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Wild Edible Flowers of Western Himalayas: Nutritional Characterization, UHPLC-QTOF-IMS-Based Phytochemical Profiling, Antioxidant Properties, and *In Vitro* Bioaccessibility of Polyphenols

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ABSTRACT: Four edible flowers commonly consumed in the Western Himalayan region, namely, *Bauhinia variegata* (Kachnar), *Tropaeolum majus* (Nasturtium), *Matricaria chamomilla* (Chamomile), and *Tagetes erecta* (Marigold), were characterized for their nutritional and phytochemical composition. Through the UHPLC-QTOF-IMS-based metabolomics approach, 131 compounds were tentatively identified consisting of phenolic acids, flavonoid glycosides, terpenoids, amino acids, and fatty acid derivatives. Kaempferol and quercetin glycosides for Kachnar, apigenin glycosides and caffeoylquinic acid derivatives for Chamomile, patulin and quercetin derivatives for Marigold, cyanidin and delphinidin glycosides for Nasturtium were the predicted marker metabolites identified through non-targeted metabolomics. Kachnar and Chamomile scored best in terms of macronutrients and essential micronutrients, respectively. Nasturtium contained high concentrations of α-linolenic acid, anthocyanins, and lutein. Kachnar contained the highest total phenolic acids (63.36 ± 0.38 mg GAE g^{-1}), while Marigold contained the highest total flavonoids (118.90 ± 1.30 mg QUE g^{-1}). Marigolds possessed excellent free radical scavenging and metal chelation activities. Chamomile exhibited strong α-glucosidase inhibition activity, followed by Nasturtium. The *in vitro* gastrointestinal digestibility of flower extracts indicated that the bioaccessibility of phenolic acids was higher than that of flavonoids. Polyphenols from Nasturtium and Chamomile showed the highest bioaccessibility. The study is an attempt to characterize traditionally consumed edible flowers and promote their wider utilization in gastronomy and nutraceuticals.
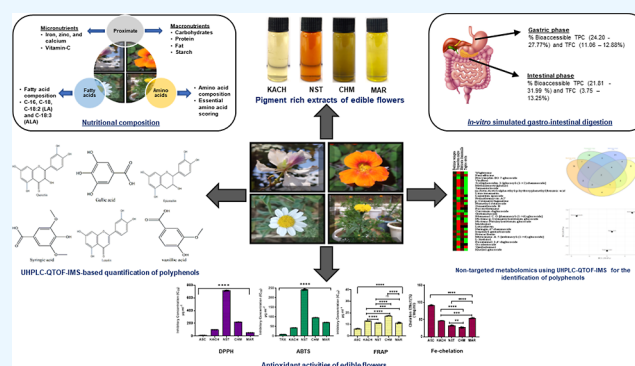
1. INTRODUCTION

The demand for edible flowers has risen sharply in the recent past. Edible flowers have been utilized for enhancing the sensory attributes of foods such as the decoration of desserts, salads, ice creams, and beverages.[1] Besides their attractive colors and culinary applications, edible flowers have been attributed with myriad bioactive properties such as antioxidant, anti-inflammatory, anti-cancer, and neuroprotective effects.[2] The wide range of bioactivities could be corroborated by the presence of phytochemicals such as polyphenols (phenolic acids and flavonoids), carotenoids, and terpenes.[3] Edible flowers have been part of wild edibles in several cultures and traditions.[4−6] Traditionally, they are used to enhance the color and aroma of foods, as condiments, and as an aphrodisiac.[7,8] Clinical evaluations of essential oils, solvent extracts, and dry powders of a few edible flower species, such as *Calendula officinalis*, *Viola* sp., *Matricaria* sp., *Achillea millefolium*, *Hibiscus* sp., *Rosa* sp., and *Crocus sativus*, have been reported to show health benefits such as anti-hypertensive, anti-depressant, anxiolytic, and sleep-enhancing properties conforming to the traditional wisdom of their consumption.[3] The medicinal effects of some of the edible flowers, such as rose, hibiscus, and

jasmine, have been mentioned in traditional medicinal systems such as Ayurveda, Unani, and Chinese medicine.[9,10] This information creates a significant interest in pursuing edible flowers as an alternative source of nutraceuticals and therapeutics.

In this context, we identified four edible flower species, namely, *Bauhinia variegata* L. (Kachnar), *Tropaeolum majus* L. (Nasturtium), *Matricaria chamomilla* L. (Chamomile), and *Tagetes erecta* var. Pusa Basanti Gainda (Marigold), that are quite commonly consumed in the Western Himalayan region.[11] There are very few reports describing in detail the nutritional, phytochemical, and antioxidant properties of the aforesaid edible flowers.[12,13] For example, Marigolds have been extensively utilized as an industrial source of lutein; however, they are a rich source of flavonoids that are seldom
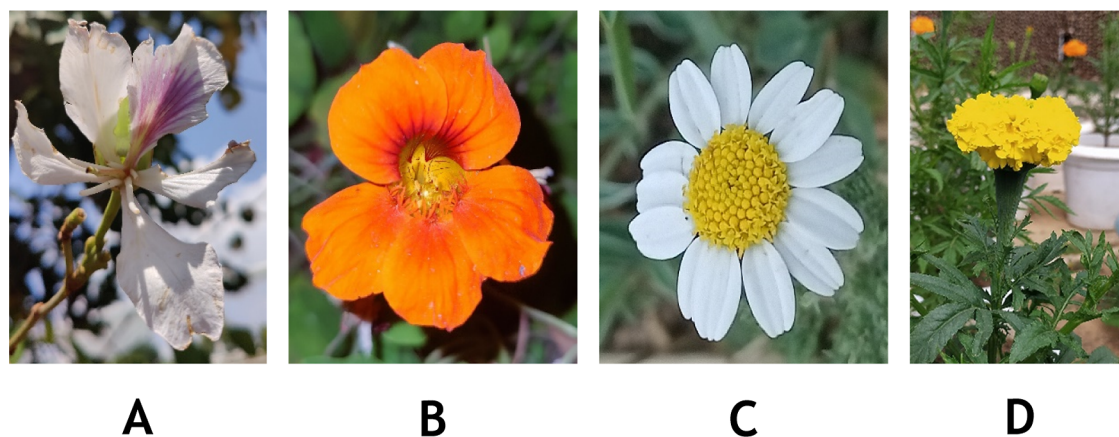
Figure 1. Photographs of wild edible flowers (A) *Bauhinia variegata*, (B) *Tropaeolum majus*, (C) *Matricaria chamomilla*, and (D) *Tagetes erecta*.

characterized and under-explored.[14] Similarly, prior studies are limited to only the phytochemical composition without clear information on their nutritional quality and bioaccessibility.[15] The data on nutritional composition and dietary value is essential, as some of the wild edible flowers selected in the present study, such as *B. variegata* and *T. majus,* are consumed as vegetables and used in mainstream food preparations in the Western Himalayan region.[16] Thus, in the present study, we have characterized in detail the nutritional components such as macronutrients, amino acids, fatty acids, minerals, and phytochemicals in the selected edible flower species. With the rise of modern techniques, such as metabolomics in food composition analysis, the scope of utilization of under-utilized crops, their value addition, and quality control in food processing and preservation has increased significantly.[13,17]

Metabolomics is an evolving and widely deployed *omics* tool for quantifying and analyzing the distribution of different metabolites.[18] In the present study, we evaluated the phytochemical composition of selected edible flowers using both targeted and non-targeted metabolomics approaches and identified several metabolites that could find use as marker compounds and understood the distribution of a different class of metabolites that were earlier not identified or reported. Further, the determination of the bioaccessibility of phytochemicals from edible flowers is important, as the gastrointestinal environment can change the structures and biological characteristics of polyphenols, thus affecting their bioactivity.[19] Therefore, in the present study, we simulated the gastrointestinal environment under *in vitro* conditions and quantified the bioaccessible total phenolic acids and flavonoid contents of the selected edible flowers. Ultimately, the present work was envisaged to highlight the nutritional and nutraceutical potential of selected edible flowers from the Western Himalayan region and promote their domestication, wider cultivation, and enhanced utilization in gastronomy.

2. RESULTS AND DISCUSSION

There have been very fewer comprehensive investigations on the nutritional and phytochemical composition of edible flowers.[4] Most of the prior reports focus only on the polyphenol composition and antioxidant properties of edible flowers.[20−23] A comprehensive analysis of nutritional and phytochemical composition is essential for enhanced utilization and value addition of edible flowers that seldom find applications in mainstream foods. In this direction, the present study, for the first time, describes the amino acid and fatty acid composition of selected edible flowers along with non-targeted metabolomics for the identification of potential marker compounds in an attempt to broaden the existing knowledge on these flowers for their nutraceutical applications. The photographs of edible flowers collected from the Western Himalayas are presented in Figure 1 and their ethnobotanical applications are provided in the Supporting Information (Table S1).

2.1. Nutritional Composition of Edible Flowers. The moisture content of the flowers ranged between 77 and 86% with the highest moisture content observed for Nasturtium. Total crude protein, starch, and total sugars were highest in Kachnar (Table 1). However, the trend was the opposite with respect to the total fat content of the flowers. Marigold and Chamomile contained the highest fat content (∼4% w/w), almost 2-fold higher compared to Kachnar. The vitamin C content was highest in Chamomile (125.08 mg 100 g^{-1}), followed by Nasturtium (100.06 mg 100 g^{-1}), Marigold (75.05 mg 100 g^{-1}), and least in Kachnar (50.03 mg 100 g^{-1}). The ash content of the flowers ranged from 4.5 to 6.75%, and essential micronutrients such as calcium, iron, and zinc were 2 to 4 folds higher in Chamomile when compared to the other three flowers. Magnesium content was highest in Marigold, while potassium and phosphorus contents were highest in Nasturtium (Table 1).

Among the selected flowers, water-extractable proteins were highest in Kachnar (12.33 ± 0.13 g 100 g^{-1}), followed by Chamomile (10.27 ± 0.30 g 100 g^{-1}), Nasturtium (6.90 ± 0.28 g 100 g^{-1}), and least in Marigold (5.23 ± 0.02 g 100 g^{-1}) (data not shown). Results suggested that almost 90% of the proteins from Kachnar and Chamomile were water extractable, while those from Marigold and Nasturtium were 50 and 58%, respectively. The total protein content of the flowers reported in the present study was relatively higher compared to other commonly consumed edible flowers such as rose, calendula, and sunflower inflorescence.[24] The high-water soluble proteins, total starch, and sugar content in Kachnar indicate its nutritional superiority with respect to macronutrients over other flowers. This suggests the flower's popularity as a vegetable and delicacy in the Western Himalayan region.[11] In general, the macronutrient composition of the edible flowers evaluated in the present study was in the range observed in the earlier reports.[21]

Table 1. Nutritional Composition Analysis of Edible Flowers[a]

