineral contents, significant differences among the marigold genotypes were noticed ($p < 0.05$) except for Na and Mg (Table 5). The content of K, Ca and Fe fluctuated within the range of 1564.00 to 1691.07, 157.67 to 225.33 and 55.00 to 109.33 mg/ 100 g, respectively. M_8 genotype had numerically the highest amount of K (1691.07 mg/ 100 g) followed by M_5, M_2, M_6 and M_7 genotype. In contrast, M_4 had the least K content (1564.00 mg/100 g) exhibiting statistical similarity with M_3 and M_1 genotype. Meanwhile, Ca content in the marigold samples was detected with the highest value (225.33 mg/100 g) in M_5 genotype which was statistically identical with M_3, followed by M_1, M_2 and M_7. Genotype M_8 had flowers with the lowest Ca content (157.67 mg/ 100 g) showing statistical unity with M_4 genotype. As for the Fe content, M_8 genotype was found with the highest mean (109.33 mg/ 100 g) followed by M_5, M_3 and M_6 genotype whereas M_1 showed the lowest value (55.00 mg/100 g) which was statistically at par with M_2 and M_4 genotype. However, Na and Mg content

Genotype	β-carotene (mg/100 g)	Vitamin C (mg/100 g)	Vitamin E (mg /100 g)
M_1	0.44 ± 0.01 b[x]	16.72 ± 0.40 c	4.00 ± 0.04[NS]
M_2	0.41 ± 0.01 c	14.07 ± 0.98 d	3.98 ± 0.06
M_3	0.34 ± 0.01 d	19.56 ± 0.50 b	3.98 ± 0.15
M_4	0.22 ± 0.01 e	12.76 ± 0.78 d	3.98 ± 0.26
M_5	0.50 ± 0.01 a	28.61 ± 0.69 a	3.97 ± 0.34
M_6	0.24 ± 0.01 e	11.00 ± 0.15 e	3.97 ± 0.28
M_7	0.43 ± 0.01 bc	7.48 ± 0.50 f	3.96 ± 0.08
M_8	0.45 ± 0.01 b	15.84 ± 0.20 c	4.03 ± 0.09

Table 4. β-carotene and vitamin (C and E) contents of marigold genotypes. NS = Non significant. [x]Data presented as means ± standard deviation in each column followed by the same letter(s) are not significantly different at $p < 0.05$ as determined by Duncan Multiple Range Test (DMRT) using the R software.

Genotype	Na (mg/100 g)	K (mg/100 g)	Ca (mg/100 g)	Mg (mg/100 g)	Fe (mg/100 g)
M_1	84.00 ± 0.70 [xNS]	1570.67 ± 0.40 b	214.33 ± 0.15 ab	104.33 ± 0.68[NS]	55.00 ± 0.70 c
M_2	84.33 ± 1.03	1610.00 ± 0.31 ab	214.33 ± 0.06 ab	102.67 ± 0.70	57.10 ± 0.75 c
M_3	83.67 ± 1.05	1567.33 ± 0.25 b	222.33 ± 0.10 a	103.67 ± 0.76	87.67 ± 0.85 ab
M_4	96.67 ± 0.71	1564.00 ± 0.41 b	167.67 ± 0.07 c	103.67 ± 0.70	58.33 ± 0.91 c
M_5	83.00 ± 0.89	1651.02 ± 0.30 ab	225.33 ± 0.06 a	104.00 ± 0.66	88.00 ± 0.80 ab
M_6	73.33 ± 0.59	1607.33 ± 0.16 ab	194.67 ± 0.06 b	103.67 ± 0.64	86.33 ± 0.93 ab
M_7	85.33 ± 0.68	1604.00 ± 0.41 ab	204.33 ± 0.05 ab	103.33 ± 1.30	70.67 ± 0.95 bc
M_8	74.00 ± 0.98a	1691.07 ± 0.30 a	157.67 ± 0.10 c	103.33 ± 0.61	109.33 ± 1.61 a

Table 5. Mineral compositions of marigold flowers of different genotypes. NS = Non significant. [x]Data presented as means ± standard deviation in each column followed by the same letter(s) are not significantly different at $p < 0.05$ as determined by Duncan Multiple Range Test (DMRT) using the R software.

Genotype	TPC (mg GAE/100 g)	TFC (mg QE/100 g)	IC_{50} (mg/ mL)
M_1	483.38 ± 3.03 e	157.77 ± 9.91 cd	1.99 ± 0.01 h
M_2	508.16 ± 2.19 d	187.13 ± 9.12 b	4.24 ± 0.01 e
M_3	535.44 ± 3.61 c	233.39 ± 7.15 a	6.81 ± 0.01 a
M_4	441.91 ± 3.27 f	159.26 ± 5.57 cd	2.59 ± 0.01 g
M_5	592.71 ± 3.72 a	172.03 ± 4.70 bc	4.72 ± 0.01 d
M_6	581.16 ± 4.92 b	232.41 ± 9.53 a	6.47 ± 0.01 b
M_7	428.58 ± 4.42 g	144.28 ± 4.97 de	3.25 ± 0.01 f
M_8	484.55 ± 4.09 e	135.06 ± 6.18 e	5.19 ± 0.01 c

Table 6. Bioactive properties of different marigold genotypes. [x]Data presented as means ± standard deviation in each column followed by the same letter(s) are not significantly different at $p < 0.01$ as determined by Duncan Multiple Range Test (DMRT) using the R software.

were detected within the range of 73.33 to 96.67 and 102.67 to 104.33 mg/ 100 g, respectively, without significant variations across the genotypes studied.

Bioactive compounds

Variations in bioactive compounds as a result of genotypic difference were also found significant ($p < 0.01$) in the present study (Table 6). While considering the TPC, M_5 genotype displayed the highest value (592.71 mg GAE/100 g), while the lowest value of 428.58 mg GAE/100 g was noted for the genotype M_7. As for TFC, maximum content belonged to the M_3 genotype (233.39 mg QE/100 g) showing statistical resemblance to the M_6 genotype whereas M_8 genotype demonstrated minimum value (135.06 mg QE/100 g) followed by M_7 genotype. Meanwhile, IC_{50} value of the marigold flower extracts ranged from 1.99 to 6.81 mg/mL and M_1 demonstrated the lowest value (1.99 mg/mL) indicating the highest activities and the least activity was associated with the genotype M_3 whose IC_{50} value was highest of 6.81 mg/mL.

