

## PHYTOCHEMICAL DIVERSITY AND NUTRITIONAL VALUE OF KENGER AT DIFFERENT LOCATIONS IN SULAIMANI REGION - IRAQ

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### ABSTRACT

Kenger (*Gundelia tournefortii* L.) plants were collected based on the availability of these plants in the local market to determine the nutrition value, some phytochemical content, and element content during March 2023 at the vegetative stage at thirteen different locations in the Sulaimani region. Some vegetative traits and chemical contents were studied. The results indicated that the highest content of carbohydrates was (12.670%) at Mawat, the protein was (32.703%) at Hazarmerd, and fiber was (35.620%) at Penjwen locations respectively. The analysis of Kenger edible parts (stem and leaves) through GC-MS revealed the presence of a complex mixture of compounds varying from 13 to 19 compounds among the locations. Compounds such as carboxylic acids ranged from 25.217% at Penjwen to 77.043% at Temar, terpenes ranged from 15.912% in Temar to 57.749% in Penjwen, and sterols ranged from 5.351% at Qara Dagh to 24.688% at Hazarmerd. In other locations, these compounds were detected in between those ranges, with differences in their concentrations. According to the results obtained from ICP-OES analysis, macro-elements (Ca, K, P, S, Mg, and Na) contents between the studied locations, the highest concentration of 3.127% occurred at Temar location, the lowest value was obtained at Gulp location with 2.468%, with an average of 2.811% for all locations.

**Keywords:** Medicinal plants, *Gundelia tournefortii* L., Phytochemical, GC-MS, Macro and microelements, ICP-OES.

احمد

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التنوع الكيميائي النباتي والقيمة الغذائية للكعوب في مواقع مختلفة في منطقة السليمانية – العراق

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المستخلص

تم جمع نباتات الكعوب (*Gundelia Tournefortii* L.) بناءً على توفرها في الاسواق المحلية خلال شهر مارس 2023 في المرحلة الخضرية في ثلاثة عشرة موقعاً مختلفاً في منطقة السليمانية لتحديد القيمة الغذائية وبعض المحتوى الكيميائي النباتي والعناصر المعدنية. تمت دراسة بعض الصفات الخضرية والمحتوى الكيميائي، بحث أشارت النتائج إلى أن أعلى نسبة بين المواقع من محتوى الكربوهيدرات كانت (12.670%) في مawat، والبروتين (32.703%) في هزرميرد، والألياف (35.620%) في بنجوين. كشف تحليل الأجزاء الصالحة للأكل (السيقان والأوراق) باستخدام جهاز GC-MS عن وجود خليط معقد من المركبات يتراوح عددها بين 13 إلى 19 مركباً بين المواقع. تراوحت نسبة المركبات مثل الأحماض الكربوكسيلية من 25.217% في بنجوين إلى 77.043% في تيمار، وتراوحت التربينات من 15.912% في تيمار إلى 57.749% في بنجوين، وتراوحت الستيرويدات من 5.351% في قره داغ إلى 24.688% في هزرميرد. وفي مواقع أخرى تم الكشف عن هذه المركبات بين تلك المدايات مع وجود اختلافات في تراكيزها. وفقاً للنتائج التي تم الحصول عليها من تحليل ICP-OES، محتوى العناصر الكبرى (Ca، K، P، S، Mg، و Na) بين المواقع المدروسة، تم الحصول على أعلى تركيز 3.127% في موقع تيمار، وتم الحصول على أقل قيمة في موقع كولب بنسبة 2.468% و بمتوسط 2.811% لجميع المواقع.

الكلمات المفتاحية: النباتات الطبية؛ *Gundelia tournefortii* L.، المركبات النباتية، GC-MS، العناصر الكبرى و الصغرى ، ICP-OES.

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## INTRODUCTION

The Asteraceae family includes *Gundelia tournefortii* L. (Kenger) genus, is a medical plant and widely distributed in the Mediterranean region, the Middle east, and Asia (20). This plant has been used for ages as a traditional food (15), either raw or cooked, in our region. Kenger has therapeutic uses in traditional medicine (9); it has been gradually increasing as an ethno- medicinal plant for pharmaceutical purposes such as anti-inflammatory, antifungal, antipyretic antibacterial, and antioxidant (21). Many plants were screened, and found that they possess antioxidant and protective effects against diabetes-induced drugs through an elevation in the concentration of antioxidant enzymes (58). Due to inadequate supply of drugs, cost of treatment, side effects, and the development of resistance to currently used drugs. Demand for and emphasis on the use of medicinal plant extracts for different ailments has increased in the population (49). Kenger is known as an herbal medicine and has an advantage in the treatment of diseases like inflammations, bronchitis, diarrhea, vitiligo, cuts, gastric pain, chewiness, splenomegaly, kidney problems, chest pain, and heart stroke. It is also used as an effective diuretic in folk medicine (35). According to several studies, the chemical components of Kenger include alkaloids, saponins, tannins, phenolic compounds, terpenoids, and flavonoids (44). It also contains compounds that have anticancer activities like lupeol, gitoxigenin,  $\alpha$ -amyrin, stigmasterol, sitosterol and artemisinin (1,14,45). The Kenger phytochemical composition consists of an antioxidant tocopherol and stigmasterol (35). Stigmasterol has thyroid inhibiting effect (24), and also hypoglycemic properties (38). In addition to its therapeutic properties, the Kenger edible stem is also a rich source of nutrients, including vitamins and minerals, crude oil, fiber, and protein (35). Rich in fatty acids like palmitic, oleic, linoleic, and stearic acid, as well (5). It consists of a broad dietary mineral spectrum, macro- minerals (Ca, K, P, S, Mg, and Na) and micro-minerals (Fe, Cu, Zn, Mn, Se, Cr, and Mo) with daily requirements limited to a few milligrams (54). Understanding the concentration of micro