Nutrient parameter	B. variegata	T. majus	M. chamomilla	T. erecta
Moisture (%)	77.27 ± 0.37[b]	86.66 ± 3.49[b]	80.45 ± 1.23[ab]	80.72 ± 2.07[ab]
Crude protein ($N \times 6.25$), g 100 g^{-1}	13.55 ± 0.07[a]	11.79 ± 0.03[b]	11.62 ± 0.33[b]	10.45 ± 0.02[c]
Crude fat, g 100 g^{-1}	1.54 ± 0.04[c]	3.53 ± 0.13[b]	3.99 ± 0.11[a]	4.03 ± 0.03[a]
Total carbohydrates, g 100 g^{-1}	77.87 ± 0.12[a]	74.46 ± 0.64[b]	78.98 ± 0.26[a]	78.71 ± 0.31[a]
Total starch, g 100 g^{-1}	16.43 ± 1.24[a]	13.15 ± 1.08[b]	10.68 ± 1.0[b]	10.17 ± 1.84[b]
Total sugars, g 100 g^{-1}	34.06 ± 0.60[a]	24.38 ± 0.59[d]	30 ± 0.69[b]	26.88 ± 1.35[c]
Total ash (%)	5.675 ± 0.07[b]	6.75 ± 0.45[a]	4.9 ± 0.10[c]	4.65 ± 0.15[c]
Vitamin-C, mg 100 g^{-1}	50.03 ± 1.12[c]	100.06 ± 2.23[b]	125.08 ± 2.77[a]	75.05 ± 1.68[b]
Energy, kcal 100 g^{-1}	379.50 ± 0.36[b]	376.77 ± 1.44[b]	393.05 ± 0.99[a]	392.86 ± 1.04[a]
Mineral Profile (mg 100 g^{-1})				
Calcium	172.81 ± 1.72[d]	314.78 ± 6.45[c]	759.45 ± 5.34[a]	372.43 ± 1.59[b]
Iron	18.28 ± 0.35[c]	30.73 ± 0.90[b]	48.11 ± 0.62[a]	14.86 ± 0.16[d]
Magnesium	160.36 ± 1.16[d]	202.01 ± 2.14[b]	192.62 ± 0.77[c]	219.09 ± 2.12[a]
Zinc	3.36 ± 0.05[b]	3.86 ± 0.15[b]	8.29 ± 0.28[a]	3.65 ± 0.06[b]
Potassium	1979.93 ± 7.49[b]	2539.13 ± 2.03[a]	1864.41 ± 3.12[c]	1550.80 ± 4.54[d]
Phosphorus	276.07 ± 2.40[c]	497.65 ± 4.00[a]	342.93 ± 2.32[b]	353.06 ± 2.80[b]
Amino Acid Composition (g 100 g^{-1})				
Essential AA				
Histidine	0.42 ± 0.03[a]	0.41 ± 0.008[a]	0.36 ± 0.01[b]	0.21 ± 0.01[c]
Leucine	1.08 ± 0.04[c]	1.19 ± 0.02[a]	1.11 ± 0.008[b]	0.56 ± 0.005[d]
Isoleucine	0.61 ± 0.01[b]	0.72 ± 0.03[a]	0.66 ± 0.003[b]	0.29 ± 0.02[c]
Lysine	0.90 ± 0.005[b]	1.02 ± 0.02[a]	0.95 ± 0.002[b]	0.52 ± 0.005[c]
Methionine	0.43 ± 0.01[a]	0.30 ± 0.01[b]	0.30 ± 0.005[b]	0.20 ± 0.01[c]
Phenylalanine	0.65 ± 0.02[b]	0.82 ± 0.008[a]	0.69 ± 0.04[b]	0.35 ± 0.008[c]
Threonine	0.68 ± 0.02[b]	0.85 ± 0.02[a]	0.74 ± 0.03[b]	0.37 ± 0.01[c]
Tryptophan	0.13 ± 0.01[b]	0.15 ± 0.05[b]	0.09 ± 0.05[c]	0.18 ± 0.01[a]
Valine	0.63 ± 0.02[b]	0.90 ± 0.04[a]	0.79 ± 0.02[a]	0.31 ± 0.01[c]
Non-essential AA				
Arginine	0.68 ± 0.01[b]	0.77 ± 0.01[a]	0.71 ± 0.008[b]	0.43 ± 0.02[c]
Aspartic acid	4.31 ± 0.04[a]	2.54 ± 0.02[b]	1.94 ± 0.02[c]	1.24 ± 0.01[d]
Cysteine	0.12 ± 0.005[a]	0.07 ± 0.005[ab]	0.15 ± 0.01[a]	0.04 ± 0.01[b]
Glutamic acid	1.79 ± 0.05[c]	2.48 ± 0.01[b]	2.59 ± 0.04[a]	1.65 ± 0.02[d]
Glycine	0.71 ± 0.01[c]	0.91 ± 0.01[a]	0.80 ± 0.01[b]	0.52 ± 0.01[d]
Proline	0.92 ± 0.01[b]	0.93 ± 0.02[b]	1.03 ± 0.03[a]	0.59 ± 0.02[c]
Serine	0.75 ± 0.008[b]	1.01 ± 0.02[b]	0.80 ± 0.04[a]	0.48 ± 0.01[c]
Tyrosine	0.46 ± 0.01[a]	0.49 ± 0.01[a]	0.48 ± 0.01[a]	0.28 ± 0.02[b]
Alanine	0.78 ± 0.008[b]	0.97 ± 0.03[a]	0.81 ± 0.01[b]	0.47 ± 0.01[c]
$\sum$TAA	15.90 ± 0.18[b]	16.40 ± 0.17[a]	14.92 ± 0.06[c]	8.67 ± 0.06[d]
$\sum$EAA	6.09 ± 0.16[b]	7.05 ± 0.06[a]	6.33 ± 0.06[b]	3.47 ± 0.07[c]
Fatty Acid Composition (Relative %)				
Dodecanoic acid (C-12:0)	0.47 ± 0.007[bc]	4.01 ± 0.17[a]	0.88 ± 0.12[b]	0.44 ± 0.04[c]
Tetradecanoic acid (C-14:0)	1.86 ± 0.23[a]	nd	nd	3.30 ± 0.36[b]
Hexadecanoic acid (C-16:0)	27.05 ± 1.34[a]	31.10 ± 1.69[a]	30.30 ± 0.56[a]	27.15 ± 1.62[a]
Octadecanoic acid (C-18:0)	7.11 ± 0.12[b]	8.41 ± 0.36[a]	5.23 ± 0.15[c]	6.91 ± 0.40[b]
6-Octadecenoic acid (C-18:1, n6)	0.42 ± 0.01[a]	nd	nd	nd
9-Octadecenoic acid (C-18:1, n9)	Nd	nd	nd	25.95 ± 4.03[a]
10-Octadecenoic acid (C-18:1, n10)	Nd	nd	nd	1.16 ± 0.41[a]
9,12-Octadecadienoic acid (C-18:2, n9, 12)	37.80 ± 1.14[a]	22.75 ± 0.77[c]	30.05 ± 0.49[b]	33.05 ± 3.18[ab]
9,12,15-Octadecatrienoic acid (C-18:3, n9, 12, 15)	24.52 ± 0.01[c]	33.28 ± 0.36[a]	27.60 ± 0.51[b]	nd
$\sum$SFA	36.50 ± 1.23[b]	43.51 ± 1.15[a]	36.41 ± 0.84[b]	37.80 ± 1.55[b]
$\sum$MUFA	0.42 ± 0.01[b]	nd	nd	27.11 ± 4.44[a]
$\sum$PUFA	24.52 ± 0.01[c]	33.28 ± 0.37[a]	27.59 ± 0.51[b]	nd
IA	0.55 ± 0.01[a]	0.62 ± 0.03[a]	0.54 ± 0.01[a]	0.67 ± 0.06[a]
IT	0.39 ± 0.01[b]	0.35 ± 0.01[b]	0.36 ± 0.02[b]	1.24 ± 0.07[a]
HPI	1.79 ± 0.05[a]	1.60 ± 0.10[a]	1.85 ± 0.04[a]	1.48 ± 0.14[a]
HH	0.85 ± 0.03[a]	0.95 ± 0.05[a]	0.88 ± 0.03[a]	0.88 ± 0.20[a]

[a]nd—not detected; AA—amino acids; $\sum$TAA—sum of total amino acids; $\sum$EAA—sum of essential amino acids; $\sum$SFA—sum of saturated fatty acids; $\sum$MUFA—sum of mono-unsaturated fatty acid; $\sum$PUFA—sum of poly-unsaturated fatty acid; IA—index of atherogenicity; IT—index of thrombogenicity; HPI—health-promoting index; HH—hypocholesterolemic/hhypercholesterolemic ratio. Values are the mean of three replicates ±SD (standard deviation). Values followed by different letters in the same row are significantly different ($p < 0.05$).
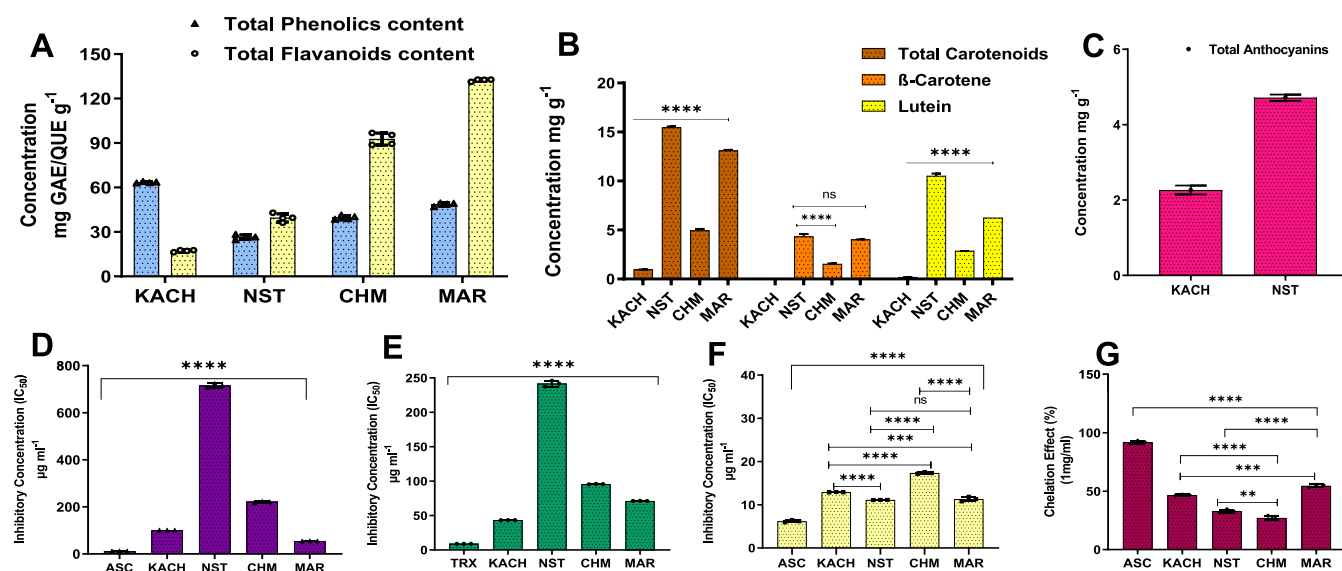
Figure 2. Total phenolics and flavonoid contents (A), total carotenoids, β-carotene, and lutein composition (B), total anthocyanin content (C), and antioxidant activities DPPH (D), ABTS (E), ferric reducing power (F), Fe-chelation effect (G) of four edible flowers. KACH = Kachnar; NST = Nasturtium; CHM = Chamomile; MAR = Marigold; ASC = Ascorbic acid; TRX = Trolox. Values are the mean of three individual experiments expressed as mean ± SD. ****$p < 0.0001$, ***$p = 0.0002$, **$p = 0.0021$, and ns = 0.8776 (analyzed using one-way ANOVA).

The total amino acid content (TAA) ranged between 14.9 and 16.4 g 100 g^{-1} protein among Kachnar, Nasturtium, and Chamomile flowers, while the TAA content was two-fold lower in Marigold flowers (Table 1). A similar trend was observed for essential amino acid (EAA) content (Table 1). Amongst EAA, lysine content was highest in Nasturtium, while methionine content was highest in Kachnar. The branched-chain amino acids (BCAA) viz., leucine, isoleucine, and valine contents were quantitatively highest in Nasturtium, followed by Chamomile, Kachnar, and least in Marigold (Table 1).