Genotype	Phytate (%)	Alkaloid (%)	Tannin (mg TAE/g)	Saponin (%)
M_1	1.30 ± 0,21 a[x]	10.04 ± 0.67 e	135.90 ± 0.15 a	8.67 ± 0.58 c
M_2	0.74 ± 0.14 b	29.26 ± 0.84 b	132.60 ± 0.53 b	18.00 ± 1.00 a
M_3	0.19 ± 0.03 c	4.44 ± 0.58 f	128.54 ± 0.33 d	10.00 ± 1.00 bc
M_4	0.14 ± 0.02 c	9.81 ± 0.83 e	129.73 ± 0.80 c	12.33 ± 0.58 b
M_5	0.74 ± 0.09 b	26.46 ± 0.63 c	118.27 ± 0.41 f	18.33 ± 0.58 a
M_6	0.14 ± 0.04 c	1.52 ± 0.10 g	136.58 ± 0.32 a	12.00 ± 1.00 b
M_7	0.14 ± 0.03 c	37.23 ± 0.96 a	127.44 ± 0.43 e	4.33 ± 0.58 d
M_8	0.23 ± 0.09 c	18.44 ± 0.46 d	126.73 ± 0.32 e	6.00 ± 1.00 d

Table 7. Antinutrient factors of different marigold genotypes. [x]Data presented as means ± standard deviation in each column followed by the same letter(s) are not significantly different at $p < 0.01$ as determined by Duncan Multiple Range Test (DMRT) using the R software.

Genotype	Phy: K	Phy: Ca	Phy: Mg	Phy: Fe
M1	0.05	0.37	0.46	2.00
M2	0.03	0.21	0.27	1.10
M3	0.01	0.05	0.07	0.18
M4	0.01	0.05	0.05	0.20
M5	0.03	0.20	0.26	0.71
M6	0.01	0.04	0.05	0.14
M7	0.01	0.04	0.05	0.17
M8	0.01	0.09	0.08	0.18
Critical values	0.50	0.24	1.00	1.00

Table 8. Molar ratios of phytate to minerals of marigold flowers. *Phy* Phytate

Antinutritional factors

Assessment of antinutritional factors of the selected marigold genotypes revealed the existence of phytate, alkaloid, tannin and saponin in varying concentrations as depicted in Table 7. The concentration of phytate in marigold flower extracts was determined to be low, ranging from 0.14 to 1.30%. Flowers from genotypes M_4, M_6, and M_7 exhibited the minimum phytate content (0.14%) being statistically indistinguishable from that of genotypes M_3 and M_8. Conversely, genotype M_1 displayed the highest phytate percentage (1.30%). Among the genotypes, M_7 was found to be alkaloid rich with the highest level of 37.23% while M_6 exhibited the lowest concentration (1.52%). With respect to the tannin content, the utmost quantity was detected in M_6 genotype (136.58 mg TAE/100 g), which showed no statistical difference from M_1, while the lowest value was observed in the M_5 genotype (118.27 mg TAE/100 g). The saponin composition of the samples showed significant variation and the values fluctuated between 4.33 and 18.33%. M_5 genotype stood out as having the highest amount of saponin (18.33%) being statistically identical with M_2 genotype whereas the lowest amount (4.33%) was recorded in M_7, which statistically paralleled with the genotype M_8. Oxalate was not detected in any of the studied marigold flower samples.

Bioavailability of minerals

There are numerous methods for evaluating mineral bioavailability in the presence of antinutrients in food, but the phytate: mineral and oxalate: Ca ratios are widely recognized as effective models[24]. Hence, the molar ratios of phytate to the studied minerals were calculated and compared with the acceptable critical values[25,26] (Table 8). The molar ratios of phytate to K (Phy: K), phytate to calcium (Phy: Ca), phytate to magnesium (Phy: Mg) and phytate to iron (Phy: Fe) ranged from 0.01 to 0.05, 0.04 to 0.37, 0.05 to 0.46 and 0.14 to 2.00, respectively. Phy: K and Phy: Mg ratios of all the genotypes were below the critical thresholds. Nevertheless, Phy: Ca ratio for genotype M_1 (0.37) and Phy: Fe ratio for genotypes M_1 (2.00) and M_2 (1.10) exceeded the critical values whereas these ratios for the remaining genotypes fell below their respective critical limits.

Multivariate analyses

Pearson's correlation analysis was employed to assess the relationships between the studied dependent variables of color parameters, pigments, nutritional composition, bioactive properties and antinutrient content in response to the independent variables of the marigold genotypes (Fig. 1). Positive correlations were designated by red hues, while blue shades indicated negative correlations in the correlation matrix stated in the right correlation scale bar (Fig. 2A). The intensity of the color signified the degree of correlation strength between the variables, while empty cells were deemed to represent no significant relationships at a 5% level of significance. The correlation matrix revealed varying degrees of association between the color parameters. As observed, lightness (L*) had a strong positive correlation with h* ($r = 0.93$) and a moderate positive correlation with color