elements in medicinal plants is crucial to comprehending their pharmacological effects (48). The chemical constituents metal ions in plants, are particularly responsible for medicinal and nutritional properties and also for their toxicity. In the Kurdistan region, the Kenger plant is considered one of the delicious vegetables found in the local markets during spring, and people collect this plant in different locations which grown naturally. Because of the lack of research on this plant in this region, this research aims to provide a comprehensive understanding of the phytochemical constituents and nutritional values of Kenger and provide insights into the safety of consuming Kenger plants based on their elemental content, which may have a significant role and implications for its use in medicine and agriculture.

## MATERIALS AND METHOD

### Plant collection and identification

Kenger plant material was collected during March 2023 in different locations at the Sulaimani region during the growth stage when the plants were at the edible stage, i.e.; before flowering (stem and leaves), (Table 1). The selection of the collected locations was based on the presence of this plant in the local market. Using the Global Positioning System (MGRS UTM GPS version 1.9.4) to recode the information about the locations. Sample specimens were identified and deposited in the University of Sulaimani, College of Agricultural Engineering Sciences. Some morphological traits of Kenger plant were studied at all of the locations, stem length (cm), number of leaves/plants, and dry stem weight/plant (g). Stem dry matter of the plant samples was determined by drying the plant material at 105°C for 24 hours in an oven. Then, the oven dried samples were weighted and recorded.

**Chemical analysis:** Analysis of Kenger samples for carbohydrate protein and fiber was carried out with the “Reflection Infrared Spectroscopy FT-IR Techniques IRA finitty-1 SHIMADZU- KYOTO JAPAN as an important technique in chemistry (Kurdistan, Sanandaj, Iran - Faculty of Basic Science - Chemistry Department), (51).

**Plant Extract Preparation:** The 50g of the fine Kenger plant powder were dissolved in to 500 ml hexane (99%) in an 1L Erlenmeyer flask. The Kenger samples solution was sonicated for 2 h at 50 °C, to ensure complete extraction of the compounds. Then, the flask covered with aluminum foil and kept in a dark place for 24 hrs., the mixture was filtered using 0.4µm filter. The hexane solution was preserved in a sealed glass container and kept at -20 °C until use.

**GC-MS analysis:** Agilent Technologies 7890A gas chromatograph with a mass selective detector and Agilent Technologies 5975C inert XL MSD mass spectrometer was used to analyze the aerial part of Kenger plant samples at University of Kurdistan, Faculty of Basic Science, Chemistry Department, Sanandaj, Iran. The Agilent 190915-433:

325°C (30 m × 250 µm × 0.25 µm) GC-MS column was designed to start at 40°C and increased by 10°C per minute to a maximum temperature of 280°C. The temperature of the heater front inlet was 250°C, the injector port was set to 290 °C, and helium was utilized as the carrier gas at a flow rate of 1 mL/min. The injection mode was split at a ratio of 5:1. At 70 eV, mass spectrometry was used in the electron impact mode (EI). Using the normalization of the GC peak areas, the percentages of the phytochemical components were calculated. The software automatically normalized the peaks by dividing each peak area by the total area and then multiplying by 100. Using the Wiley library, the relative proportions of the constituents were calculated (19).

**Table 1. Collection places information**

Locations	Elevations (MASL)	Lat.	Long.
Qaramani	566	35.10.23 N	45.51.49 E
Bezhawa	591	35.11.39 N	45.54.19 E
Gulp	716	35.15.44 N	46.5.7 E
Bnary Qandil	730	36.17.14 N	45.0.41 E
Mawat	803	35.54.7 N	45.24.52 E
Azmar	857	35.38.5 N	45.28.4 E
Temar	870	35.51.21 N	45.4.38 E
Ahmad Awa	929	35.17.50 N	46.4.3 E
Hazarmerd	1040	35.30.17 N	45.19.37E
Kani Tu	1236	35.59.53 N	45.11.36 E
Nalparez	1313	35.34.28 N	45.51.20 E
Qara Dagh	1419	35.18.38 N	45.23.31 E
Penjwen	1505	35.36.51 N	45.57.10 E

**ICP-OES analysis of the macro and microelements:** The analysis of element content was carried out using a Spectro Arcos model ICP-OES instrument supplied by SPECTRO Analytical Instruments in Kleve, Germany. This device included an automated sampler, a fixed 3.0 mm injector tube, and a quartz torch with radial viewing. The ICP-OES instrument's operating parameters comprised of an RF power of 1450 W, a flow rate of 13.5 L/min for plasma gas, 1.0 L/min for auxiliary gas, 0.8 L/min for nebulizer, 2.0 ml/min for sample aspiration, and a temperature of 15 °C for the polychromator. Three replicates were obtained for each measurement, with a 60-second read period for each replication. Using standard solutions at concentrations of 100, 200, 400, 800, 1000, 1200, 1600, and 2000 µg/L, the calibration curve for mineral analysis was constructed.