The amino acid composition of the selected edible flowers was scored against the reference amino acid pattern for children aged between 3 and 10 years as recommended by WHO/FAO/UNU.[25] The chemical scoring indicated that tryptophan was the first limiting amino acid in Kachnar, Nasturtium, and Chamomile, whereas leucine was the first limiting amino acid in Marigold (Table S2). Most of the EAA, except tryptophan, had a chemical score ratio >1.00 with respect to Kachnar, Nasturtium, and Chamomile. However, this was not the case with Marigold, where most of the EAA had a chemical score ratio <1.00. With respect to sulfur amino acids, Kachnar and Chamomile had the similar and highest score (1.56), followed by Nasturtium (1.35) and the least in Marigold (1.14). With respect to branched-chain amino acids, viz., leucine, isoleucine, and valine, the sum of scores was highest in Chamomile (6.13), closely followed by Nasturtium (5.89), while almost two-fold lesser in Marigold (2.65). In the case of aromatic amino acids (phenylalanine and tyrosine), Chamomile scored highest (1.81) closely followed by Nasturtium (1.76) while almost two folds lesser in Marigold (0.95). The study indicated that the protein quality of the Marigold flower was poor in comparison with the other flowers. To the best of our knowledge, the present study is the first report to provide a detailed account of the total amino acid composition and its quality for the selected edible flowers. The high-water soluble protein content of Kachnar and Chamomile can be effectively utilized in preparing beverages

and floral infusions for enriching both the macronutrient and phytochemical contents of the food products.

The relative composition of the fatty acids of edible flowers is presented in Table 1. Kachnar, Nasturtium, and Chamomile possessed a similar composition with palmitic (C-16:0), linoleic (LA, C-18:2, $n - 6$), and α-linolenic acid (ALA, C-18:3, $n - 3$) being the predominant fatty acids. In the case of the Marigold, ALA was not detected, and oleic acid (C-18:1, $n - 9$) was the major fatty acid (25% w/w) along with LA and palmitic acid. Whereas, oleic acid was a minor fatty acid (<3% w/w) in the other three flowers, while stearic acid was present in lower amounts between 5 and 8% among all the flowers with the highest content in Nasturtium. The fatty acid composition of Kachnar flowers observed in the present study with high concentrations of LA and ALA was earlier reported by Villavicencio et al.[15] Barring this, there is no detailed characterization available on Kachnar flowers. Similarly, the fatty acid composition of Nasturtium and Chamomile flowers is not characterized in detail. In the case of Marigold, the results obtained in the present study were similar to an earlier report on the fatty acid composition of marigold oleoresin obtained from supercritical CO_2 extraction.[26]

The nutritional quality of the fatty acid composition of the selected edible flowers was assessed by the determination of various nutritional indices such as IA, IT, HH, and HPI. The atherogenic index of the fatty acids ranged between 0.54 and 0.67 and was statistically not significant. The thrombogenicity index of Kachnar, Nasturtium, and Chamomile flowers was less than 0.4 and was statistically similar; however, in the case of Marigold, the value was three folds higher (1.24). This could be attributed to the absence of ALA and high concentrations of oleic acid. Despite the low occurrence of ALA in marigold lipids, the HPI and HH are at par with the other edible flowers. The presence of high quantities of SFAs such as lauric, myristic, and palmitic acids in dietary fats is considered to be proatherogenic and thrombogenic, which promotes adhesion of lipids to the cells and clot formation in vascular tissues.[27] There is evidence that fat sources with lower IA and IT scores

reduce the total cholesterol and LDL cholesterol levels in blood plasma.[28] The IA and IT scores obtained for edible flowers in the present study were in the range observed for marine foods such as seaweeds, fish, and certain crops such as cumin, guar seeds,[27] and the pharmacologically important flower *Scabiosa stellata*.[29] The HH ratio was below 1, indicating lower total PUFAs in edible flowers compared to marine foods. The HPI is an inverse of IA, and the values ranged between 1.48 and 1.85 and were statistically insignificant. Thus, foods with relatively higher unsaturated fatty acids are beneficial and promote cardiovascular health. The present study adds new information with respect to the fatty acid composition of the selected edible flowers. The comparative evaluation of the fatty acid composition of flowers (petals) indicated potential applications of Kachnar, Nasturtium, and Chamomile flowers as a source of ALA. The plant-derived ALA has been attributed with cardio-protective and cognition improvement properties, as evidenced from clinical studies.[30] Further, it may suggest that lipid extracts of these edible flowers may be utilized for enriching the fatty acid composition of high SFA foods, such as dairy products that have usually higher IA, IT, and lower HPI scores.[31]

2.2. Total Phenolic Acids and Flavonoids Content.

Flowers are known for their characteristic colors (pigments) and phytochemical constituents such as phenolic acids and flavonoids that impart various health benefits when consumed.[32] Extraction with water resulted in two folds higher solids yield (>300 mg g^{-1}) compared to aqueous methanol for all the flowers. However, extraction with aqueous methanol yielded significantly higher phytochemical constituents, $1.2-3$ fold higher TPC, and $1.4-11$ fold higher TFC when compared to water extracts (Table S4). The water extracts predominantly contained soluble proteins. Among the four flowers, TPC content in the aqueous methanolic extracts was highest in Kachnar (63.36 mg GAE g^{-1}), followed by Marigold (48.55 mg GAE g^{-1}), Chamomile (39.65 mg GAE g^{-1}), and least in Nasturtium (Figure 2A). In case of total flavonoids, the trend was opposite, with Marigold containing the highest TFC with 132.42 ± 0.61 mg QUE g^{-1}, followed by Chamomile (92.62 ± 3.33 mg QUE g^{-1}), Nasturtium (39.46 ± 2.25 mg QUE g^{-1}), and least in Kachnar (17.21 ± 0.52 mg QUE g^{-1} flower) in aqueous methanolic extracts (Figure 2A).

The data obtained for the selected flowers in the present study significantly varied with respect to TPC and TFC contents when compared with the earlier reports published by Navarro-González *et al.*[21] for Marigold and Nasturtium, Zheng *et al.*[22] for Kachnar and Nasturtium flowers, Chensom, Okumura and Mishima; Barros *et al.*[4,23] for Nasturtium. The high degree of variations in the TPC and TFC contents of flower samples between the earlier reports and the present study could be attributed to the effect of differences in sample origin, environmental conditions, and physiological characteristics of the plant varieties.[22] Further, few authors express TPC in catechin (mg CE g^{-1})[33] or ferulic acid equivalents (mg FE g^{-1}) and flavonoids in rutin equivalents (mg RU g^{-1}),[34] making it difficult to compare the present results with the earlier reports. However, the TPC and TFC values obtained for selected edible flowers in the present study were much higher compared to commonly consumed vegetables and fruits (Table S4). Similar observations of higher TPC and TFC in edible flowers over vegetables and fruits have been made by Cai *et al.*[35] Thus, it is recommended to include edible flowers in the diet for enhanced intake of polyphenols.

2.3. Antioxidant Activity.

The selected edible flowers have naturally high concentrations of bioactive compounds such as polyphenols, anthocyanins, and carotenoids and thus can be considered as natural sources of antioxidants. With respect to DPPH radical scavenging activity, Marigold showed the highest scavenging activity, with an IC$_{50}$ value of 54.59 ± 0.37 μg mL^{-1}. In contrast, the poorest activity was exhibited by Nasturtium with an IC$_{50}$ value of 715.59 ± 2.53 μg mL^{-1} (Figure 2D). The IC$_{50}$ values shown by each of the flower extracts were statistically significant at $p < 0.0001$ (Figure 2D). In the case of ABTS scavenging activity, Kachnar showed the highest scavenging activity (IC$_{50}$—43.31 ± 0.04 μg mL^{-1}), followed by Marigold (IC$_{50}$—71.22 ± 0.09 μg mL^{-1}) (Figure 2E). Similar to the DPPH scavenging activity, Nasturtium showed the poorest ABTS radical scavenging activity (IC$_{50}$—241.66 ± 3.71 μg mL^{-1}) (Figure 2E).

The reducing power of the flower extracts was highest in Nasturtium and Marigold, with an IC$_{50}$ value of 11.10 ± 0.02 and 11.31 ± 0.47 μg mL^{-1}, respectively. However, the values were statistically not significant ($p = 0.877$) between them. This was followed by Kachnar (IC$_{50}$—12.90 ± 0.04 μg mL^{-1}), while the lowest reducing power was exhibited by Chamomile (IC$_{50}$—17.3 ± 0.15 μg mL^{-1}) (Figure 2F). With respect to ferrous chelation activity, Marigold showed the highest percentage of chelation at $54.54 \pm 1.29\%$, followed by Kachnar at $42.45 \pm 0.52\%$. Chamomile and Nasturtium showed 2-fold and 1.7-fold lower ferrous chelation activity compared to Marigold (Figure 2G).

The poor antioxidant activity of Nasturtium with respect to radical scavenging activity could be attributed to the low cumulative polyphenol content (sum of phenolic acids and flavonoids) when compared to other flowers (Figure 2A). Polyphenols scavenge free radicals by donating H atoms through their hydroxyl groups (mainly 3-OH) and catechol moieties.[36] The efficiency of scavenging could be directly attributed to the number of H-donating groups in the extracts,[23] thus explaining the lower scavenging activity of Nasturtium. Further, the results obtained in the present study with respect to the radical scavenging properties of Nasturtium corroborated with an earlier report by Garzón and Wrolstad.[20] The authors reported that ABTS scavenging properties were five-fold higher compared to DPPH, similar to our observation where the ABTS scavenging activity was three folds higher compared to that of DPPH.

In the case of the FRAP assay, the highest reducing power exhibited by Nasturtium and Marigold extracts could be attributed to the presence of hydroxy carotenoids such as lutein in significant amounts compared to the other flowers (Figure 2B). It has been reported that hydroxy carotenoids such as lutein, zeaxanthin, and β-cryptoxanthin were the most effective in reducing ferric ions owing to the presence of hydroxyl functional groups in 3′ positions at the ring system.[37] The dual presence of a conjugated double-bond system and hydroxyl functional groups makes lutein an effective reducing agent similar to phenolic acids and flavonoids.[37] In the case of Marigold, the excellent ability to scavenge free radicals and reduce ferric ions and chelate metals could be correlated with the presence of both polyphenols, specifically flavonoids, and carotenoids in high concentrations. Some characteristic flavonoids, such as patuletin present in Marigold, have been identified with antiproliferative, apoptosis-inducing properties and exhibit the strongest antioxidant property in comparison

Table 2. UHPLC-QTOF-IMS-Based Quantification of Phenolic Acids and Flavonoids Using Targeted Metabolomics[a]

	Flower extracts (μg g^{-1})			
	B. variegata	*T. majus*	*M. chamomilla*	*T. erecta*
Gallic acid	0.61 ± 0.01[a]	3.17 ± 0.31[b]	0.08 ± 0.01[a]	0.42 ± 0.008[a]
Protocatechuic acid	4.35 ± 0.07[b]	3.14 ± 0.10[c]	0.41 ± 0.04[d]	6.13 ± 0.10[a]
Vanillic acid	0.83 ± 0.01[a]	6.38 ± 0.42[b]	0.30 ± 0.04[a]	0.25 ± 0.01[a]
Caffeic acid	0.70 ± 0.10[b]	1.56 ± 0.38[a]	0.05 ± 0.02	0.07 ± 0.002[c]
Syringic acid	0.71 ± 0.02[a]	9.28 ± 0.47[b]	0.32 ± 0.01[a]	0.15 ± 0.01[a]
Epicatechin	3.21 ± 0.09[b]	3.69 ± 0.71[a]	0.19 ± 0.006[c]	nd
p-Coumaric acid	0.09 ± 0.003[a]	1.21 ± 0.24[b]	0.06 ± 0.005[a]	0.33 ± 0.008[a]
Rutin	0.62 ± 0.02[b]	1.14 ± 0.11[a]	0.12 ± 0.008[c]	nd
Ferulic acid	12.87 ± 1.19[a]	11.59 ± 0.16[a]	0.35 ± 0.02[b]	0.27 ± 0.01[b]
Quercetin	25.96 ± 2.2[a]	15.70 ± 0.73[b]	0.56 ± 0.009[c]	0.42 ± 0.005[c]
Luteolin	4.02 ± 0.05[a]	1.80 ± 0.59[b]	0.14 ± 0.014[c]	0.14 ± 0.008[c]

[a]nd—not detected. Values represented are mean of triplicates ±SD (standard deviation). Values followed by different letters in the same row are significantly different ($p < 0.05$).

to quercetin due to the presence of the methoxyl group in the C6 position.[38]

2.4. UHPLC-QTOF-IMS-Based Metabolomics.

2.4.1. UHPLC-QTOF-IMS-Based Quantification of Phenolics and Flavonoids in Edible Flowers Using Targeted Metabolomics. A total of 11 phenolic acids and flavonoids were identified and quantified in the aqueous methanolic extracts through a targeted approach (Table 2). In the case of Kachnar, the predominant phenolic acids were ferulic acid (12.86 ± 1.19 μg g^{-1}), pro-catechuic acid (4.35 ± 0.07 μg g^{-1}), and epicatechin (3.21 ± 0.09 μg g^{-1}). In Nasturtium, ferulic acid (11.59 ± 0.16 μg g^{-1}), syringic (9.28 ± 0.47 μg g^{-1}), and vanillic (6.38 μg g^{-1}) acids were predominant, followed by gallic acid, pro-catechuic acid, and epicatechin in similar concentration ranges (3.14−3.69 μg g^{-1}). The levels of aforesaid phenolic acids were very low, ranging between 0.05 and 0.42 μg g^{-1} in Chamomile flowers, while pro-catechuic acid was found to be in the highest concentration in Marigold (6.13 ± 0.10 μg g^{-1}). Among the flavonoids, quercetin was the predominant one, with Kachnar containing the highest levels (25.5 ± 2.26 μg g^{-1}), followed by Nasturtium (15.70 ± 0.73 μg g^{-1}). Luteolin levels were highest in Kachnar (4.025 ± 0.05 μg g^{-1}), while rutin concentration was highest in Nasturtium (1.14 ± 0.11 μg g^{-1}). A similar trend to phenolic acids was observed in both Chamomile and Marigold, with lesser concentrations of individual flavonoids.