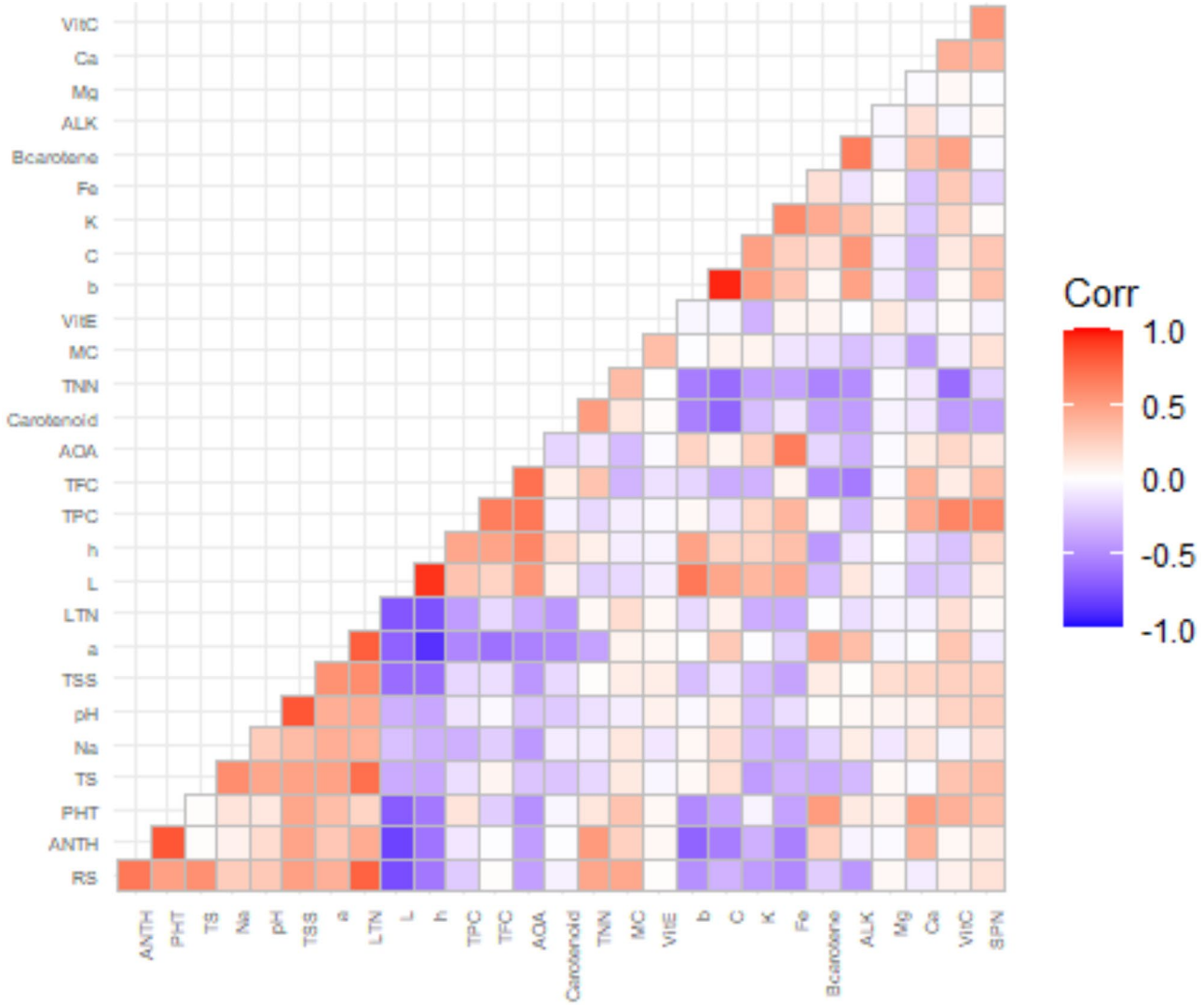

Fig. 1. Correlation coefficient for variables related to color attributes, nutritional content, bioactive properties and antinutritional compositions of marigold genotypes. [L = Lightness, a* = green–red components, b* = blue–yellow components, C* = chroma, h* = hue, ANTH = anthocyanin, LTN = lutein, MC = moisture content, TSS = total soluble solids, TS = total sugar, RS = reducing sugar, VitC = vitamin C, VitE = vitamin E, TPC = total phenol content, TFC = total flavonoid content, AOA = antioxidant activity, PHT = phytate, ALK = alkaloid, TNN = tannin, SPN = saponin].

value b* ($r = 0.68$). Conversely, both L* ($r = 0.68$) and h* value ($r = 0.88$) correlated negatively with a* value. The results also showed a robust positive link between parameter b* and C* ($r = 0.96$), implying that yellowness was linked to increased level of chroma. Regarding pigments, anthocyanin displayed a negative correlation with L* ($r = 0.81$) and b* (0.67) but strong positive correlation with phytate content ($r = 0.82$). Meanwhile, carotenoids associated negatively with the chroma ($r = 66$) meaning that as carotenoid content increase, color saturation would decrease. Further, lutein demonstrated a strong negative correlation with both L* ($r = 0.73$) and h* value ($r = 0.75$) but positive association with the total sugars and reducing sugars with correlation coefficients of 0.72 and 0.77, respectively. Among the nutritional traits, pH and TSS exhibited a strong positive correlation with each other ($r = 0.82$). Concerning bioactive compounds, moderately positive association was observed between TPC and TFC ($r = 0.65$), both of which were positively correlated with the IC_{50} value (AOA).

Principal component analysis (PCA) was performed on the analyzed compositions (dependent variables) to examine the relative variability within the marigold genotypes. It was noted that the first two principal components (dimension 1 and dimension 2) adequately accounted for the majority (45.2%) of the pattern variations (Fig. 2A). Among the variables, color parameters along with reducing sugar and tannin demonstrated strong contributions; lutein, anthocyanin, carotenoids, TSS, pH, β-carotene, vitamin C, K, Fe, TFC, AOA, phytate and alkaloid exhibited intermediate contributions, while moisture content, total sugar, vitamin E, Na, Ca, Mg, TPC and saponin were found to contribute less. The PC loading plot reveals that variables such as a*, TSS, reducing sugar, lutein, anthocyanin and phytate exhibited higher positive loadings while L, b*, h*, antioxidant