Multi-element stock solutions (1000 µg/L) were diluted in a 0.1 mol/L HNO<sub>3</sub> solution to create these standard solutions. During the ICP-OES measurements, the rinse solvent was a 2 mol/L HNO<sub>3</sub> solution.

**Statistical analysis:** This study was conducted using Completely Randomized Design (CRD) with three replications. The (ANOVA) analysis of variance as a general test for the experiment was used to determine the significant differences between Kenger plant samples at different locations, while the mean comparisons were performed according to the LSD value at both significant levels of 0.05 and 0.01. confidence intervals for the macro and micro element concentration depending on the sample means (34).

## RESULTS AND DISCUSSION

Results in table 2 show the mean of some traits and the mean squares of analysis of variance

for the Kenger plant samples at all studied locations. Highly significant effects ( $p \leq 0.01$ ) were obtained for the traits stem length and stem dry weight, while a number of leaves responded significantly ( $p \leq 0.05$ ). The Gulp location recorded the longest stem 17.13 cm, while the Hazarmerd location had a minimum value of 5.333 cm. Regarding number of leaves gave a significant effect at the Bnary Qandil location with a maximum value of 9.000. In comparison, the minimum value was recorded by the Azmar location with a value of 5.333. Ahmad Awa location surpassed all other locations for the trait of a dry stem weight with a value of 4.713g, while the Azmar location gave a minimum value of 1.113g. Plants from low altitudes invest about twice as much as dry matter per unit and total leaf dry matter in leaf petioles, whose absolute length is 4 to 7 times greater than in high-altitude plants. Altitude plants are typically small in size and produce fewer cells. According to Körner *et al.*, (29), at a high altitudes plant cell size did not reduce, instead, their organs become smaller due to a decreases in cell production. Esbati *et al.*, (16), reported that during the vegetative stage, the mean dry matter increases. Data in Table 2 shows the mean carbohydrate percentage was higher in the Mawat location at 12.670% compared with the other locations, which was statistically

significant ( $p < 0.01$ ). The location shows a variation in the amount of protein percentage content which was statically significant ( $p \leq 0.01$ ), ranging from 17.427 % in the Kani Tu location and 32.703% in the Hazarmerd location. Hoy *et al.*, (23) concluded that in the mature stage, the content of crude protein decreased. A study by Fang *et al.*, (18) emphasized that carbohydrate and protein content could be increased at the maturity stage, meanwhile, carbohydrate contents decreased. In terms of Fiber content, Kenger samples from Hazarmerd location displayed the lowest value 20.560%, with the highest value 35.620% for the Penjwen location which was statistically significant ( $p \leq 0.01$ ). The amount of solid tissues (sclerenchyma tissue) and preservatives increases when plant age is increased (31), which leads to an increase in the amount of crude fiber at the maturity stage. The results obtained in this study were found to be partially in line with the study conducted by Tuncturk *et al.*, (54), which reported that Kenger had abundant active compounds among the plant species studied. The amount of crude protein and crud fiber, were higher than other species. The dry matter content of Kenger plant was 17.46%, the total crude protein was 21.00% and the crude fiber was 36.44%.

**Table 2. Means of the growth traits and chemical contents of Kenger at different locations**

Locations (MASL)	Stem length (cm)	No. of leaves/plant	Stem dry weight/plant (g)	Carbohydrate (%)	Protein (%)	Fiber (%)
Qaramani (566)	9.833	7.333	2.087	8.267	30.320	32.093
Bezhawa (591)	14.500	6.667	3.853	7.380	20.237	33.547
Gulp (716)	17.133	6.000	2.540	8.657	23.970	28.497
Bnary Qandil (730)	9.500	9.000	2.500	9.357	27.467	30.353
Mawat (803)	13.200	8.000	2.751	12.670	22.430	27.840
Azmar (857)	7.000	5.333	1.113	10.213	18.183	30.643
Temar (870)	5.500	7.333	1.148	7.393	21.820	29.623
Ahmad Awa (929)	11.167	6.667	4.713	6.750	29.560	34.690
Hazarmerd (1040)	5.333	5.667	1.673	7.590	32.703	20.560
Kani Tu (1236)	9.633	6.667	1.123	3.503	17.427	31.383
Nalparez (1313)	7.333	6.000	2.667	5.497	23.187	20.563
Qara Dagh (1419)	7.867	8.000	2.677	6.943	19.790	21.510
Penjwen (1505)	9.667	6.667	1.553	11.277	20.753	35.620
LSD ( $P \leq 0.05$ )	1.750	1.900	0.478	0.243	0.692	0.973
LSD ( $P \leq 0.01$ )	2.366	n.s	0.646	0.328	0.935	1.315

**Phytochemical diversity of Kenger using GC-MS:** Phytochemical compounds were screened using GC-MS at all locations as it has been shown in (Table 3 a, b, c). Along with the total area for each detected chemical class in Kenger plants, each study location revealed the presence of a complex mixture of compounds ranged from 13 to 19 compounds with corresponding retention times, relative peak areas, and percentages of similarity relative to the reference library in the GC-MS. Compounds such as carboxylic acids, terpenoids, and sterols were detected in the Kenger plant extracts, which were relatively similar to those detected in all locations, with differences in their concentrations.