The present study is one of the first reports to characterize individual phenolic acids and flavonoids through a targeted approach for Kachnar flowers. Barros et al.[23] reported the presence of rutin, quercetin, ferulic acid, kaempferol, and *p*-coumaric acid in Nasturtium flowers, with rutin and chlorogenic acid being the major compounds. All these compounds were identified in the present study, however, with significant variations in their concentrations. In addition, the present study reported the presence of vanillic acid in Nasturtium, which was not detected by Barros et al.[23] The variations in the individual concentrations of different polyphenols in the present study in comparison to the earlier reports could be attributed to the effect of environmental conditions on the physiological characteristics of plants, consequently affecting the chemical composition of flowers.[22]

2.4.2. Non-targeted Metabolomics Using UHPLC-QTOF-IMS. A total of 48 metabolites were identified in positive ion mode using UHPLC-QTOF-IMS based on their peak identification, MS/MS spectra, and reported literature.

Furthermore, raw files (.d format) generated were used to search the non-targeted metabolites using the METLIN database (scoring accuracy of >95%). It is easy, less time-consuming, and mainly used for the determination of plant metabolite profiling, and also provides univariate statistical information and pathway analysis.[39]

2.4.2.1. Kachnar. In Kachnar, eleven flavonoid glycosides (peak **1−11**) were identified based on their mass fragmentation pattern. Four peaks (**1**, **3**, **6**, and **7**) were identified as kaempferol-3-*O*-glucosyl-7-*O*-glucoside, kaempferol-3-*O*-rutinoside, kaempferol-3-*O*-glucoside, and kaempferol-3-*O*-robinoside at retention times (rt) 5.399, 7.954, 9.653, and 9.962 with measured mass *m/z* 609.25 [M + H]$^{+}$, 595.23 [M + H]$^{+}$, 449.16 [M + H]$^{+}$, and 595.23 [M + H]$^{+}$, respectively. Similar to kaempferol, quercetin derivatives such as quercetin-3-*O*-rhamnoside, quercetin-3-*O*-glucosyl-7-*O*-glucoside, and quercetin-3-*O*-rutinoside were identified at peaks 4, 9, and 10. Apart from this, glycosidic derivatives of apigenin, myricetin, and luteolin were identified. The detailed identification of different classes of metabolites is given in Table 3 and Figure S2. Prior to this, there were no detailed reports on the polyphenol composition of Kachnar commonly consumed in the Western Himalayan region. However, the polyphenol composition of white flowers of *B. variegata* L. var. *Candida alba* Buch-Ham from Brazil locally known as "pata-de-vaca" was evaluated by Villavicencio et al.[15] The group reported the presence of phenolic acids such as chlorogenic, caffeic, and *p*-coumaric acid derivatives and flavonoids quercetin, kaempferol, and myricetin glycosides, apigenin, and luteolin glycosidic derivatives in the flower extracts. A comparison of the polyphenol profile obtained for Kachnar with that of the *pata-de-vaca* variety indicated that these flavonoid glycosides could be used as marker compounds in the identification of *B. variegata*. Similar compounds have been reported in other *Bauhinia* species, such as *Bauhinia forficata* and *Bauhinia galpinii*, from Egypt.[40]

2.4.2.2. Nasturtium. In the case of Nasturtium, five flavonoid glycosides, five phenolic acids, one flavanone, one flavone, and one phenolic aldehyde were identified (Table 3, Figure S2). Five phenolic acids (peaks **12**, **13**, **18**, **19**, and **21**) at rt 1.269, 2.059, 5.393, 6.735, and 8.492 with measured masses *m/z* 155.07 [M + H]$^{+}$, 339.11 [M + H]$^{+}$, 359.23 [M + Na]$^{+}$, 375.12 [M + Na]$^{+}$, and 299.10 [M]$^{+}$ were identified as protocatechuic acid, *cis*-3-*O*-*p*-coumaroylquinic acid, coumaroylquinic acid, caffeoylquinic acid, and ellagic acid, respec-

Table 3. UHPLC-QTOF-IMS-Based Identification of Compounds in Edible Flower Extracts Using Non-targeted Metabolomics[a]

Peak no.	t_R	Molecular formula	Actual mass	Measured mass	Major fragments	Expected compound	Major class	References
				B. variegata				
1	5.399	$C_{27}H_{30}O_{15}$	608.5	609.25 $[M + H]^+$	447.19	kaempferol-3-O-glucosyl-7-O-glucoside	flavonoid glycoside	15, 40
2	6.436	$C_{21}H_{20}O_{13}$	480.44	480.19 $[M]+$	317.17	myricetin-3-glucoside	flavonoid glycoside	
3	7.954	$C_{27}H_{30}O_{15}$	594.52	595.23 $[M + H]^+$	279.16	kaempferol-3-O-rutinoside	flavonoid glycoside	
4	8.017	$C_{21}H_{20}O_{11}$	448.41	449.17 $[M + H]^+$	303.13	quercetin-3-O-rhamnoside	flavonoid glycoside	
5	9.352	$C_{21}H_{20}O_{10}$	432.21	433.17 $[M + H]^+$	341.28	apigenin-6-C-glucoside	flavonoid glycoside	
6	9.653	$C_{21}H_{20}O_{11}$	448.62	449.16 $[M + H]^+$	287.10	kaempferol-3-O-glucoside	flavonoid glycoside	
7	9.962	$C_{27}H_{30}O_{15}$	594.15	595.23 $[M + H]^+$	301.16	kaempferol-3-O-robinoside	flavonoid glycoside	
8	12.693		594.25	617.34 $[M + Na]^+$	431.23	apigenin-C-hexoside-O-hexoside	flavonoid glycoside	
9	13.308		626.19	627.37 $[M + H]^+$	393.16	quercetin-3-O-glucosyl-7-O-glucoside	flavonoid glycoside	
10	14.704	$C_{27}H_{30}O_{16}$	610.25	611.37 $[M + H]^+$	212.06	quercetin-3-O-rutinoside	flavonoid glycoside	
11	15.436	$C_{21}H_{20}O_{11}$	448.39	469.34 $[M + Na]^+$	287.10	luteolin-6-C-glucoside	flavonoid glycoside	
				T. majus				
12	1.269	$C_7H_6O_4$	154.12	155.07 $[M + H]^+$	127.05	protocatechuic acid	phenolic acid	19, 21, 23
13	2.059	$C_{16}H_{18}O_8$	338.31	339.11 $[M + H]^+$	104.13	*cis*-3-O-*p*-coumaroylquinic acid	phenolic acid	
14	2.302	$C_8H_8O_3$	152.15	153.09 $[M + H]^+$	138.08	vanillin	phenolic aldehyde	
15	2.972	$C_{15}H_{10}O_5$	270.03	271.18 $[M + H]^+$	91.08	apigenin	flavone	
16	3.703	$C_{15}H_{12}O_5$	272.26	273.18 $[M + H]^+$	252.16	naringenin	flavanone	
17	4.246	$C_{15}H_{12}O_5$	433.23	433.24 $[M]^+$	292.16	quercetin pentoside	flavonoid glycoside	
18	5.393	$C_{16}H_{18}O_8$	336.41	359.23 $[M + Na]^+$	298.17	coumaroylquinic acid	phenolic acid	
19	6.735	$C_{16}H_{18}O_8$	352.21	375.12 $[M + Na]^+$	191.04	caffeoylquinic acid	phenolic acid	
20	7.826	$C_{27}H_{31}O_{15}^+$		595.23 $[M + H]^+$	448.24	cyanidin hexosyl deoxyhexoside	flavonoid glycoside	
21	8.492	$C_{14}H_6O_8$	299.10	299.10 $[M]^+$	122.11	ellagic acid	Phenolic acid	
22	8.617	$C_{27}H_{31}O_{17}^+$	627.14	627.23 $[M + H]^+$	303.10	delphinidin dihexoside	flavonoid glycoside	
23	9.223	$C_{27}H_{22}O_{18}$	633.37	633.21 $[M + H]^+$	287.10	HHDP-galloyl hexoside	flavonoid glycoside	
24	10.017	$C_{30}H_{33}O_{19}^+$	697.16	697.23 $[M + H]^+$	287.10	cyanidin (malonylhexosyl)-hexoside	flavonoid glycoside	
				M. chamomilla				
25	2.053	$C_{19}H_{18}O_8$	374.2	375.16 $[M + H]^+$		dihydroxy tetramethoxy flavone	flavone	41, 42
26	3.082	$C_{16}H_{22}O_{10}$	356.12	357.17 $[M + H]^+$	193.15	ferulic acid glucose	phenolic acid derivative	
27	4.366	$C_{19}H_{18}O_9$	464.15	464.18 $[M]^+$	304.15	pentahydroxyflavon-7-O-hexoside	flavonoid glycoside	
28	5.515	$C_{27}H_{30}O_{16}$	610.25	611.28 $[M + H]^+$	303.19	quercetin-3-O-rutinoside	flavonoid glycoside	
29	6.067	$C_{25}H_{24}O_{12}$	520.18	521.19 $[M + H]^+$	167.10, 352.22	4,5-dicaffeoylquinic acid	phenolic acid	
30	6.008	$C_{15}H_{10}O_8$	480.21	481.19 $[M + H]^+$	313.14	hexahydroxyflavone-3-O-hexoside	flavonoid glycoside	
31	6.552	$C_{25}H_{24}O_{12}$	518.52	519.15 $[M + H]^+$	351.21	1,5-dicaffeoylquinic acid	phenolic acid	
32	7.280	$C_{24}H_{22}O_{13}$	518.39	519.15 $[M + H]^+$	267.13	apigenin-7-O-molonylglucoside	flavonoid glycoside	
33	7.706	$C_{16}H_{18}O_9$	354.52	355.15 $[M + H]^+$	167.10, 179.07	3-O-caffeoylquinic acid	phenolic acid	
34	8.923	$C_{21}H_{19}O_{12}$	462.35	463.18 $[M + H]^+$	303.10	quercetin-3-O-glucoside	flavonoid glycoside	
				T. erecta				
35	2.010	$C_{16}H_{11}O_8^+$	331.25	332.16 $[M + H]^+$	258.18	patuletin	flavonol	21, 44
36	3.344	$C_{21}H_{21}O_{12}^+$	466.40	467.14 $[M + H]^+$	268.15	delphinidin-3-O-hexoside	flavonoid glycoside	
37	4.549	$C_{16}H_{17}O_9^+$	354.31	355.15 $[M + H]^+$	127.05, 191.98	3-O-caffeoylquinic acid	phenolic acid	
38	5.703	$C_{16}H_9O_8^+$	330.24	331.18 $[M + H]^+$	202.22, 230.14	dimethylellagic acid	phenolic acid	
39	6.678	$C_{15}H_{17}O_9^+$	342.30	343.15 $[M + H]^+$	135.11	caffeic acid hexoside II	flavonoid glycoside	
40	7.225	$C_{17}H_{13}O_7^+$	330.29	331.33 $[M + H]^+$	299.15, 315.12	5,7-dimethyl quercetin	flavonoid glycoside	
41	7.655	$C_{27}H_{29}O_{14}^+$	578.12	579.20 $[M + H]^+$	244.28	kaempferol-3-O-rhamnoside-7-O-rhamnoside	flavonoid glycoside	
42	8.505	$C_{21}H_{17}O_{13}^+$	478.25	501.46 $[M + Na]^+$	244.28, 272.28	methylellagic acid hexoside	phenolic acid derivative	
43	9.412	$C_{24}H_{27}O_{14}^+$	540.19	541.22 $[M + H]^+$	182.09, 183.09	disyringoyl hexoside I	flavonoid glycoside	
44	10.634	$C_{29}H_{25}O_{17}^+$	646.89	647.53 $[M + H]^+$	495.17	patuletin-7-O-(6″-galloyl) hexoside	flavonoid glycoside	
45	11.363	$C_{40}H_{56}O_2$	568.87	569.40 $[M + H]^+$	533.40	lutein	xanthophyll	