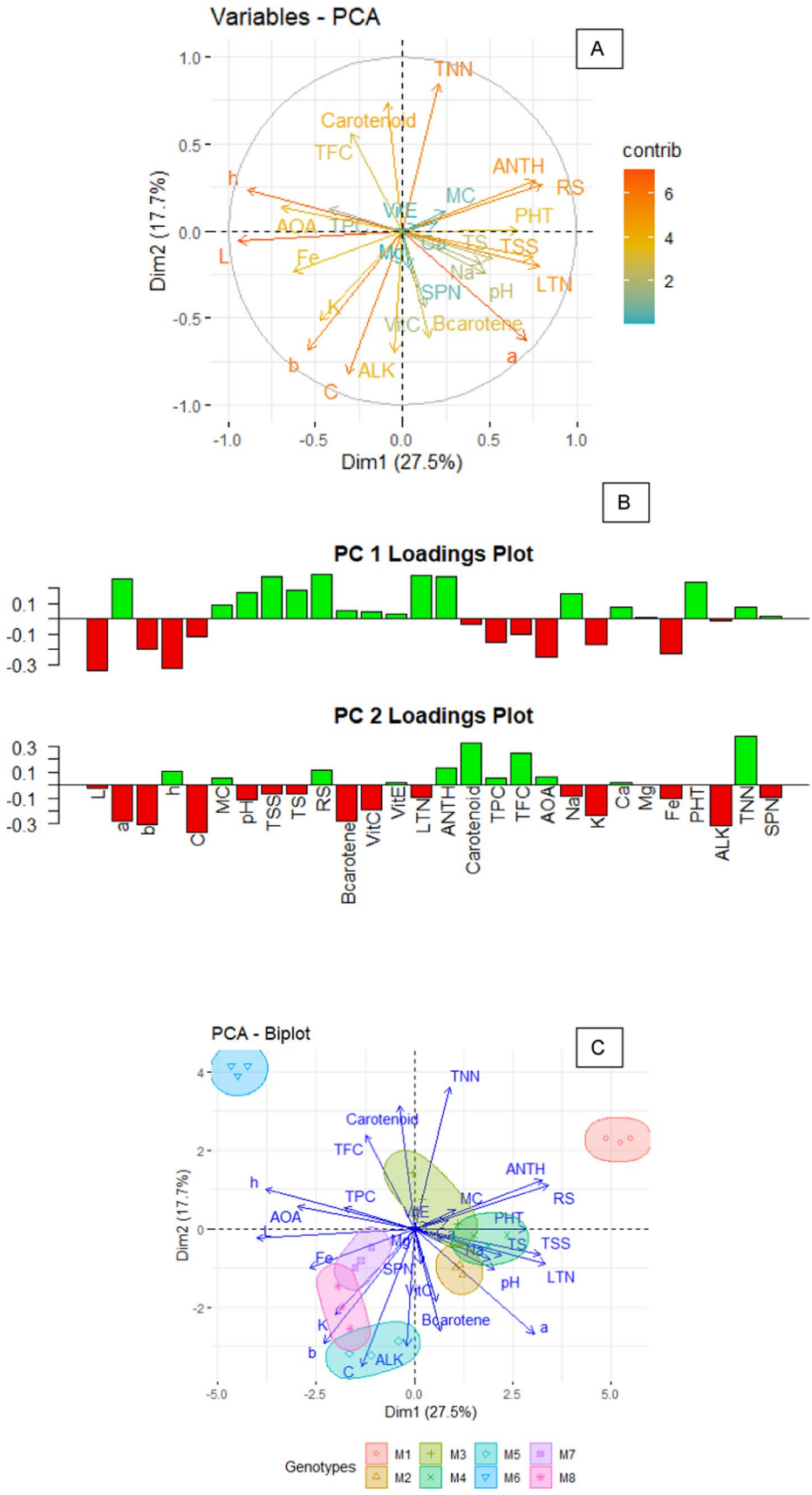

Fig. 2. Principal components analysis (PCA) of the variables showing their major contribution (**A**); factor loadings for the first two principal components (**B**); PCA–biplot analysis representing the performance of marigold genotypes regarding colorimetric, nutritional, bioactive and antinutritional attributes (**C**).

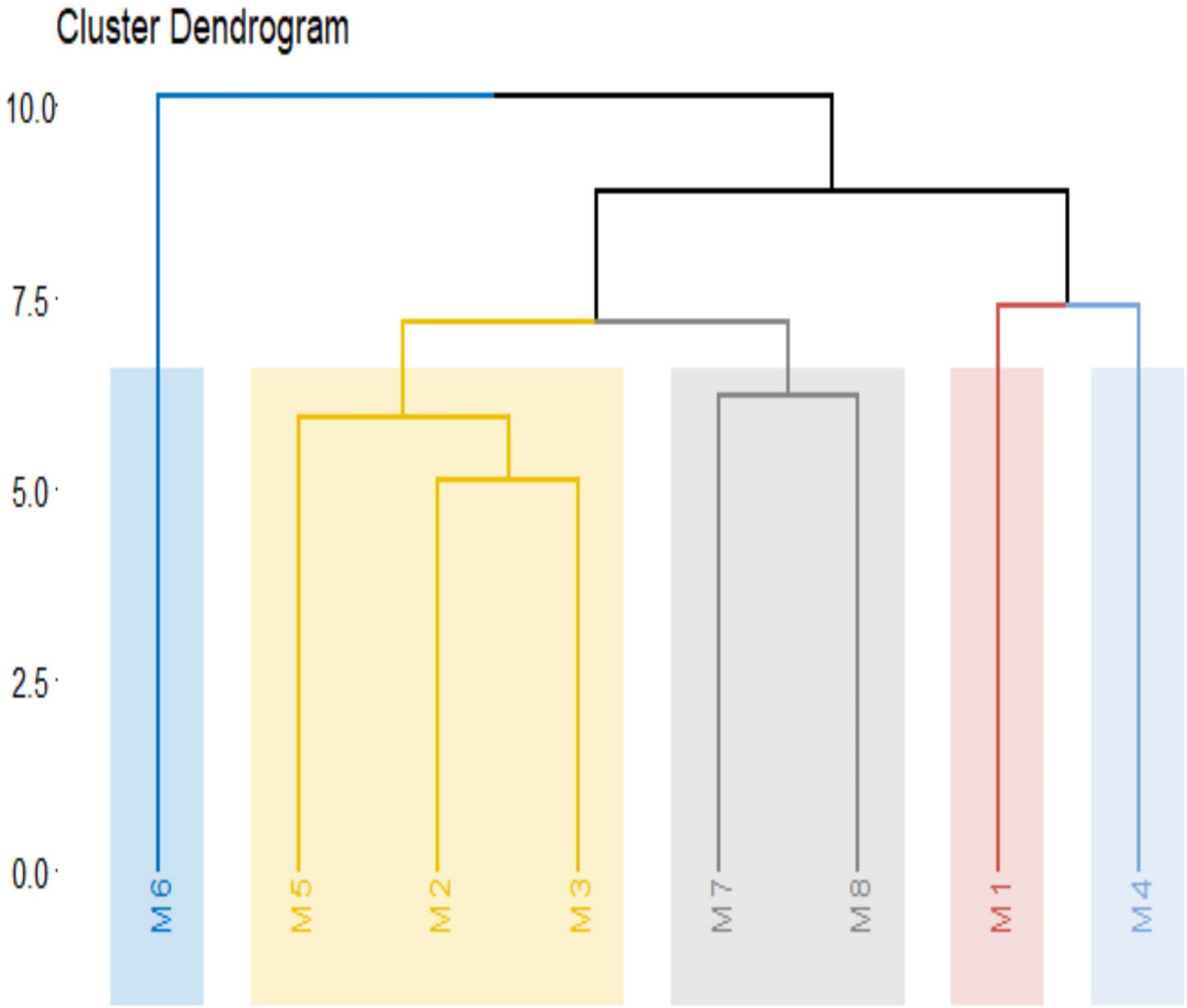

Fig. 3. Dendrogram showing cluster analysis of eight marigold genotypes based on nutritional, bioactive and antinutritional attributes.

and Fe showed higher negative loadings on PC1 (Fig. 2B). Conversely, on PC2, higher positive scores were linked to carotenoid, TFC and tannin, with higher negative scores for a*, b*, C*, vitamin C, β-carotene, K, and alkaloid. However, moisture content, reducing sugar, vitamin E, anthocyanin, Ca and tannin demonstrated positive loadings for both plots.