Fatty acids and their derivatives (lipophilic content) made up the majority of the extracts, which are dodecanoic acid, tetra decanoic acid, palmitic acid, methyl palmitate, linoleic acid, methyl linoleate, lauric acid, oleic acid, (9Z)-9,17-octadecadienal. Methyl palmitate was the highest among the detected compounds and ranged from 4.464% in the Penjwen location to 45.389% in the Qara Dagh location, followed by linoleic acid, which ranged from 18.752% in the Gulp location to 1.558% in Nalparez location. Lauric acid was detected in all locations and ranged from 15.11% at the Ahmad Awa location to 1.413% at the Bezhawa location. Dalar *et al.*, (12), found seven fatty acids in which linoleic acid was the most abundant fatty acid followed by oleic, palmitic, and stearic acid. Another study by Al-Saadi *et al.*, (5), reported GC-MS analysis of the Kenger extract. They detected fatty acids such as linoleic acid methyl ester, oleic acid methyl ester, palmitic acid methyl ester, and 8- octadecenoic acid methyl ester. The GC-MS analysis of Kenger detected the major compounds that were palmitic acid, lauric acid,  $\alpha$ -ionene, myristic acid, 1-hexadecanol, 2-methyl, phytol, and  $\beta$ -turmeron (19). Likewise, Kadan *et al.*, (26), studied the chemical composition of the Kenger plant in their study and they reported that the plant contained palmitic acid, glycerol and linoleic acid. Artemisinin, an active and promising photochemical, was also identified and ranged from 23.050% in the Bezhawa location to 6.848 in the Ahmad Awa location. This compound had potential anticancer activities

(53). Matricarin is a natural compound found in certain plants; its abundance ranged from 5.071% in the Kani Tu location to 0.945% in the Gulp location. This compound is found particularly in species of chamomile; it is a precursor to the formation of chamazulene (2). Stigmasterol and sitosterol were detected to constitute 8.616% in Nalparez, 2.924% in Azmar, 15.383% in Bezhawa, and 3.578% in Qaramani, its components respectively. Sitosterol was found to act in vivo as a tumor-promotion inhibitor and prevent carcinogenesis (56). A study by Ali *et al.*, (7) revealed that stigmasterol acts as a tumor-promotion inhibitor in mice at two different stages of carcinogenesis. Topical application of a mixture of sitosterol and stigmasterol was observed to have anti-inflammatory action. They conclude that the anticancer properties of Kenger may be influenced by the presence of these sterols. A number of investigations conducted on Kenger plant revealed that it contains a high amount of  $\alpha$ -amyrin, lupeol,  $\beta$ -sitosterol, and 3-acetyl-lupeol (1,26) 5-avenasterol, 7-stigmasterol, campesterol, 7-avenasterol, and stigmasterol (45).  $\alpha$ -amyrin composed 13.121% in the Mawat location and 1.483% in the Ahmad Awa location, while it was not detected in each of the Tamar and Gulp locations. This compound was previously identified as having significant anticancer activity on four cancer cell lines (30). Hop-22(29)-en-3 $\beta$ -ol triterpenes detected in four locations, the highest abundance was detected in Bezhawa location 12.715% and the lowest was 3.124% in Hazarmerd location. This substance has been described as natural product with antiparasitic properties (32), in prokaryotes, it is widely distributed, while in plants, it is relatively rare. Lupeol, an additional phytochemical, was detected in some locations; the sample weighed between 7.25% in Mawat and 1.631% in Qara Dagh. It has been discovered to function as a new androgen receptor that targets  $\beta$ -catenin signaling during carcinogenesis to prevent human prostate cancer cells from proliferating (46). Olean-12-en-3-yl acetate is one of the derivatives of oleanolic acid, a naturally occurring triterpenoid compound found in various plants. Their major abundance in the Kenger plant is 6.871% in Bnary Qandil.

Thunbergol is a monocyclic diterpene alcohol (17). Its abundance was 13.393% in the Ahmad Awa location. Gitoxygenin, which weighed 17.017% of the Kenger plant sample in Hazarmerd. It has anti-cancer properties, especially for renal adenocarcinoma (33). A-neogammacer- 22(29)- en-3-ol, acetate (3.β.,21β.) detected in some sample locations, the highest peak area was detected at Penjwen location with 18.95%. The compound is a triterpenoid found in medicinal plants (283). The results obtained above were in agreement with the finding by Abu-Lafi *et al.*, (1), the GC-MS analysis detected 27 phytochemical constituents, such as gitoxygenin,  $\alpha$ -amyrin, artemisinin, sitosterol, stigmasterol, and lupeol, which have been reported to have anticancer properties. As it was noticed from Tables (4a, b, and c) the detected phytoconstituents varied among the locations, resulting in discrepancies in the total number of phytoconstituents. Kani Tu and Penjwen locations gave the maximum phytochemical compounds in comparison with the other locations; this is due to specific biotic and abiotic biological conditions present in their location. Despite their varying proportions and quantities across all locations, certain of the chemicals discovered in the current investigation were common (42). Such variations in the concentration of common plant secondary metabolites in plants could be the consequence of particular biosynthetic variations that are either up- or down-regulated (36). Geographical location is one of the most important factors that causes variations in phytoconstituents. Studies have revealed that the concentration of a specific secondary metabolite varies among plants of the same species that grow in various environments. This is because, to combat environmental stress, the plant must produce secondary metabolites in a specific quantity and quality. As a result, research on each environmental element is crucial to understanding plant availability and adaptability in a given area (39). The average temperature is lower at higher elevations than at the lowest points of the mountains. An important factor in the growth and development of plants is elevation. Sunlight exposure, water absorption, and nutrient availability vary with altitude (27).