Table 3. continued

Peak no.	t_R	Molecular formula	Actual mass	Measured mass	Major fragments	Expected compound	Major class	References
				T. erecta				
46	12.700	$C_{28}H_{23}O_{16}^+$	616.12	617.25 [M + H]$^+$	301.34	quercetin-3-O-(6″hexosyl) hexoside	flavonoid glycoside	
47	12.947	$C_{27}H_{29}O_{15}^+$	594.22	595.422 [M + H]$^+$	301.34	quercetin-3,7-di-O-rhamnoside	flavonoid glycoside	
48	14.465	$C_{26}H_{27}O_{15}^+$	580.21	581.40 [M + H]$^+$	301.34	quercetin-3-O-rhamnoside-7-O-pentoside	flavonoid glycoside	

[a]t_R—retention time.
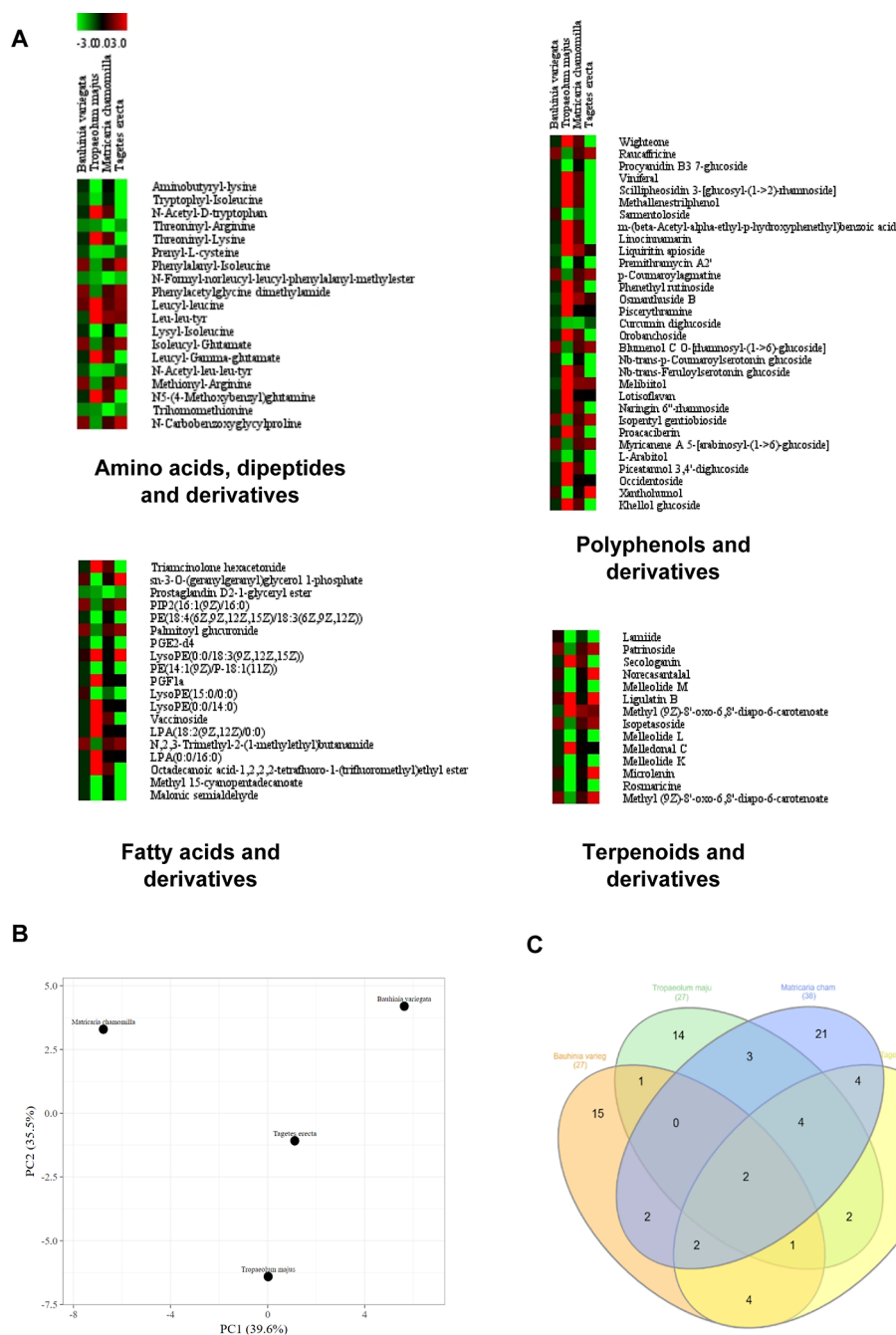
Figure 3. Visualization of metabolites classified into four categories (A), PCA analysis of edible flowers (B), Venn-diagram depicted the differential and common metabolites among the four edible flower species (C).

tively. Among flavonoid glycosides (peaks **17**, **20**, **22**, **23**, and **24**), quercetin pentoside, cyanidin hexosyl deoxyhexoside, delphinidin dihexoside, HHDP-galloyl hexoside, and cyanidin-

(malonylhexosyl)-hexoside were identified (Table 3). Identification of cyanidin and delphinidin derivatives in the extracts could be corroborated with the total anthocyanin content of

the Nasturtium extracts (Figure 1C). Other metabolites identified include apigenin (flavone) and naringenin (flavonone). Some of the flavonoids identified in the present study were previously reported in the native Brazilian Nasturtium species called *capuzin* and varieties from Spain.[21,23] *Capuzin* was identified with the presence of flavonols such as kaempferol and quercetin glycosides, phenolic acids, such as chlorogenic, ellagic, and protocatechuic acids, in addition to flavones and flavanones.[23] Nasturtium flowers have been identified with the presence of anthocyanins such as cyanidin, delphinidin, and pelargonidin earlier.[19,20] However, in the present study, we could not detect pelarogonidin or its derivatives.

2.4.2.3. Chamomile. Similar to the above samples, Chamomile was also identified with five flavonoid glycosides *viz.*, pentahydroxyflavon-7-O-hexoside, quercetin-3-O-rutinoside (rutin), hexahydroxyflavone-3-O-hexoside, apigenin-7-O-molonylglucoside, and quercetin-3-O-glucoside at peaks **27**, **28**, **30**, **32**, and **34**. Caffeoylquinic acids (4,5-dicaffeoylquininc acid, 1,5-dicaffeoylquininc acid, and 3-caffeoylquinic acid) were the predominantly identified phenolic acids in the Chamomile (peaks **29**, **31**, and **33**). In addition, a flavone, dihydroxy tetramethoxy flavone, was identified at peak **25** with a measured mass of 375.16 $[M + H]^+$. The polyphenolic composition obtained in the present study matched with earlier reports on Chamomile infusions.[41,42] The group reported that rutin trihydrate, ferulic acid, caffeoylquinic acids, and apigenin-7-O-glucoside were predominant compounds in Chamomile infusions. Based on the data obtained from past and current research, rutin, caffeoylquinic acids, and apigenin derivatives, mainly apigenin-7-O-glucoside, could be considered as marker compounds of *Matricaria* spp. As these compounds were identified as predominant metabolites in *Matricaria recutita* and commercial teas made of Chamomile.[41−43]

2.4.2.4. Marigold. Nine flavonoid glycosides (peaks **36**, **39**, **40**, **41**, **43**, **44**, **46**, **47**, and **48**) were tentatively assigned for delphinidin-3-O-hexoside, caffeic acid hexoside II, 5,7-dimethyl quercetin, kaempferol-3-O-rhamnoside-7-O-rhamnoside, disyringoyl hexoside I, patuletin-7-O-(6″-galloyl) hexoside, quercetin-3-O-(6″hexosyl) hexoside, quercetin-3,7-di-O-rhamnoside, and quercetin-3-O-rhamnoside-7-O-pentoside, respectively in the extract. Apart from flavonoid glycosides, flavonol and 3 phenolic acid derivatives were identified with measured masses m/z of 332.16 $[M + H]^+$, 355.15 $[M + H]^+$, 331.18 $[M + H]^+$, and 501.46 $[M + Na]^+$ and assigned for patuletin, 3-O-caffeoylquinic acid, dimethylellagic acid, and methylellagic acid hexoside, respectively.

The identification of different types of flavonoid glycosides through a non-targeted metabolomics approach corroborates the highest TFC content in Marigold among the tested edible flowers. The presence of flavonoid glycosides listed in our study has been previously reported in the ethanolic extracts of *T. erecta*.[21,44] Two flavonoid compounds have been predominantly identified, *viz.*, patuletin and laricitrin and their derivatives in the *Tagetes* genus.[44] Patuletin is a quercetagetin (trimethoxyflavone) and is generally considered a marker compound in *T. erecta*, *Tagetes patula* (French marigold)[26,38] while laricitrin is a methyl derivative of myricetin (monomethoxyflavone) identified in *T. erecta*.[21,44] In our study, we identified patuletin; however, we did not detect laricitrin in the aqueous methanolic extracts.

Apart from these, 31 flavonoids and their derivatives, 19 amino acids, dipeptides, and their derivatives, 19 fatty acid derivatives, and 14 terpenoids were identified. Visualization was performed based on z-score values with heat maps for the four edible flower extracts. Dark red and bright green showed higher and lower z-scores, respectively. Although heat map matrices are useful for large data sets to display the underlying information in two-dimensional mode, PCA is a more popular metabolomics method useful for describing the distribution behavior of a group of a large number of metabolites after dimensional reduction.[45] PCA analysis revealed that the four edible flower samples had distinctive behavior with a clear separation with a total variance of 75.1% in normalized data. PC1 and PC2 contributed to 39.6 and 35.5% of the variance, respectively. Kachnar and Chamomile lie in opposite quadrants of PCA due to significant differences in the metabolites. The METLIN database identified unique and common metabolites, as depicted in the Venn diagram (Figure 2). Leucyl-leucine was commonly present in all four edible flowers, followed by threoninyl-arginine, *N*-formyl-norleucine-leucyl-phenylalanyl-methyl ester, trihomomethionine, and prostaglandin D2-1-glyceryl ester identified in Nasturtium, Chamomile, and Marigold. An amino acid (tryptophyl-isoleucine) and sugar alcohol (*L*-arabitol) were noticed in Nasturtium and Marigold (Figure 3).