The PCA-Biplot exhibited four clusters for M_1 to M_8 genotypes, indicating noticeably varied characters of the genotypes (Fig. 2C). Marigold genotypes in the right quadrants (M_1 and M_3 genotypes) were characterized by higher content of tannin, anthocyanin, reducing sugar, phytate and moisture content. Here, M_1 formed a distinct cluster at the positive end of dimension 1. Meanwhile, M_2 and M_4 genotypes were overlapping each other and located in the 2nd quadrant showing higher properties for pH, TSS, TS, lutein, β-carotene, Na and vitamin C. Genotypes in the third quadrant (M_5, M_7 and M_8 genotypes) were found overlapped for b*, C*, K, Fe and alkaloid content while M_6 genotype formed another distinct cluster dominated by h*, carotenoids, antioxidant (IC_{50}), TPC and TFC.

The cluster dendrogram illustrated the grouping of eight marigold genotypes into two primary clusters (Fig. 3). Cluster 1 contained only the M_6 genotype, distinct from the other genotypes whereas cluster 2 was subsequently divided into two subclusters. Subcluster 1 included genotypes M_1 and M_4, indicating their similarity, while subcluster 2 split further into two smaller clusters with genotypes M_5, M_2, and M_3 showing notable similarities and M_7 and M_8 forming another distinct group.

Discussion

Ornamental flowers with beautiful colors play a pivotal role in enhancing the aesthetic appeal and ambiance of gardens, landscapes and indoor spaces. The vivid colors of flower petals hold allure not only for pollinators but

also for human consumers, who value them for their decorative appeal and ability to enhance the color and flavor of dishes. Moreover, petal color of the edible flowers is considered as an important quality trait greatly affecting their consumption[27]. Among the flowers, marigold stand out as an economically important ornamental plant worldwide showcasing a diverse array of flower colors. In the present study, the selected marigold genotypes produced flowers of various colorations varied from yellow, orange, bicolor to deep maroon performing differently with respect to color attributes. Colorimetric measurements indicated that among the genotypes observed, those with yellow flowers displayed greater lightness compared to others, with M_6 registering the highest mean, consistent with its light yellow flowers and the most elevated hue angle. In contrast, the flowers of genotype M_1 appeared darker and redder, characterized by lower lightness (L*) and higher redness (a*) values. Additionally, the vibrant orange flowers of genotype M_8 showcased the highest levels of yellowness (b*) and color intensity (C*). In a prior study[28], also measured color parameters of common marigold (L* 68.56, a* 14.22, b* 42.44) and African marigold (L* 54.44, a* 22.49, b* 28.45) and their results closely paralleled the findings within our investigation, highlighting consistency across studies.

The attractive natural display of flower colors primarily arises from pigmentation and is largely attributed to three major groups of pigments such as flavonoids (including anthocyanins), carotenoids, and betalains[29]. The capacity to produce and accumulate these pigments varies among plant species. Beyond their role in creating vibrant colors, pigments also offer health benefits and may reduce the risk of diseases[30].

Anthocyanins are plant pigments that impart a spectrum of appealing colors to flowers in shades of orange, red, pink and blue[6]. Various studies have highlighted the diverse beneficial properties of anthocyanins, including anti-inflammatory, antioxidant, anticancer, antimicrobial, and antiallergic effects rendering them valuable ingredients for incorporation into functional foods and cosmetics[31]. In this study, it was noted that marigold flowers exhibiting red coloration (M_1, M_2, and M_3) contained a higher total anthocyanin content compared to those with orange and yellow flowers, corroborating the reports of[32] Additionally, their reported anthocyanin levels for orange *Tagetes erecta* (0.75 mg/100 g), white *Viola* × *wittrockiana* (0.35 mg/100 g), white *Dianthus* × *barbatus* (0.73 mg/100 g) and orange *Calendula officinalis* (0.47 mg/100 g) aligned with our findings, but they observed much higher levels in red *Dianthus barbatus* (13.35 mg/100 g), blue *Viola wittrockiana* (13.6 mg/100 g) and red *Petunia hybrida* (14.44 mg/100 g).

The coloration of flowers is also significantly influenced by carotenoids commonly found in vegetables, fruits and flowers contributing to yellow, orange and red tints[6]. The composition of carotenoids in flowers varies widely among plant species and cultivars, playing a crucial role in human nutrition as they cannot be synthesized de novo and must be obtained through natural foods and supplements[5]. In marigold, carotenoids serve as major pigments with their varying levels across different genotypes significantly contributing to the diversity of flower colors[33]. The total carotenoid content among the examined marigold genotypes ranged from 5.02 to 11.08 mg/100 g on a fresh weight basis, reflecting variability attributable to both flower color and genetic makeup of the genotypes. The values obtained in this study were, however, lower than those reported for chrysanthemum[34] and pumpkin flowers[35].

Among the carotenoids, lutein is a yellow plant pigment belonging to the xanthophyll group usually found in egg yolks, fruits, dark green vegetables, grains and flowers. The consumption of lutein-rich foods can lower the risk of non-communicable diseases such as coronary heart disease, cancer, and age-related macular degeneration[36]. These health benefits have spurred the incorporation of lutein as a functional food ingredient. Lutein dominates the pigmentation of marigold petals, comprising around 90% of the total carotenoids, influencing differences in petal colors[33]. Therefore, the marigold flower emerges as the premier commercial source of pure lutein, making it one of the most significant sources of this xanthophyll. In the present study, quantitative differences were found among the marigold genotypes evaluated having a total content of lutein ranging from 0.19 to 9.78 µg/g. The orange marigold cultivar displayed higher values for lutein content which was in conformity with the research finding of[37]. Although the values estimated in the present study was found lower than the values stated for several marigold cultivars (8.31 to 20.59 mg /g dry marigold petal)[37] and certain other edible flowers (11.78 to 1217.2 µg/g)[6], they are comparable with some vegetables like green pepper (8.8 µg/g) and carrot (2.5–5.1 µg/g)[38]. Therefore, the vibrant marigold petals would justify their use in foods as both natural coloring pigments and functional food ingredients.