Other research has found that altitude influences the chemical composition of various plants (22). Ali-Shtayeh *et al.*, (6), discovered that a number of both internal and external factors, including plant genetic structure, growing conditions, soil characteristics, and used plant parts, have an impact on the chemical composition of plant. **The analysis of macro and micro elements** The results obtained from the ICP-OES analysis, macro-elements (Ca, K, P, S, Mg, and Na) content varied from 3.127% in Temar to 2.468 in Gulp, with an average of % for all locations, while micro-elements (Fe, Cu, Zn, Mn, Se, Cr, and Mo) were relatively rare in the Kenger plant samples from all of the locations (Table 3). Among the determined elements, calcium (Ca) has the highest concentration in the whole Kenger plant sample. The calcium contents ranged from 7843 mg/kg in Gulp to 11968 mg/kg in Temar and accounted for 0.946% as an average of all locations. High dietary calcium consumption, particularly from plant sources, lowers the chance of osteoporosis and increases bone mineral density. In addition to being a potential source of calcium, vegetables may also include vitamins and minerals that have extra positive effects on bone health (40). Potassium (K) was the second most prevalent element found in plant samples, with concentrations ranging from 5864 mg/kg in the Gulp location to 9974 mg/kg in the Ahmad Awa location. Potassium is found in both plant and animal tissues since it is a necessary element for all living cells to operate properly. It is particularly found in fruits and plants (8). Phosphor (P) is the third most abundant macro element detected in Kenger plant samples. Phosphor is an important macro element in plants; the content varied from 1442 mg/kg in the Azmar location to 4919 mg/kg in the Bnary Qandil location. Sulfur (S) is a macro element. It is one of the most abundant minerals in the human body. It is the third most important mineral in the body, after calcium and phosphorus (37). The average in plant samples was 3270 mg/kg, and it ranged from 2342 mg/kg in Bezhawa to 4716 mg/kg in Temar.

Table 3 a. Phytochemicals of Kenger at extract verified by GC/MS from all locations

Peaks	Compounds	Qaramani (566)			Bezhawa (591)			Gulp (716)			Bnary Qandil (730)		
		RT (min.)	Abundance (%)	Similarity* (%)	RT (min.)	Abundance (%)	Similarity* (%)	RT (min.)	Abundance (%)	Similarity* (%)	RT (min.)	Abundance (%)	Similarity* (%)
1	Dodecanoic acid	2.472	0.614	92	/	/	/	2.475	0.417	87	2.455	0.257	89
2	Tetra decanoic acid	2.574	0.718	89	/	/	/	2.594	0.502	75	2.579	0.334	75
3	Palmitic acid	3.772	1.358	79	/	/	/	3.776	0.722	86	3.770	1.750	89
4	Methyl palmitate	4.328	25.38	92	4.513	12.169	90	4.278	23.551	92	4.385	7.980	92
5	Linoleic acid	4.695	11.415	87	4.567	4.849	88	4.692	18.752	91	4.694	6.545	80
6	Methyl Linoleate	/	/	/	4.920	4.188	87	/	/	/	4.918	3.903	83
7	Lauric acid	5.619	4.01	71	5.611	1.413	84	5.618	10.197	82	5.614	8.703	74
8	Oleic acid	/	/	/	5.707	4.615	89	5.756	6.191	99	5.667	16.813	86
9	Artemisinin	6.248	3.225	77	6.249	1.855	97	/	/	/	/	/	/
10	Matricarin	6.460	20.269	80	6.451	23.050	89	6.463	17.319	80	6.453	17.226	80
11	Heptadecane	6.846	3.886	70	6.827	3.524	92	6.928	0.945	91	6.837	3.783	78
12	Stigmasterol	7.100	5.515	96	7.096	4.784	65	7.103	7.541	94	7.096	3.039	88
13	$\beta$ -sitosterol	7.538	3.578	98	7.532	15.383	96	/	/	/	7.542	4.779	98
14	$\alpha$ -amyrin	7.710	7.787	97	7.695	6.059	97	7.713	6.217	85	7.698	5.652	97
15	Hop-22(29)-en-3 $\beta$ -ol	/	/	/	8.04	12.715	93	/	/	/	8.050	7.836	87
16	Lupeol	8.304	4.134	91	8.289	4.709	97	/	/	/	/	/	/
17	Olean-12-en-3-yl acetate	8.421	3.021	96	/	/	/	8.438	3.588	78	8.413	6.871	90
18	Thunbergol	9.798	5.09	88	9.762	0.687	85	/	/	/	9.785	2.031	86
19	Gitoxigenin	/	/	/	/	/	/	9.932	2.11	82	9.891	2.066	83
20	A-neogrammacer-22(29)-en-3-ol	/	/	/	/	/	/	10.555	1.948	90	10.451	0.432	96

\* % of similarity relative to the reference library in the GC/MS

Table 3 b. Phytochemicals of Kenger at extract verified by GC/MS from all locations

