Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/81a03h38

ICP-OES ANALYSIS OF ESSENTIAL, HEAVY, AND TRACE ELEMENTS IN THE MEDICINAL PLANTS OF UMERKOT DISTRICT, SINDH, PAKISTAN

Khadim Ali Gishkori[1,2*], Saba Naz[2], Aftab Ahmed Kandhro[2], Aijaz Panhwar[3], Mohammad Younis Talpur[2], Muhammad Yousif Seelro[2], Nusrat Jalbani[3].

[1*]Pakistan Standard Quality Control Authority, Karachi-Pakistan
[2]Dr. M. A. Kazi Institute of Chemistry, University of Sindh,76080, Jamshoro-Pakistan
[3]PCSIR, Laboratories Complex, Off University Road, 75280, Karachi- Pakistan.

***Corresponding author:** Khadim Ali Gishkori
*email: khadimaligishkori@gmail.com

ABSTRACT
In this study, samples of medicinal plants from District Umerkot, Sindh, were analyzed for determining the concentrations of 17 essential elements, heavy metals, and trace metals such as Al, As, B, Ca, Cd, Co, Cr, Cu, Fe, Mg, Mn, Na, Ni, P, Pb, Se, and Zn. Determination of elemental profile of medicinal plants which are being used for therapeutic purposes in different regions. The analysis employed a 700 series Agilent inductively coupled plasma optical emission spectrometer (ICP-OES) coupled with ultrasonic CETAC U-6000AT + Nebulizer, following microwave-assisted acid digestion of the plant samples with MDS-6G closed microwave digester. The findings revealed significant concentrations of trace elements in medicinal plant extracts from the Umerkot, Sindh. Notably, *Ipomoea Carnea* samples exhibited elevated concentrations of essential elements, heavy metals, and trace metals such as 386.1, 0.0320, 0.8079, 167.4, 0.194, 0.0016, 0.376, 1.932, 5.787, 33.72, 2.242, 15.56, 0.0115, 0.4936, 0.2371, 0.0494, 0.4785 mg/Kg, respectively. *Fagonia indica* samples displayed concentrations of 5.127, 0.0004, 0.6850, 121.7, 0.0036, 0.0024, 0.0181, 1.879, 1.844, 32.83, 0.3300, 11.83, 0.0019, 0.4054, 0.0054, 0.0202, and 0.0202 mg/Kg, respectively. However, *Tinospora malabarica* exhibited concentrations of 69.03, 0.0361, 1.555, 384.8, 0.0121, 0.0040, 0.0427, 0000, 7.100, 80.33, 3.242, 17.45, 0.0250, 0.14080, 9.550, 0.0181, 2.572 mg/Kg for Al, As, B, Ca, Cd, Co, Cr, Cu, Fe, Mg, Mn, Na, Ni, P, Pb, Se, and Zn, respectively. These elements can play a pivotal role in the plants' curative effects on human health.

Keywords: Elements, *Fagonia indica*, *Ipomoea carnea*, *Tinospora malabarica*, Medicinal Plants, ICP-OES

1. INTRODUCTION
Centuries of human history have witnessed the widespread utilization of medicinal plants for health promotion and the treatment of diverse ailments. These plants house compounds endowed with therapeutic properties, offering manifold benefits to the human body. Throughout the ages, numerous cultures have incorporated plants and plant-based materials into various aspects of daily life, encompassing food, medicine, ornaments, as well as tools for hunting, building, religious practices, and the creation of agricultural and musical instruments, household appliances, and dwellings (1,4). Recently, there has been a global surge in the use of plants as herbal or natural health products,

particularly in developed countries, underscoring their potential health benefits (5). Globally, the market for medicinal plant products is estimated to be around $400 billion, experiencing a growth rate of 15 to 25% annually (6). In 2000, the approximate global market value for medicinal plants, encompassing herbal products and raw materials, was about US $65 billion (7).

Many of the mineral elements found in plants are essential to human nutrition (8). Several of these elements, which are regarded as micronutrients and are normally present in plants in small amounts, include iron, manganese, zinc, copper, and selenium. Others serve as trace elements, which are necessary for the body's many metabolic activities (9). Humans with severe disease symptoms have been related to deficiencies in or toxicity from these trace elements, which are frequently caused by imbalances in plant-based diets or exposure to metal toxicities (10,11).

A trace amount of a few mineral elements, including Al, B, Se, Fe, Zn, and Mn, is needed is needed for the transportation process, function as channels, and support normal growth and development. The complex link between these trace elements and many physiological activities in metabolism makes it difficult to exactly quantify microelements. To shed light on deficits or toxicities, it is imperative to examine the amounts of these micronutrients in different plants. This is in line with the suggestions made by European regulation (1881/2006/EC) about the maximum amounts that can be tolerated (12,13).