Due to changes in dietary habits, external quality attributes like color, shape, and size of food no longer fully satisfy consumer informational needs for decision-making. Therefore, understanding the composition of edible flowers is essential and provides a strong rationale for their consumption. The pH assessment of the petal sap unveiled an acidic pH reaction in marigold flowers with no significant variances ($p > 0.05$) detected among them. Our findings aligned with those reported for herbaceous peony (5.84 and 5.05) by[39]. The nutritional traits of marigold flowers reported in this work demonstrated a wide range of variations across the genotypes. Moisture content in food is a critical factor that affects its quality, preservation, and resistance to deterioration while also promoting hydration and supporting proper intestinal function, thus enhancing digestion after consumption[35]. The evaluated data highlighted the presence of relatively high moisture content (85.68–88.52%) in the marigold flowers which was in consonance with the values reported for some other edible flowers[40]. Nevertheless, the relatively high moisture contents in the studied genotypes revealed the necessity of care for appropriate preservation as they would be prone to deterioration. TSS are often associated with sweeter and more flavorful produce, thereby enhancing consumer sensory appeal. The TSS measurements of fresh marigold flowers exhibited significant variation, ranging from 3.90 to 6.10 °Brix which were corroborated with the findings of previous researches on pansy and snapdragon flowers (2.97 and 5.33 °Brix)[4]. Sugar plays a vital role in plants and a higher total sugar content makes them more palatable[41]. The estimated quantity of total and reducing sugar of the selected eight marigold genotypes varied from 13.66 to 43.43 mg/100 g and 8.01 to 21.63 mg/100 g, respectively, which were fairly lower compared to those documented by[41] for five edible flowers.

Vitamins are organic compounds considered as essential constituents of our diet that help the body to grow and function properly by boosting the immune system[21]. In scientific reports, β-carotene, a precursor of vitamin A, is often mentioned for its bioactive properties in edible flowers[6]. In our study, different genotypes showed varying content of β-carotene ranging from 0.22 to 0.50 mg/100 g and the findings indicated substantially higher concentrations compared to the previously reported values for *Tagetes tenuifolia* Cav. (0.992 µg/g)[34]. Moreover, our values were comparable with those obtained in chrysanthemum (0.05–5.51 µg/g)[34] and cultivars of *Rosa hybrida* L[6]. Vitamin C is another essential nutrient in the human diet that participates in various biological processes like collagen synthesis, iron absorption, cholesterol regulation, immune system enhancement and free radical scavenging[11]. Vitamin C content detected in the flowers of studied marigold genotypes were within the range (2.6 to 44.9 mg/100 g) stated by[40] in some wild edible flowers. Moreover, the vitamin C contents observed in this study were comparable to those of some fruits and vegetables such as apples (5 mg/100 g)[40] and tomatoes (32.5 mg/100 g)[11] offering their potentiality as a supplement of vitamin C in the diet. Vitamin E, widely used in medicine, cosmetics, and various industries, serves as a vital nutritional supplement and is renowned for its antioxidant properties[42]. The vitamin E content of studied marigold flower samples did not show significant differences ($p > 0.05$) with 4.03 mg/100 g being the highest content. Our observed results exceeded those reported in a previous study for the edible flower species borage (2.2 mg/100 g), centaurea (1.24 mg/100 g) and red pansies (0.67 mg/100 g) but were considerably lower compared to camellia (9.27 mg/100 g) as well as white (8.64 mg/100 g) and yellow (22.21 mg/100 g) pansies[43]. Despite not being considered as a good source of vitamin E, marigold flowers could contribute to the supply of vitamin E to the consumer.

Mineral elements are the vital components of the human diet. They play a critical role in maintaining balanced human nutrition as well as normal body development and maintenance[21,23]. The results of mineral composition obtained in the current study revealed the presence of considerable amounts of Na, K, Ca, Mg and Fe in the marigold flowers of various genotypes. Moreover, it is noteworthy to mention that K was the most abundant mineral in all the genotypes evaluated confirming the previous reports on some edible flowers[4,35]. In addition, our study showed higher values for all the minerals than the respective values stated for various edible flower species in previous observations by[4,14,35]. Furthermore, mineral contents except Mg recorded for all the genotypes were remarkably higher than those reported for quinoa grains, often regarded as a super-food[44]. Fe content was also quite higher than that found in some leafy vegetables like quinoa leaves (11.55), spinach (23.65), amaranth (16.77) and bathua (15.20 mg/ 100 g)[45]. As a result, the selected marigold flowers could be served as the natural sources of these essential minerals specially K and Fe and could supply them in adequate quantity that might aid in the prevention of diseased conditions linked with their deficiencies.