Peaks	Compounds	Mawat (803)			Azmar (857)			Temar (870)			Ahmad Awa (929)		
		RT (min.)	Ab. (%)	Similarity* (%)	RT (min.)	Abundance (%)	Similarity* (%)	RT (min.)	Abundance (%)	Similarity* (%)	RT (min.)	Ab. (%)	Similarity* (%)
1	Dodecanoic acid	2.464	0.195	93	/	/	/	2.475	0.517	93	2.481	0.38	93
2	Tetra decanoic acid	/	/	/	2.574	0.894	85	2.583	0.94	95	2.588	0.275	82
3	Palmitic acid	3.771	0.346	84	3.769	0.408	88	3.777	0.926	92	3.779	0.347	92
4	Methyl palmitate	4.321	6.803	78	4.248	21.406	90	4.287	40.938	78	4.291	9.259	78
5	Linoleic acid	4.705	12.036	85	4.692	17.072	89	4.691	16.534	94	4.696	4.686	87
6	Methyl Linoleate	4.926	1.566	79	4.917	5.25	93	/	/	/	4.930	1.618	85
7	Lauric acid	5.617	9.423	88	5.613	1.64	91	5.621	11.273	94	5.620	15.11	91
8	Oleic acid	5.715	7.137	75	5.71	3.801	81	5.758	5.915	83	/	/	/
9	Artemisinin	/	/	/	6.25	0.898	78	6.375	1.932	88	6.251	1.798	81
10	Matricarin	6.470	18.338	89	6.462	20.605	83	6.485	8.707	73	6.460	6.848	80
11	Heptadecane	6.847	5.574	86	6.828	2.842	86	/	/	/	6.849	4.263	93
12	Stigmasterol	7.101	4.213	90	7.096	2.924	89	7.111	3.169	89	7.098	2.781	88
13	$\beta$ -sitosterol	7.431	5.205	95	/	/	/	/	/	/	/	/	/
14	$\alpha$ -amyrin	7.703	13.121	89	7.694	12.535	97	/	/	/	7.696	1.483	97
15	Hop-22(29)-en-3 $\beta$ -ol	/	/	/	/	/	/	/	/	/	/	/	/
16	Lupeol	8.304	7.25	93	8.298	5.084	93	8.326	3.588	86	/	/	/
17	Olean-12-en-3-yl acetate	8.420	3.534	87	8.419	2.551	84	8.468	1.685	84	8.409	4.704	89
18	Thunbergol	/	/	/	/	/	/	/	/	/	9.717	13.393	92
19	Gitoxigenin	9.814	2.231	90	/	/	/	9.975	3.876	92	9.858	5.265	90
20	A-neogrammacer-22(29)-en-3-ol	10.398	3.028	84	10.584	2.090	90	/	/	/	10.415	27.79	88

\* % of similarity relative to the reference library in the GC/MS



Table 3 c. Phytochemicals of Kenger at extract verified by GC/MS from all locations

Peaks	Compounds	Hazarmerd (1040)			Kani Tu (1236)			Nalparez (1313)			Qara Dagh (1419)			Penjwen (1505)		
		RT (min.)	Ab. (%)	Sim.* (%)	RT (min.)	Ab. (%)	Sim.* (%)	RT (min.)	Ab. (%)	Sim.* (%)	RT (min.)	Ab. (%)	Sim.* (%)	RT (min.)	Ab. (%)	Sim.* (%)
1	Dodecanoic acid	2.474	0.405	98	2.467	0.306	86	2.470	0.551	89	/	/	/	2.477	0.66	89
2	Tetra decanoic acid	2.581	0.564	92	2.576	0.467	82	2.583	0.791	87	/	/	/	2.585	0.798	75
3	Palmitic acid	3.778	3.406	83	3.769	0.532	92	3.773	1.356	92	/	/	/	3.772	0.793	82
4	Methyl palmitate	4.258	26.75 3	79	4.214	27.78 3	93	4.282	31.096	93	4.210	45.389	89	4.432	4.464	88
5	Linoleic acid	4.684	4.925	87	4.691	6.091	85	4.683	1.558	79	4.719	7.657	78	4.705	4.036	93
6	Methyl Linoleate	/	/	/	4.926	10.14 3	78	4.949	0.965	88	/	/	/	4.953	1.987	92
7	Lauric acid	5.620	6.073	74	5.617	5.521	91	5.614	11.189	84	5.617	4.277	92	5.616	3.23	74
8	Oleic acid	5.758	7.091	69	5.763	3.573	75	5.668	15.866	90	5.761	10.098	85	5.742	4.79	80
9	Artemisinin	6.253	2.715	93	6.248	2.932	86	6.248	2.63	78	6.257	0.948	81	/	/	/
10	Matricarin	6.467	14.84	73	6.474	11.56 4	88	6.465	16.206	93	6.441	15.624	93	6.467	8.48	93
11	Heptadecane	/	/	/	6.851	5.071	90	/	/	/	6.860	3.114	79	6.846	4.459	94
12	Stigmasterol	7.108	7.651	94	7.100	3.583	81	7.097	8.616	80	7.106	3.203	65	7.095	5.037	89
13	$\beta$ -sitosterol	/	/	/	7.557	5.536	79	/	/	/	/	/	/	7.531	3.877	90
14	$\alpha$ -amyrin	7.716	5.426	90	7.708	2.495	93	/	/	/	7.726	3.771	80	7.701	5.279	88
15	Hop-22(29)-en-3 $\beta$ -ol	8.090	3.124	92	8.062	2.452	88	/	/	/	/	/	/	8.040	5.29	74
16	Lupeol	/	/	/	8.317	2.837	86	/	/	/	8.347	1.631	83	8.281	5.98	78
17	Olean-12-en-3-yl acetate	/	/	/	8.428	5.986	72	8.418	3.597	76	8.440	0.73	90	8.411	5.82	87
18	Thunbergol	/	/	/	9.637	1.386	84	9.783	5.58	90	9.753	1.41	82	9.758	7.95	83
19	Gitoxigenin	9.883	17.01 7	85	9.820	1.742	89	/	/	/	9.984	2.148	90	9.877	8.12	90
20	A-neogrammacer-22(29)-en-3-ol	/	/	/	/	/	/	/	/	/	/	/	/	10.526	18.95	91