Non-targeted metabolomics in the present study suggested that the kaempferol and quercetin glycosides could be the characteristic marker compounds for Kachnar, while cyanidin and delphinidin glycosides could be the markers for Nasturtium flowers. In the case of Chamomile, apigenin glycoside and caffeoylquinic acid derivatives could be the marker compounds, while patuletin and quercetin glycosides could be the characteristic markers for Marigold. These suggestive marker compounds have been associated with a myriad of health benefits, owing to their free radical scavenging and anti-inflammatory properties.[46]

Flavonoid derivatives identified in edible flowers, such as kaempferol and quercetin glycosides, have been shown to inhibit the oxidation of low-density lipoproteins by scavenging reactive oxygen species (ROS) and preventing platelet aggregation, thereby reducing the thrombogenic risk and coronary heart disease.[47] Further, kaempferol has been associated with the reduction of hyperglycemia and associated complications such as diabetic neuropathy, nephropathy, and retinopathy through the enhancement of insulin sensitivity and glucose uptake.[48] Apigenin glycosides are known to improve the antioxidant status by scavenging free radicals, inhibiting of pro-inflammatory cytokines and lipid peroxidation, downregulation of cyclooxygenase and lipoxygenase enzymes, and enhancing the glutathione, superoxide dismutase, and catalase levels.[49] Likewise, patuletin and its derivatives have been attributed with anti-proliferative properties along with strong antioxidant and anti-inflammatory activities.[38] Phenolic acids such as caffeoylquinic acid derivatives possess nutraceutical value, especially in treating metabolic syndromes such as obesity, dyslipidemia, and hypertension.[50,51] Further, they have been demonstrated with excellent neuroprotection and enhanced cognitive effects, along with improvement of memory and learning deficits.[50] Anthocyanins such as cyanidin and delphinidin derivatives have been shown to inhibit pro-inflammatory cytokine production and NF-κB by the down-regulation of mitogen-activated protein kinase pathway (MAPK), inhibition of angiotensin-converting enzymes, and

enhanced nitric oxide synthesis.[52] These properties accord them with various health benefits like cardioprotective, anti-obesity, and improved visual acuity.

2.5. Pigment Composition of Edible Flowers.
2.5.1. Carotenoids. The total carotenoid content of the flower samples was determined in the 70% aqueous methanolic extracts. Among the selected flowers, Nasturtium contained the highest total carotenoid content (15.46 mg g^{-1}), followed by Marigold (13.10 mg g^{-1}), while Kachnar contained the least total carotenoids (0.95 mg g^{-1}) (Figure 2B). The total carotenoid content observed for Nasturtium and Marigold flowers in the present study was higher when compared to earlier published reports for Brazilian Nasturtium sp., which contained 7.6 to 8.5 mg g^{-1} total carotenoids,[23] *T. erecta* L. var. "Deep Orange" containing 4.39 mg g^{-1}, and *T. erecta* var. Pusa Basanti containing 7.8 mg g^{-1}.[33,53] HPLC-DAD analysis of saponified extracts revealed that lutein and β-carotene were the major pigments in Marigold and Nasturtium, contributing 75 and 95% of the total carotenoid composition, respectively. Traditionally, marigolds are known to be a rich source of lutein; however, in the present study, Nasturtium contained a higher lutein content (10.52 mg g^{-1}) compared to marigold (6.24 mg g^{-1}). However, the β-carotene contents were statistically similar among these two flowers. In Marigold, the lutein and β-carotene constituted nearly 80% of the total carotenoids. The remaining components of Marigold carotenoid extracts could be other xanthophylls such as zeaxanthin, cryptoxanthin, and vialoxanthin and lutein esters.[53] Earlier literature has reported the presence of esterified lutein such as lutein monoesters (3'-O-lauroyl/myristoyl/palmitoyl lutein) and di-esters containing different combinations of fatty acids, which constitute more than 60% of marigold extracts.[54] In the present study, we have not characterized the lutein esters or other carotenoid esters in the extracts of selected edible flowers.

The present study reiterates the fact that edible flowers are a richer source of carotenoids (5 to 10 folds higher) compared to commonly consumed vegetables such as carrot, pumpkin, spinach, kale, and other plants belonging to Umbelliferae.[55] The health benefits of carotenoids in ameliorating oxidative stress, chronic disorders, and non-communicable diseases have been well understood.[56] This implies that edible flowers could be used for enriching the carotenoid content in foods and act as effective dietary sources of carotenoids. According to the National Health and Medical Research Council (2006),[57] issued by the Commonwealth Department of Health and Aging, Australia, and the National Institute of Health, USA, the estimated daily requirement of β carotene is 4−18 mg per day for various age groups. Similarly, it has been reported that consumption of 6 mg of lutein per day could reduce the risk of age-related macular degeneration.[58] From the present study, it is evident that supplementation of 3 to 5 g of dry Nasturtium powder per day could satisfy the daily β-carotene requirements, and supplementation of 2 to 3 g of dried Nasturtium and Marigold flowers could meet the aforesaid lutein requirements.

2.5.2. Anthocyanins. Anthocyanins were detected in two of the selected edible flowers, Nasturtium and Kachnar. Nasturtium contained 4.71 ± 0.08 mg g^{-1} TAC, followed by Kachnar (2.26 ± 0.11 mg g^{-1}) (Figure 2C). The TAC of Nasturtium observed in the present study was higher in comparison to the earlier report of Bortolini *et al.*,[19] who reported concentrations ranging between 0.66 and 3.79 mg

cyanidin equivalents g^{-1} d.w. in the Nasturtium flowers of Brazil. In the case of Kachnar, the TAC content observed in the present study was 3 to 7 folds lower when compared with different *Bauhinia* sp., such as including *B. variegata* as reported by Ahmed *et al.*[59] Prior reports on the anthocyanin composition of Nasturtium suggest that cyanidin and its glycosidic derivatives, delphinidin, pelargonidin were predominant anthocyanins,[19,20] while Kachnar consisted of cyanidin glucosides as the predominant anthocyanin.[59] Anthocyanins are used as natural colorants in the food processing industry as an alternative to artificial colorants and have been attributed with health benefits owing to their high antioxidant and anti-inflammatory properties.[2] The present study indicated that Nasturtium flowers could be a potential source of both carotenoids and anthocyanins despite their relatively lower total phenolic acids and flavonoid contents. It has been reported that Nasturtium exists in a wide range of colors from yellow to orange to a deep red which could be associated with the presence of high concentrations of both anthocyanins and carotenoids.[23]

2.6. α-Glucosidase Inhibition Activity. Polyphenols have been reported to possess anti-diabetic properties by their ability to inhibit key enzymes involved in carbohydrate metabolism, *viz.*, pancreatic α-amylase and intestinal α-glucosidase.[60−62] The polyphenol extracts of the four edible flowers were screened for their α-glucosidase inhibitory property through an *in vitro* assay. The ability to inhibit is expressed as an IC$_{50}$ value, and a lower IC$_{50}$ value indicates a better inhibitory potential. A dose-dependent α-glucosidase inhibitory activity was observed in the polyphenol extracts of the four edible flowers. Among the four edible flowers, Chamomile extracts showed the best inhibitory activity with the lowest IC$_{50}$ value (181.98 μg mL^{-1}), followed by Nasturtium (226.10 μg mL^{-1}). The Marigold extract exhibited the least inhibitory property with nearly 15-fold higher IC$_{50}$ values compared to Chamomile and Nasturtium (Figure 4).
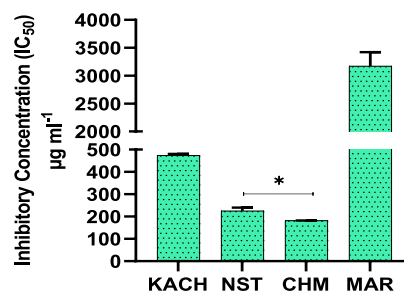

Figure 4. α-Glucosidase inhibitory activity of edible flower extracts. Values are the mean of three individual experiments expressed as mean ± SD.

The ability of Chamomile flavonoids to inhibit digestive enzymes such as lipase, α-glucosidase has been reported earlier.[60] The group reported that enzymatic modification of flavonoid-rich infusions exhibited nearly 60% inhibition of α-glucosidase activity at 60 μM compared to raw infusions, which exhibited about 37% of inhibition at similar concentrations. The group reported that the polyphenols play a main role in the α-glucosidase inhibition in Chamomile, such as apigenin derivatives, mainly apigenin-7-O-glucosides, caffeic acid, and caffeoylquinic acids. These metabolites were detected in the present study (Table 3). In the case of Marigold, the inhibitory

Table 4. *In Vitro* Simulated Gastro-Intestinal Digestibility of Phenolic Acids and Flavonoids[a]

Edible flowers	Crude		G-phase		% Bioaccessibility after gastric phase		I-phase		% Bioaccessibility after intestinal phase	
	TPC (mg GAE g⁻¹ flower)	TFC (mg QUE g⁻¹ flower)	TPC (mg GAE g⁻¹ flower)	TFC (mg QUE g⁻¹ flower)	TPC	TFC	TPC (mg GAE g⁻¹ flower)	TFC (mg QUE g⁻¹ flower)	TPC	TFC
B. variegata	63.36 ± 0.38[a]	14.24 ± 0.16[d]	15.05 ± 0.19[a]	1.82 ± 0.03[d]	24.23 ± 0.31[b]	12.88 ± 0.24[a]	13.50 ± 0.30[a]	1.77 ± 0.09[d]	21.81 ± 0.49[b]	12.48 ± 0.67[a]
T. majus	29.33 ± 0.6[d]	40.12 ± 0.10[c]	7.15 ± 0.21[d]	4.94 ± 0.14[c]	24.38 ± 0.72[b]	12.31 ± 0.35[a]	9.38 ± 0.42[c]	5.31 ± 0.21[b]	31.99 ± 1.45[a]	13.25 ± 0.72[a]
M. chamomilla	33.12 ± 0.51[c]	74.48 ± 0.37[b]	9.18 ± 0.33[c]	7.58 ± 0.15[b]	27.73 ± 0.99[a]	10.18 ± 0.20[b]	10.29 ± 0.75[c]	6.13 ± 0.2[a]	31.06 ± 2.27[a]	8.23 ± 0.37[b]
T. erecta	41.36 ± 0.36[b]	118.90 ± 1.30[a]	10.09 ± 0.19[b]	13.15 ± 0.25[a]	24.40 ± 0.46[b]	11.06 ± 0.21[b]	12.06 ± 0.35[b]	4.46 ± 0.24[c]	29.17 ± 0.85[a]	3.75 ± 0.20[c]

[a]G-phase—gastric phase; I-phase—intestinal phase; TPC—total phenolic acid content; TFC—total flavonoids content; GAE—gallic acid equivalent; QUE—quercetin equivalent. Values represented are mean of triplicates ±SD (standard deviation). Values followed by different letters in the same column are significantly different ($p < 0.05$).

capacity was much lower compared to a few earlier reports on African Yellow Marigold.[61,62] The polyphenolic extracts in these studies exhibited 10-fold lower IC_{50} values compared to the values observed in the present study. Contrasting results of very low α-glucosidase inhibition activity with IC_{50} values ranging between 3.12 and 7.40 mg mL⁻¹ were reported by Parklak *et al*.[63] The wide variations in the bioactivity could be attributed to the varying polyphenol composition arising due to varietal differences, agro-climatic conditions, nutrient availability, and the method of extraction. The α-glucosidase inhibitory activity of Nasturtium flowers from the Mediterranean region has been previously reported, and the activity has been attributed to the presence of polyphenols.[64]

The present study, for the first time, reports the α-glucosidase inhibitory activity for Kachnar flowers. Previously, the anti-diabetic activity, specifically α-glucosidase inhibition, of *B. variegata* L. leaves from Egypt was reported, wherein the IC_{50} value was 139 μg mL⁻¹. The inhibitory activity was attributed to the presence of flavonoids (quercetin, rutin, and hesperidin) and phenolic acids (chlorogenic and *p*-coumaric acid). Similar observations of α-glucosidase inhibition by leaves of different *Bauhinia* sp., such as *B. forficata*, *B. galpinii*, *B. variegata*, and *B. variegata* var. *Candida*, have been reported by Farag *et al*.[40] The anti-diabetic properties of polyphenols are mediated through multiple mechanisms, such as inhibition of digestive enzymes, as performed in the present study, stimulation of glycogen storage, activation of insulin signaling, and inhibition of advanced glycation end products.[65] Thus, the present study offers scope for more detailed work on the anti-diabetic potential of edible flowers of the Western Himalayas owing to their high polyphenol content.