Apart from the nutritional value, edible flowers have gained attention recently due to their potential health benefits from bioactive compounds, making them excellent candidates for functional foods[5]. Hence, knowing the bioactive compounds and their functional properties is needed to diversify their utilization and reach broader consumer base. Plant phenolic compounds are natural bioactive molecules noted for their antioxidant activities. Accordingly, the food industries are keenly interested in phenolic compounds of edible flowers which provide natural alternatives to artificial additives and preservatives currently prevalent in the market[6]. In our study, the TPC varied from 428.58 to 592.71 mg GAE/100 g among the different marigold genotypes which were in line with that of *Tagetes patula* (4.78 g GAE/kg of FM) and various rose genotypes (241.87 to 533.18 mg GAE/100 g)[14,46]. Mlcek et al. [46] also stated similar values of TPC in *Begonia* × *tuberhybrida* (4.82 g GAE/kg) and *Rosa* (4.45 g GAE/kg). Moreover, our findings surpass those observed in various fruits and vegetables like strawberry (363.7 mg GAE/100 g), loquat (199.4 mg GAE/100 g), leafy vegetables (23.0-136.0 mg GAE/100 g)[47], pumpkin (199 mg GAE/100 g)[35] and broccoli (391.71 mg GAE/100 g)[48]. Hence, the selected marigold flowers could stand out for their TPC and had great potential in preventing diseases associated with free radicals. Flavonoids are likely the most significant phenolics naturally occurring in various parts of plant and are well known for their beneficial effects on health. In addition to their crucial role in pigmentation, they serve as main active substances in flowers, showcasing robust antioxidative properties[41]. The TFC obtained for the examined marigold genotypes (135.06 to 233.39 mg QE/100 g) were in agreement with that of *Tagetes erecta* (1.92 mg/g)[49] and within the reported values in the edible rose genotypes (1.61 to 5.58 mg QE/g)[31]. In addition, our study revealed a higher TFC compared to some leafy vegetables, which ranged from 15.50 to 50.10 mg Q/100 g[47]. Antioxidants, known for inhibiting free radicals, are crucial for assessing the nutritional value of foods and diagnosing oxidative stress-related diseases[16]. Natural antioxidants are typically derived from plants, with their activity varying based on species, extraction methods, and growing conditions[50]. Several possible sources of natural antioxidants have been discovered, including edible flowers[5]. In the DPPH assay, the marigold genotypes showed IC_{50} values ranging from 1.99 to 6.81 mg/mL, indicating considerable antioxidant activity. This was in contrast to the findings of[11] who observed IC_{50} values of 13.9 to 25.3 µg/mL for methanolic extract of *Tagetes erecta* samples. However, our results aligned with the values of *Ixora coccinea* flower (6.6 mg/mL) observed in previous research[51]. These findings imply that having appreciable antioxidant activity, selected marigold flower petals could be exploited as a potential source of natural antioxidants for utilization in the food industries in place of artificial antioxidant compounds.

Antinutrients, referred to as secondary metabolites in plants, are highly biologically active chemical compounds synthesized in natural food or feed stuffs during the natural metabolism. True to their name, antinutrients hinder optimal exploitation of the nutrients present in a food substance when present beyond a certain amount and thus decrease the nutritional value of the food[22]. Various kinds of anti-nutritional factors with toxic potential such as saponins, cyanogenic, glucosides, tannins, phytic acid, protease inhibitors, amylase inhibitors, antivitamin factors, alkaloids, etc. have been documented in food[5]. However, subjecting the foods to processes like soaking, boiling, cooking, etc. removes excess antinutrient sufficiently, rendering them suitable for consumption[42]. Apart from the positive impacts on human health that edible flowers may have, there are several safety issues related to the presence of potential toxic or poisonous compounds. Hence, emphasis is being given

on assessing the antinutritional factors of edible flowers to find whether they are safe for consumption or not, particularly the novel species that are not commonly or traditionally utilized in culinary purposes[6].

Phytate is recognized as one of the most ubiquitous antinutrient as it interferes with bioavailability of essential minerals like calcium, iron, magnesium and zinc[22]. As a result, its excessive accumulation may lead to health problems. The composition of phytate identified in this study (0.14 to 1.30%) was higher than the values reported for edible flowers of *Allium cepa*, *Carica papaya* and *Cucurbita maxima*[22], yet it aligned with the range (720–1300 mg/100 g) observed in several commonly consumed leafy vegetables[52]. However, the highest content recorded in marigold genotypes was within the safe limit, since the inhibition of mineral absorption by phytate typically occurs at levels exceeding 10% in a diet[21], thus establishing them as food sources within the safe limits of phytate intake.

Alkaloids are plant secondary metabolites that affect the nervous system, disrupt electrochemical transmission and can also induce gastrointestinal and neurological disorders when consumed in high concentrations[22]. Alkaloids are found in various species with edible flowers[6]. The estimated alkaloid content of the studied flower was far higher than those reported for the edible flowers of *Allium cepa* (0.88 mg/100 gm) and *Carica papaya* (0.18 mg/100 gm), *Cucurbita maxima* flowers (0.35 mg/100 gm[22], but was comparable with those of different rose genotypes (1.24 to 14.64 g/100 g)[14]. The elevated alkaloid content in the selected marigold genotypes raised significant concern as it exceeds the designated safe consumption threshold of 0.02%[53]. Nevertheless, addressing this concern through appropriate processing methods such as boiling, soaking, etc. could be effective to decrease the partially water-soluble alkaloid content substantially[42].

Tannins are nutritionally undesirable because they precipitate proteins, hinder digestive enzymes and impair iron absorption[53]. The tannin content in marigold flowers ranged from 118.27 to 136.58 mg TAE/100 g, exceeding the stipulated maximum limit of tannin in food (12 mg/100 g)[54]. However, the estimated tannin content was less than that found in various food legumes such as green beans, black gram and field peas[55] as well as certain commonly consumed vegetables in Bangladesh (*Centella asiatica* leaves and jute leaves)[56] and West Ethiopia[52]. In addition, it was found lower compared to two Bangladeshi dragon fruit varieties, BARI Dragon Fruit-1 (335.04 mg TAE/100 g) and BAU Dragon Fruit-1 (345.80 mg TAE/100 g)[57]. However, tannin intake below 1.5–2.5 g daily is considered safe[22], allowing processed marigold flowers to be consumed without side effects.

Saponins, found in foods like soybeans, sugar beets, peanuts, spinach, are surface-active secondary metabolites with soap-like properties and a bitter taste[53,58]. In high concentrations, they can inhibit nutrient absorption by affecting enzymes and binding with nutrients like zinc[21]. However, in small amounts, they can be beneficial, with concentrations below 10% posing negligible health risks[42]. Among the eight tested marigold genotypes, four (M_1, M_3, M_7 and M_8) had saponin levels within 10%, while the others exceeded this threshold limit. Despite higher saponin content than certain edible flowers[22], they were comparable to the flowers of several rose genotypes (4.03–14.0 g/100 g) and *Amaranthus caudatus* leaves (4.20-35.62%), a widely used vegetable in West Africa known for its potential functional properties[14,58]. Therefore, proper processing of marigold flowers before consumption is crucial.