\* % of similarity relative to the reference library in the GC/MS

Magnesium (Mg) is a necessary element, and deficiencies in it are recognized to have typical symptoms in people, animals, and plants. Magnesium is a prevalent intracellular element found in all living cells, second only to potassium. Plants contain 1 to 8 g/kg (52). In the present study, Mg contents ranged from 1547 mg/kg in Mawat to 2455 mg/kg in Qaramani and were within the reference value of plant content. Sodium (Na) is an important element for the maintenance of acid-base equilibrium and the osmotic pressure of body liquids (10). The sodium contents of all the samples were found to be about 957 mg/kg in Azmar and 1403 mg/kg in Mawat. Two minerals that are necessary for human life are sodium and potassium. These ions play a role in numerous physiological functions. Their equilibrium is crucial to the operation of numerous organs and/or physiological systems. The nerve impulse is one of the main functions of the body's potassium/sodium balance (41). Significant element amounts in plants fall within a limited range, and high concentrations of metals may be toxic to people. These results are consistent with those of the researchers when taking into account earlier research on the macroelement concentrations of a few edible wild and medicinal plants. It is commonly known that a variety of factors, such as soil properties and water availability, have an impact on plant composition and significantly alter the mineral content of plants (47). In this study, there was also a wide variation in a macroelements content among the studied location plant samples. Determining the microelements content of the Kenger plant for consumers is very important because it is used as a food and medicinal plant. The miner element Fe was the highest among microelements and ranged from 4 mg/kg in Bezhawa, Mawat and Azmar to 109 mg/kg in Qara Dagh, with an average of 47 mg/kg. The Fe content in some samples reached the highest reference values (17–50 mg/kg) (25). The copper (Cu) element ranged from 27 mg/kg in Azmar to 56 mg/kg in Tamar samples; it was the second highest among the microelements. According to a study by Akguc *et al.*, (4) the typical range for copper in plants was between 4 and 15 mg/kg. When copper concentrations were higher than

these ranges, they were found to have harmful impacts on human health. In this study, it was determined that the Cu content of the studied samples was observed differently and was below the specified limit values. In addition, the Cu amount of the studied species is higher than the limit value determined by FAO's standard, which is 30 mg/kg. The minimum and maximum levels of Zn measured were 26 in Mawat and 58 mg/kg in Tamar, respectively. The amount of Mn varies in the range of 22 mg/kg in Azmar to 38 mg/kg in Tamar. The findings showed that of all the plant samples, the Mn content was the lowest. The World Health Organization (WHO) states that there are set permitted levels for zinc and manganese, also known as micronutrients, that allow for sufficient dietary consumption. Adult Zn and Mn recommendations range from 5.0 to 22.0 mg and 2.0 to 20.0 mg, respectively (50). Both humans and animals require selenium as a trace element. It has immunological, anti-inflammatory, and antioxidant qualities in addition to defense against cancer, toxins, and cardiac issues (11). Its recommended daily uptake was 0.15–0.4 mg/kg (13). In Kenger the Se concentrations of plant species diversified between 3 mg/kg in Bezhawa, Gulp, Ahmad Awa and, Qara Dagh and 23 mg/kg in Penjwen, with an average of 8 mg/kg. According to WHO data, there are no acceptable limit values for the Cr element. In the study Yener *et al.*, (57), the amount of chromium varies in the range of 0.15–4.8 mg/kg. In this study, the chrome contents of thirteen plant samples from different locations were measured. According to our findings, chrome content was determined to be lowest at 2 mg/kg in Hazarmerd and then highest at 11 mg/kg in Tamar location. The molybdenum contents of samples varied from 2 mg/kg in Azmar, Ahmad Awa, and Nalparez to 5 mg/kg in Bnary Qandil. The minimum daily requirement for Mo by adults is estimated at about 0.02 mg/kg (55). The total element content ranges from 24854 mg/kg at Gulp to 31491 mg/kg at Tamar. These components, together with additional chemical substances, are crucial pharmacodynamic building blocks for medicinal plants (43).