2.7. *In Vitro* Digestibility of the Edible Flower Extracts. The concentration of metabolites available for absorption after gastrointestinal digestion is called bioaccessibility. The bioaccessibility of total phenolic acids and flavonoids in the selected edible flowers after simulated gastrointestinal digestion is presented in Table 4. The percentage bioaccessibility of TPC after the gastric phase was in the range between 24 and 27%, which was four folds lesser compared to the initial TPC content in crude aqueous methanolic extracts. Between the flower samples, the percent bioaccessibility of TPC showed very little variations, with the highest percent bioaccessibility in Chamomile (27.73 ± 0.99%), while the remaining samples showed similar values (24.30−24.40%) which were statistically not significant ($p >$

0.99). The percent bioaccessibility of TPC after the intestinal phase ranged between 21.81 and 31.99%, with statistically similar and highest percent bioaccessibility observed for Nasturtium, Chamomile, and Marigold and least in Kachnar. The data indicates that the percent bioaccessibility of TPC significantly increased for Nasturtium, Chamomile, and Marigold from the gastric phase to the intestinal phase, while that of Kachnar decreased.

The flavonoid content (TFC) after the gastric phase reduced by 10-fold in comparison with crude extracts with percent bioaccessibility ranging between 10.18 and 12.88%. However, after the intestinal phase, the bioaccessibility of TFC varied among the flowers, with Kachnar and Nasturtium showing statistically similar and highest percent bioaccessibility of 12.48 and 13.25%, respectively. The percent bioaccessibility of TFC in Marigold was the lowest (3.75 ± 0.20%). It can be inferred that the flavonoids from Kachnar and Nasturtium are more bioaccessible when compared with Marigold, despite later containing significantly higher TFC.

The bioaccessibility of polyphenols under simulated gastrointestinal digestion is species-specific and varies widely with different flower species. These variations could be attributed to the change in the structure of phenolics by oxidation or enzymatic.[12] In the present study, the TPC and TFC content significantly decreased after gastrointestinal digestion in comparison to crude extracts for all the flowers. The increase in the percentage bioaccessibility after the intestinal phase when compared to the gastric phase in flowers such as Nasturtium, Chamomile, and Marigold could be attributed to the differences in the hydrolysis of bound and complex phenolic acids. For example, the bioaccessibility of certain phenolic acids such as chlorogenic, coumaric, caffeic, gallic, and caffeoylquinic acids increased after gastrointestinal digestion in a few edible flowers from Brazil.[12] This was attributed to the hydrolysis of gallic acid from gallotannins, coumaric acid from coumarylquinic conjugates, and the hydrolysis of di- and tricaffeoylquinic acids, leading to the increased concentration of the aforesaid phenolic acids. Similar observations of enhanced bioaccessibility of aforesaid phenolic acids after gastrointestinal digestion were observed in edible Rose and Nasturtium flowers by Bortolini *et al*.[19] Further, the increase in the percentage bioaccessibility of TPC after the intestinal phase when compared to the gastric phase could be attributed to the interaction of digestive enzymes and the release of phenolic compounds linked to other moieties in the

matrix such as proteins, carbohydrates, and fibers.[12] In general, phenolic acids exist in the glycosylated forms in the matrix, as observed both in the present and earlier studies.[19] It can be hypothesized that during the simulated digestion in the intestinal phase, the alkaline pH conditions and presence of glucosidase enzymes in the intestinal fluid could hydrolyze the glycosidic bonds, thereby releasing the phenolics.[66]

3. MATERIALS AND METHODS

3.1. Standards and Reagents. Phenolic acids and flavonoids standards (gallic acid, *p*-catechuic acid, caffeic acid, syringic acid, vanillic acid, *p*-coumaric acid, rutin, quercetin, luteolin, ferulic acid) and carotenoid standards (lutein, *β*-carotene) were procured from Sigma-Aldrich, St. Louis, USA. Bovine serum albumin (BSA), anthrone, D (+) glucose, L-ascorbic acid, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Hi-Media Laboratories Private Limited, Mumbai, India. Analytical grade solvents were used for the extraction of plant materials such as acetone, methanol, hexane, acetyl chloride, and ethanol, and acids such as sulfuric acid, hydrochloric acid, and perchloric acids were procured from S.D. Fine Chem Ltd., Mumbai, India.

3.2. Collection of Flowers and Sample Preparation. All the samples were collected between March and May 2022 from the district of Kangra, Himachal Pradesh, India. Flowers of *B. variegata* L. (Kachnar) were collected from village Hangloh, Kangra, located in the GPS: 32.2119366° N and 76.180429° E, while *T. maju* L. (Nasturtium) were collected from village Mansimbal, Kangra, located in the GPS: 32.0717° N, 76.4982° E, and *M. chamomilla* L. (Chamomile) were collected from village Bharmat Uparli, Kangra, located in the GPS: 32.107542250264096° N, 76.5678324262151° E. Seeds of *T. erecta* var. Pusa Basanti Gainda (Marigold) were procured from the seed production unit, ICAR-Indian Agriculture Research Institute, New Delhi, and were cultivated at the floriculture farm of CSIR-IHBT, Palampur, Himachal Pradesh, India (GPS: 32.10530366572456° N, 76.5569041553071° E). All the plants were taxonomically validated at the CSIR-IHBT herbarium. The geographical distribution of the selected plants is presented in the Supporting Information (Table S1). The flowers were collected at the full bloom stage and washed in running water, and the petals were separated from the flower, blotted, and frozen at −20 °C. The frozen samples were freeze-dried (FDTA-50100, Operon Co., Ltd., Korea), and the dried petals were pulverized and sieved (50 mesh, ASTM standards) to achieve particle sizes of less than 300 *μ*m and stored in airtight containers at 4 °C.

3.3. Proximate and Nutrient Analysis of Edible Flowers. The proximate analysis of freeze-dried powders of flowers was determined using standard procedures of the Association of Official Analytical Chemists (AOAC 2012).[67] The crude protein was determined by the micro-Kjeldahl method, total fat was determined using *n*-hexane in a Soxhlet apparatus while ash was determined by igniting samples at 550 °C for 3 h in two cycles until constant weight. The total carbohydrate content was determined by the difference method. Total starch content was determined by enzymatically digesting samples with *α*-amylase and amyloglucosidase and determining the liberated glucose and total sugars were estimated by digesting samples in an acidic solution (2.5 N HCl), followed by determination using the phenol−sulfuric acid method as described in Sareen, Bhattacharya, and

Srivatsan.[68] The mineral compositions, such as calcium, magnesium, sodium, potassium, iron, zinc, and copper, were determined from the ash samples using atomic absorption spectrophotometry.[67] The total energy was determined by applying Atwater factors, where 1 g of protein and carbohydrate contributes 4 kcal, while 1 g of fat contributes 9 kcal of energy. All analyses were performed in triplicate.

3.4. Amino Acid Analysis. The amino acid composition was determined by digesting the samples (equivalent to 5.0 mg protein) in 6 N HCl followed by derivatization with *o*-phthaldialdehyde. The amino acid derivatives were analyzed and quantified using reverse-phase high-performance liquid chromatography (RP-HPLC). The amino acid content was expressed per 100 g of sample.[68]

3.5. Fatty Acid Analysis. The fatty acid composition of the flower samples was determined as per the method described by Christie.[69] The crude fat was converted to fatty acid methyl esters (FAME) using 5% (w/v) methanolic hydrogen chloride, followed by extraction with *n*-hexane. The FAME extracts were washed with 5% NaCl and 2% KHCO$_3$ solutions and dried over anhydrous Na$_2$SO$_4$, followed by vacuum concentration. The FAMEs were dissolved in HPLC-grade *n*-hexane and analyzed using a GC−MS (Agilent 7890 series) equipped with a flame-ionization detector and a fused silica HP-5 column (30 m length, 0.32 mm width, and 0.25 *μ*m film thickness). The temperature program for the separation of fatty acids was followed as described by Vidyashankar *et al.*[70] The injection volume was 0.5 *μ*L of the FAME extract. The FAMEs were identified by comparing the retention times with the standard FAME mixture (C$_8$−C$_{24}$, FAME mix, Sigma-Aldrich, USA) and their fragmentation patterns. The data were expressed as the relative percentage composition of the fatty acids in each sample.

3.5.1. Nutritional Indices for Assessing the Fatty Acids of Edible Flowers. Nutritional indices of dietary components, specifically fats, offer a method to evaluate the quality of fatty acid composition. Some of the most commonly used parameters are the index of atherogenicity (IA), the index of thrombogenicity (IT), the hypocholesterolemic/hypercholesterolemic (HH) ratio, and the health promoting index (HPI). These indices were calculated using the eqs 1−4 recommended by Chen and Liu[27] but originally developed by Ulbricht and Southgate.[71]

IA was calculated using eq 1.

$$IA = \frac{[(C12:0 + (4 \times C14:0) + C16:0)]}{\sum UFA} \tag{1}$$

where C12:0—lauric acid; C14:0—myristic acid; C16:0—palmitic acid; $\sum$UFA—sum of unsaturated fatty acid.

IT was calculated using eq 2.

$$IT = [C14:0 + C16:0 + C18:0]$$
$$/\left[\left(0.5 \times \sum MUFA\right) + \left(0.5 \times \sum n - 6 \, PUFA\right)\right.$$
$$\left. + \left(3 \times \sum n - 3 \, PUFA\right) + (n - 3/n - 6)\right] \tag{2}$$

where, C14:0—myristic acid; C16:0—palmitic acid; C-18:0—stearic acid; $\sum$MUFA—sum of mono-unsaturated fatty acid; $\sum n - 6 \, PUFA$—linoleic acid; $\sum n - 3 \, PUFA$—*α*-linoleic acid.

HPI was calculated using eq 3.

$$HPI = \frac{\sum UFA}{[(C12:0 + (4 \times C14:0) + C16:0)]} \qquad (3)$$

where C12:0—lauric acid; C14:0—myristic acid; C16:0—palmitic acid; $\sum$UFA—sum of unsaturated fatty acid.

HH ratio was calculated using eq 4.

$$HH = \frac{cis\text{-}C18:1 + \sum PUFA}{C12:0 + C14:0 + C16:0} \qquad (4)$$

where C12:0—lauric acid; C14:0—myristic acid; C16:0—palmitic acid; cis-C18:1—cis Oleic acid $\sum$PUFA—sum of unsaturated fatty acid.

3.6. Solvent Extraction of Flowers for Phytochemical Analysis. The freeze-dried flower samples were extracted in deionized water and 70% aqueous methanol for the determination of phytochemical composition. Briefly, 1 g of the samples was extracted with 10 mL of aforesaid solvents for 30 min in the dark. The extracts were centrifuged at 10,000 rpm for 15 min, and the supernatants were stored at 4 °C while the pellets were re-extracted till the supernatants turned colorless with respective solvents. The supernatants were pooled and filtered using polyvinylidene difluoride membranes (PVDF, Ø 13 mm, thickness 0.45 μm) and stored in the dark at 4 °C. Based on the results obtained, such as the yield of the phytoconstituents like phenolic acids and flavonoids between two solvents, 70% aqueous methanolic extracts were selected for detailed characterization.