Antinutritional factors often present in plant-based foods tends to limit the absorption of minerals by disrupting their intake, digestion, and absorption processes[53]. Hence, the amount of these complexes and the molar ratio of phytate to minerals may consequently impact the bioavailability of minerals[25]. Compared to the critical thresholds, molar ratios of the assessed minerals to phytate fell below the critical values confirming their adequate bioavailability across all the genotypes except for Ca in M_1 genotype and Fe in M_1 and M_2 genotypes. Owing to the elevated phytate levels in genotype M_1, both Phy: Ca and Phy: Fe ratios were notably high, surpassing the recommended critical thresholds. This suggests potential interference in the availability of calcium and iron in the presence of phytate. Furthermore, genotype M_2 also exceeded the cutoff value (> 1) for the Phy: Fe ratio, indicating limited bioavailability of iron in this genotype. Nonetheless, the Phy: mineral ratios could be significantly diminished through processing methods such as soaking or cooking[53,58]. Additionally, certain vitamins have been noted for their ability to facilitate mineral absorption even when consumed in diets rich in phytates. Notably, vitamin C assists in enhancing iron absorption by reducing the susceptibility of iron to complexation with phytates, consequently boosting its bioavailability[24]. The presence of vitamin C in these genotypes were, therefore, beneficial in this regard. Hence, it can be inferred that the flowers of the examined marigold genotypes could be suitable for use in human diet following suitable processing methods rather than being consumed as fresh or raw.

The correlation matrix highlighted significant relationships between the studied variables displaying wide variability among the marigold genotypes. The positive alliances of color value L* with parameter b* and its inverse relationship with a* indicated that brighter flowers tend to be more yellowish while increased redness decreases the lightness. These observed relationships aligned with the previous researches on color analysis in *Helleborus thibetanus* flowers[59]. The correlations observed between color parameters and pigment contents suggested that these compounds played a crucial role in determining the color characteristics of the marigold flowers. The anthocyanin content displayed negative association with L* value which was in conformity with the research finding of[59]. It was also verified that higher anthocyanin content leads to redder hues, which would be reflected in lower 'b*' values. The relationships between lutein and certain color parameters suggested a potential role of lutein in enhancing specific color attributes. The increased lutein was responsible for increased redness but decreased lightness of the evaluated marigold flowers. Studies relating colorimetric values with lutein revealed similar correlation in pumpkins and squash[60]. However, the positive association between anthocyanin content and phytate content was intriguing and might warrant further investigation. The strong positive correlation between pH and TSS was in line with[61] who reported that higher TSS levels could coincide with increased acidity. While focusing on bioactive compounds, positive link between TPC and TFC was noticed indicating potential synergistic effects between them, being consistent with the findings reported by[61,62]. Although phenolic

compounds are recognized for their effective antioxidant properties, yielding lower IC_{50} values as anticipated, in the current study, TPC along with TFC exhibited a positive correlation with IC_{50} values, contradicting previous findings[63]. This discrepancy is likely due to the fact that TPC are not the sole contributing factors providing higher antioxidant activity and the presence of other substances cannot be ignored, especially at lower TPC (< 10 mg GAE/g)[64]. Based on the correlation, the studied dependent variables were grouped into two main clusters with distinct deviations from each other. The PCA-Biplot analysis conducted on the M_1 to M_8 genotypes of marigold revealed distinct clusters, each characterized by unique chemical compositions and traits. These wide variations among the studied parameters were further generalized in the PCA-biplot analysis where M_1 and M_6 genotypes were revealed in a distinct position than others considering most of the variables contributing in variances. These findings were successively confirmed in the dendrogram cluster analysis where these two genotypes belonged to two different clusters.

Conclusion

In conclusion, our comparative analysis sheds light on the noteworthy diversity within the marigold genotypes regarding color attributes, pigments, nutritional aspects, bioactive properties, and antinutritional compositions. The selected genotypes presented a diverse array of flower colors, exhibiting distinct performances with respect to their color attributes. Differences in flower color were closely linked to the pigment content and meaningful correlations between color coordinates and pigment concentrations were identified. Furthermore, the genotypes exhibited substantial nutritional composition and bioactive properties with potential health benefits, particularly notable for their concentrations of vitamin C, minerals and TPC. The results pertaining to antinutrient properties underscored the existence of elevated levels of alkaloid, tannin and saponin which could be reduced through appropriate processing methods such as soaking, boiling and cooking, thereby alleviating their negative impacts upon consumption. The molar ratios of the evaluated minerals to phytate confirmed sufficient bioavailability across all the genotypes, except for Ca in M_1 genotype and Fe in M_1 and M_2 genotypes which could be notably improved through pre-consumption processing methods. Besides, the presence of vitamin C in them would enhance the Fe absorption, consequently boosting its bioavailability. Among the genotypes, M_1 was identified as being enriched with the highest quantity of anthocyanin, reducing sugar content and antioxidant activities accompanied by notable concentrations of TSS and Mg. Additionally, genotype M_5 was featured with the highest levels of TSS, β-carotene, vitamin C, Ca and TPC whereas M_6 possessed the highest amount of carotenoids and TFC. Hence, the incorporation of these three marigold genotypes after processing in the food products as natural coloring agents and potential sources of functional food could be justified, thereby diversifying flower-based dietary options to address growing health demands. These findings offer valuable insights for consumers, chefs, nutritionists and the food industry, potentially opening avenues for new business opportunities to enhance the production and market availability of edible flowers as functional food ingredients, as well as for the extraction of bioactive compounds.

Data availability

Data is provided within the manuscript or supplementary information files.

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Author contributions

H.S performed the experiments and laboratory analysis, curated the data, wrote the original draft, reviewed and edited the manuscript. J.H conceived the idea of the study, data processing, laboratory analysis, statistical analysis and interpreted the data, wrote, reviewed and edited the manuscript, provided mentoring, supervision, valuable support and guidance. M.Z and E.K provided supervision and investigation, and critically reviewed and revised the manuscript. M.Z, K.A.A, MMB and JG assisted with data interpretation and manuscript writing. Y.O, A.T.A and S.A validated the work and improved the manuscript. All authors have reviewed and approved it for submission.

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Declarations

Conflict of interest

The authors declare no conflict of interest.

Ethical approval

The present study utilized Marigold (*Tagetes sp.*) flowers as the plant material. The cultivated marigold genotypes were obtained from the flower garden of Bangabandhu Sheikh Mujibur Rahman Agricultural University, located in Gazipur-1706, Bangladesh. The marigold genotypes are cultivated and conserved in the university's flower garden for utilization in research endeavors. The field and laboratory investigation were conducted using established advance protocols and adhering to the scientific ethics rules and regulations for handling plants.

Additional information

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