Table 4. Macro and microelements (mg/kg) content in Kenger plant samples in all locations

Locations (MASL)	Macro elements (mg/kg)							
	Ca	K	P	S	Mg	Na		
Qaramani (566)	11533	9803	2635	2982	2456	1347		
Bezhawa (591)	9555	8231	2114	2343	2047	1255		
Gulp (716)	7843	5864	4352	3588	1776	1255		
Bnary Qandil (730)	8605	8715	4919	3649	2192	1243		
Mawat (803)	9023	9753	2726	2518	1548	1403		
Azmar (857)	9783	8567	1442	2451	1905	957		
Temar (870)	11968	7864	3247	4716	2248	1225		
Ahmad Awa (929)	8554	9975	3573	2956	2137	1248		
Hazarmerd (1040)	10554	8910	4214	4059	2337	1173		
Kani Tu (1236)	11486	7887	2929	3954	2019	1129		
Nalparez (1313)	8148	8779	4432	3029	2293	1376		
Qara Dagh (1419)	8554	9750	4629	3455	2178	1028		
Penjwen (1505)	7431	6670	4754	2814	2268	1044		
LSD <sub>(P≤0.05)</sub>	18	628	87	55	25	36		
LSD <sub>(P≤0.01)</sub>	24	849	118	74	34	48		
Mean ± SD	9464 ± 1500	8520 ± 1239	3535 ± 1112	3270 ± 707	2107 ± 248	1206 ± 136		
L <sub>1</sub> ≤ μ ≤ L <sub>2</sub>	8193 ≤ μ ≤ 10735	7470 ≤ μ ≤ 9570	2593 ≤ μ ≤ 4478	2671 ≤ μ ≤ 3869	1897 ≤ μ ≤ 2318	1090 ≤ μ ≤ 1321		
Locations (MASL)	Micro elements (mg/kg)							
	Fe	Cu	Zn	Mn	Se	Cr	Mo	Total
Qaramani (566)	31	46	40	31	5	5	4	30918
Bezhawa (591)	4	38	28	30	3	4	4	25656
Gulp (716)	48	44	40	31	3	7	3	24854
Bnary Qandil (730)	102	38	41	31	7	4	5	29554
Mawat (803)	4	45	26	29	4	5	4	27087
Azmar (857)	4	27	49	22	5	5	2	25220
Temar (887)	45	56	58	38	12	11	4	31491
Ahmad Awa (929)	56	40	42	29	3	4	2	28618
Hazarmerd (1040)	52	49	54	33	10	2	4	31451
Kani Tu (1236)	43	43	39	26	16	4	4	29578
Nalparez (1313)	78	40	39	35	4	4	2	28260
Qara Dagh (1419)	109	38	40	32	3	10	4	29831
Penjwen (1505)	37	31	30	25	23	5	4	25136
LSD <sub>(P≤0.05)</sub>	1.4	0.4	0.5	0.6	6.8	0.3	0.6	632
LSD <sub>(P≤0.01)</sub>	1.9	0.5	0.7	0.8	9.2	0.4	0.8	855
Mean ± SD	47 ± 33	41 ± 7	40 ± 9	30 ± 4	7 ± 6	5 ± 2	3 ± 0.9	28280 ± 2457
L <sub>1</sub> ≤ μ ≤ L <sub>2</sub>	18 ≤ μ ≤ 75	34 ≤ μ ≤ 47	32 ≤ μ ≤ 48	26 ≤ μ ≤ 33	2 ≤ μ ≤ 12	3 ≤ μ ≤ 7	2 ≤ μ ≤ 4	26199 ≤ μ ≤ 30362

Based on the obtained results, it is noteworthy that most of the Kenger plant samples exhibited concentrations of microelements exceeding the recommended daily intake, potentially reaching levels considered toxic for human consumption. However, it is crucial to underscore that the concentrations, as presented in the table, are expressed in milligrams per kilogram (mg/kg), and, therefore, the ratio itself may not be a sole

determinant of safety. Instead, the overall quantity consumed by an individual holds paramount significance in assessing the potential health risks associated with these elevated microelement concentrations.

#### CONCLUSIONS

It can be concluded that the GC-MS analysis of phytochemical compounds in Kenger plant extract across various locations has revealed a diverse and rich source of chemical

compounds such as fatty acids, sterols, and terpenoids with the potential for various medicinal and therapeutic applications. Macro elements contents across locations in Kenger shows that the grand mean of Calcium content was 9464 with a standard deviation of 1500 mg/kg and an interval of (8193 – 10735). Potassium recorded  $8520 \pm 1239$  mg/kg with a range of (7470 – 9570). A range of (2593 – 4478) was obtained for Phosphorus content with  $3535 \pm 1112$  mg/kg, whilst Sulfur gave  $3270 \pm 707$  with a range of (2671 – 3869). The means of Magnesium and Sodium contents were 2107 and 1206 with a standard deviation of 248 and 136 mg/kg and the range of (1897 – 2318) and (1090 – 1321), respectively. Concerning the Micro elements contents, Ferus gave  $47 \pm 33$  mg/kg with an interval of (18 – 75) across locations. Copper recorded  $41 \pm 7$  mg/kg with a range of (34 – 47), while a range of (32 – 48) was recorded for Zinc content with  $40 \pm 9$  mg/kg. Manganese content was  $30 \pm 4$  mg/kg with a range of (26 – 33). Selenium recorded  $7 \pm 6$  mg/kg with a wide interval of (2 – 12). The means of Chromium and Molybdenum contents were 5 and 3 with a standard deviation of 2 and 0.9 mg/kg and the range of (3 – 7) and (2 – 4), respectively.

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