3.7. Phytochemical Composition Analysis. *3.7.1. Estimation of Total Phenolics, Flavonoids, and Antioxidant Activity.* The total phenolic content (TPC) in flower extracts was determined by using the Folin–Ciocalteu (FC) phenol method as described by Ainsworth and Gillespie.[72] Gallic acid was used as a standard, and the results were expressed as mg gallic acid equivalents (GAE) per gram dry extract (mg g^{-1}). The total flavonoid content (TFC) was determined by the aluminum chloride (AlCl$_3$) method suggested by Oh et al.[73] Quercetin was used as a reference standard, and the results were expressed as mg quercetin equivalents (QUE) per gram dry extract (mg g^{-1}). The *in vitro* antioxidant activity of the aqueous methanolic extracts of four flower samples was determined using four different assays, viz., 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging and 2,2′-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radical scavenging activity as per the protocol of Idris et al.,[74] while ferric reducing power assay (FRAP) and ferrous chelation activity were determined as per the method described by Oh et al.[73] Ascorbic acid was the reference standard for DPPH, FRAP, and ferrous chelation assays, while Trolox was used as the reference standard for the ABTS assay. The results were represented in terms of 50% inhibitory concentration (IC$_{50}$) as the assays are concentration-dependent, whereas the results of the ferrous chelation assay were expressed as percentage chelation.

3.7.2. Estimation of Total Anthocyanin Content. Total anthocyanin content (TAC) was estimated using the method described by Bhatt et al.[75] The flower samples were extracted in acidified methanol (0.1 M HCl; 10 mL) under sonication at 35 °C for 30 min. The TAC was quantified using the pH differential method. The TAC was expressed as mg g^{-1} cyanidin-3-O-glucoside and calculated using the formula

$$TAC\left(\frac{mg}{g}\right) = \frac{A \times MW \times DF \times V}{\varepsilon \times l \times W} \qquad (5)$$

where $A = (A_{510nm} - A_{700nm})_{pH1.0} - (A_{510nm} - A_{700nm})_{pH4.5}$, MW = 449.2 g mol^{-1} for cyanidin-3-glucoside, DF = dilution factor; V = total volume of the extract; ε = 26,900 molar extinction coefficient in L·mol^{-1}·cm^{-1}, for cyanidin-3-glucoside; l = path length in cm; and W = sample weight (g).

3.7.3. Estimation of Total Carotenoids Content. Carotenoids were extracted using a solvent mixture consisting of hexane, ethanol, acetone, and toluene (HEAT) in the ratio of 10:6:7:7, as described by Jing et al.[76] Briefly, 0.5 g of flower powders were extracted with 30 mL of the HEAT solvent mixture. The extracts were centrifuged at 10,000 rpm for 15 min, and the supernatant was stored at 4 °C. The pellets were re-extracted with the HEAT solvent mixture until the supernatant turned colorless. The supernatant obtained from each cycle of extraction was pooled and saponified with 2 mL of a 40% KOH−MeOH solution. The mixture was left in the dark for 16 h at room temperature. To the saponified extracts, 38 mL of a 10% Na$_2$SO$_4$ solution and 30 mL of hexane were added and vortexed. The upper hexane layer was collected and dried over anhydrous Na$_2$SO$_4$. The bottom layer was reextracted with hexane and vortexed, followed by the separation of the organic phase. The hexane fractions were pooled and concentrated under vacuum. The extracts were resuspended in methanol and filtered through 0.2 μm filter and stored in −20 °C. The samples were diluted appropriately, and the carotenoid composition was determined by HPLC-PDA analysis. The total carotenoid content was expressed as mg g^{-1} and was calculated using the eq 6 suggested by Rodriguez-Amaya and Kimura.[77]

$$Total\ carotenoids = \frac{A_{total} \times V \times 10^4}{A_{cm}^{1\%} \times sample\ weight\ (g)} \qquad (6)$$

where A_{total} = absorbance; V = total volume of extract (mL); $A_{cm}^{1\%}$ = absorption coefficient of 2500, which is recommended for mixtures.

3.8. Identification and Quantification of β-Carotene and Lutein Using HPLC-PDA. The carotenoid extracts of flower samples were analyzed for β-carotene and lutein by HPLC (6CE, Waters, Singapore) using the reverse phase C18 column (Supelco, 25 cm × 4.6 mm) as per the method described by Ranga Rao et al.,[78] following ICH guidelines.[79] An isocratic solvent system containing acetonitrile (ACN), dichloromethane (DCM), and methanol (MeOH) in the ratio of 70:20:10 was used as a mobile phase, maintaining a flow rate of 1 mL min^{-1}. The separated carotenoids were identified using a photo-diode array detector (2998, Waters, Singapore) by comparing them with authentic standards of β-carotene and lutein and quantified using a calibration curve at different concentrations. The linearity range, regression equation with r^2 values, limit of detection (LOD), and limit of quantification (LOQ) of lutein and β-carotene are presented in Table S7.

3.9. UHPLC-QTOF-IMS-Based Metabolomics. *3.9.1. Quantification of Phenolic Acids and Flavonoids Using Targeted Metabolomics.* Quantification of phenolic acids and flavonoids was performed using the UHPLC-QTOF-IMS Ion mobility 6560 system (Agilent Technologies, USA). The analytical column used for separation was Eclipse Plus C18 (RRHD 2.1 × 150 mm, 1.8 μm) (Agilent Technologies, USA). A gradient mobile phase consisting of two solutions,

viz., solution A comprising 0.1% formic acid in water and solution B comprising 0.1% formic acid in acetonitrile (ACN), was used at a flow rate of 0.30 mL/min for separation of metabolites as explained in Kumar *et al.* and Dadwal *et al.*[80,81] The peaks were monitored at 280 nm, and the quantification of phenolic acids and flavonoids was performed by plotting the calibration curve for each standard at different concentrations along with their respective retention times and UV spectra as per ICH guidelines.[79−81] The linearity range, regression equation with r^2 values, LOD, and LOQ of individual phenolic acids and flavonoids are presented in Table S6.

3.9.2. Non-targeted Metabolomics Using Liquid Chromatography−Mass Spectrometry (LC−MS). The metabolites were separated and identified using high-resolution 6560 Ion Mobility Q-TOF LC/MS (Agilent, Santa Clara, USA). The technique uses a two-solvent system with mobile phases A (0.1% formic acid in water) and B (0.1% formic acid in acetonitrile) in a gradient elution condition, as mentioned in Kumar *et al.*[80] The parameters of mass spectrometry were explained earlier.[71] Furthermore, the collective sample information was processed with MassHunter Qualitative Analysis software (version B.06.00, Agilent Technology) for extensive data mining.[82] The critical elements for data processing are alignment, normalization, a specified sample set, and frequency analysis filtering. This was performed using Agilent Mass Hunter Profinder software and Mass Profiler Professional (MPP) software. The raw files (.d format) in triplicate were used for the determination of the fragments. These fragments were searched against the METLIN database for non-targeted compound identification. The absolute abundance of metabolites with >5000 counts was matched to the retention time and precise mass.

3.10. α-Glucosidase Inhibition Activity. The capacity of polyphenolic extracts of the four edible flowers to inhibit α-glucosidase was measured in a 96-well microplate reader at 405 nm as per the method described by Núñez *et al.*[62] Each well contained a 50 μL sample at different concentrations and 100 μL enzyme (1 U/mL) dissolved in phosphate buffer (12.5 mM Na_2HPO_4, 3.3 mM NaH_2PO_4; pH = 6.9). After a 10 min incubation time at room temperature, 50 μL of the substrate, *p*-nitrophenyl-α-D-glucopyranoside (3 mM) was added and incubated at 37 °C for 15 min. The absorbance was measured every 10 min after the addition of substrate for 1 h. Acarbose was used as a positive control. The percentage of enzyme inhibitory activity was plotted against the concentration of the flower extract. The assay was performed in triplicate, and the results were expressed as the mean IC_{50}.

3.11. In Vitro Simulated Gastro-Intestinal Digestibility Assay. The *in vitro* simulated gastro-intestinal digestibility assay was performed according to the method described by Bortolini *et al.*[19] Briefly, the extracts were reacted sequentially with gastric fluid consisting of pepsin at pH 2.00, followed by intestinal fluid containing pancreatin and bile salts at pH 6.00. The aqueous methanolic extracts of the edible flower samples were incubated with gastric fluid at 37 °C for 2 h in the dark in an orbital shaker at 150 rpm (LE-TT-A, Orbitek, Scigenics Biotech, India), followed by centrifugation at 5000 rpm for 10 min. The supernatant obtained was further treated with intestinal fluid, and the mixture was incubated at 37 °C for 1 h as described above. The resulting mixture was centrifuged, and the bioaccessibility of total phenolic acids and total flavonoid content was estimated according to eq 7

$$B\ (\%) = \left(\frac{C}{C_0} \right) \times 100$$

(7)

where *B* is the bioaccessibility (%), *C* is the remaining concentration of respective phytochemicals (mg g^{-1}) after *in vitro* digestion, and C_0 is the initial concentration (mg g^{-1}) in the crude extract.[19]

3.12. Statistical Analysis. All the experiments were carried out in triplicate ($n = 3$), and the data are represented as the average ± standard deviation (SD). The data were statistically analyzed using one-way ANOVA followed by Tukey's multiple comparison test. The statistical analysis was conducted using GraphPad Prism version 8.0.2 for windows (GraphPad software, California, USA). The significant differences in mass intensities were statistically evaluated using ANOVA following the Benjamini−Hochberg FDR correction. To identify metabolites, the filtered list of compounds ($p < 0.05$; log FC cut off ≤1.2) was searched against the METLIN database. Multiple Experiment Viewer (MeV, version. 4.6.2) and Venny (version 2.1) software were used to draw heat maps and Venn diagrams, respectively. Principal component analysis (PCA) was performed using ClustVis software.[83]

4. CONCLUSIONS

The present study comprehensively evaluated the nutritional and phytochemical composition of a few edible flowers from the Western Himalayas. Kachnar flower was superior in terms of macronutrients, while the presence of over 90% water-extractable proteins in Kachnar and Chamomile makes them excellent ingredients for the preparation of beverages and infusions. Nasturtium contained ALA, an omega-3 fatty acid, in significant quantity compared to other flowers. Further, the findings suggested that Nasturtium flowers could be a dual source of anthocyanins and lutein, and 2 to 3 g of dry Nasturtium and Marigold flowers could meet the daily lutein requirements of an adult. The total phenolic acids and flavonoid content of edible flowers selected in the present study were multi-fold higher compared to common vegetables and fruits. Marigold showed the best antioxidant activity owing to the presence of both flavonoids and carotenoids in significant concentrations. The use of non-targeted metabolomics resulted in the tentative identification of 48 compounds, of which flavonoid glycosides and anthocyanins were predicted as potential marker compounds. *In vitro* simulated gastrointestinal digestion revealed that Kachnar and Nasturtium have the highest percentage bioaccessibility compared to Chamomile and Marigold, despite the latter containing higher total flavonoids. In conclusion, the present study was an attempt to reveal the nutritional and nutraceutical potential of edible flowers of the Western Himalayas for their popularization, domestication, and wider commercial utilization.

■ ASSOCIATED CONTENT

ⓈI Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.3c03861.

> Additional information on ethnobotanical applications and geographical distribution of selected edible flowers; amino acid scoring; extract yield and total phenolics and flavonoids contents of flowers with different solvents; polyphenolic composition of edible flowers compared

with commonly consumed fruits and vegetables and color value of edible flowers; chromatograms of targeted and non-targeted phenolics, carotenoids composition from UHPLC-QTOF-IMS, and chromatograms of gas chromatography (PDF)

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Notes

The authors declare no competing financial interest.

ABBREVIATIONS

UHPLC-QTOF-IMS, ultra high-performance liquid chromatography-quadrupole time of flight-ion mobility separation; HPLC, high-performance liquid chromatography; FAME, fatty acid methyl esters; TPC, total phenolic content; TFC, total flavonoid content; DPPH, 2,2-diphenyl-1-picrylhydrazyl; ABTS, 2,2′-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid; FRAP, ferric reducing power assay; EAA, essential amino acid; TAA, total amino acid; ALA, α-linolenic acid; PUFA, poly-unsaturated fatty acid; MUFA, mono unsaturated fatty acid; IA, index of atherogenicity; IT, index of thrombogenicity; HPI, health-promoting index; HH, hypocholesterolemic/hypercholesterolemic ratio

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