Essential for energy metabolism, oxidation-reduction reactions are facilitated by trace metals such as iron and copper, which act as catalysts in enzyme systems (14). On the other hand, selenium protects cellular membranes from toxicity in biological systems by acting as a natural chelator of metals and metalloids (15). Nevertheless, an overabundance of selenium in the human body can have negative consequences, such as tooth decay, skin pigmentation, and digestive issues (16). Magnesium also improves endurance and lowers blood pressure, which helps to prevent osteoporosis (17). A deficiency of magnesium in the human body can cause problems like nystagmus, muscle spasms, poor coordination, and appetite loss (18). These conditions are major contributors to cardiovascular disorders. Considering the importance of metal profile of medicinal plants, three plants have been selected in this study and medicinal importance is briefly given here.

Ipomoea carnea is believed to be origin of American tropical region but now sparsely distributed in different regions and grows worldwide by 2 to 3 meter high as erect, leafed and unbranched (19). Its different parts including leaves and flowers have been used as raw material for therapeutics to cure different ailments such as venereal and skin diseases, immunodeficiency, dysentery, gout, rheumatism, and hypertension. Extracts of *I. carnea* have also demonstrated antimicrobial, anticancer, antioxidant by reducing free radical, antidiabetic, immune modulatory, wound healing, anticonvulsant, anxiolytic, anti-inflammatory, sedative, and hepatoprotective properties (20,21). Its toxic effect has long been studies to know the main constituent responsible for this activity polyhydroxy alkaloids such as swainsonine and calystegines (22).

Malabarica is one of thirty species of genus Tinospora and included on in few of those thirty species which have been used as medicinal source due to their therapeutic importance. Like other species it also grows in tropical and subtropical regions including south Asia (23). This plant is bitter due variety of alkaloids and considered a tonic for the treatment of jaundice, rheumatism, urinary disorders, gout, leprosy, and fever (24,26). Extract of dichloromethane of whole plant revealed the presence of three compounds, including N-cis-feruloyltyramine, N-transferuloyltyramine and secoisolariciresinol. All of those compounds have shown antioxidant properties when tested as per assay protocols of *β*-carotene and 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical (27). *T. malabarica* stem is found to contain flavone O-glycosides (apigenin), picroretoside, berberine, palmatine, picroretine, resin & five flavonoids (27). Hot water extract *T. malabarica* showed protective effects on renal damage and hemolysis induced by malaria infection during experiments done on mice. Thus this plant species was supposed to be a potential source in the development of variety of herbal formulations for malarial treatment (28).

Various varieties of Fagonia are commonly found throughout Pakistan, parts of India and the Middle East (29). In Sindh, indica is the mostly found species of genus *Fagonia* which is known as "*Sachi*

Booti" that means true herb. Local medicinal uses are also reported which include treatment of cancer and issued caused due to any poisonous substances. A tumor growth inhibition has been also been observed as prominent property of *Fagonia indica* aqueous extract made from whole plant (30). This plant is found rich in saponin or triterpenoid glycoside as per isolation work carried out by different group of scientists from time to time. Fagonia species are often used in traditional medicines, such as for treatment of fever, jaundice (31), blood purification, cold, cough (32), asthma, skin infection, liver problems (33), carminative, emetic (34), and the extracts of these plants have been reported to exhibit antimicrobial, anti-inflammatory, analgesic and antipyretic activities (35). Additionally, this plant is declared to be remedy for cancer (36) and thalassemia (37).
Since metals have great potential to be source for human as well as animals as nutrients however, they may also affect the activity of active metabolites of plants to reduce or enhance the effect of active molecules. Hence it is obligatory to determine the metal concentration in those plants which are considered as medicinal and used locally. Hence the presence study is aimed at determination of the concentration of metals in three medicinal plants of Umarkot, Sindh, Pakistan which are used by local people and medical practitioners in routine to cure different ailments as these plants possess different active secondary metabolites.

2. MATERIALS AND METHODS

2.1. Sample Collection

A total of 30 samples were collected, comprising 10 samples of each of plant i.e. *Ipomoea Carnea* Jack (Local Name: Bush Morning Glory), *Fagonia indica* (Local Name: Fagonbushes/Dhamasa), and *Tinospora malabarica* (Local Name: Heart-leaved/Giloy), gathered from Umerkot district, Sindh.

2.2. Reagents and Solutions

The experiment was carried out with deionized water for sample preparation and for digestion process analytical grade HNO3 (Merck) and H2O2 (Merck) were employed. In the digesting processes, analytically pure HNO_3 (Merck) and H_2O_2 (Merck) were employed. The accuracy and precision of the process were assessed using certified reference material NCS ZC73014 Tea Leaves (reference sample) from the National Institute of Standards and Technology (NIST), Gaithersburg, MD, USA. The following elements were present in the mixed standard (1000 mg L-1) for ICP-OES measurements: Al, As, B, Cd, Cr, Cu, Fe, Mn, Ni, Pb, and Se. Calibration standard solutions (0.05-1.5 mg L^{-1}) were obtained by suitably diluting the stock mix standards (10 mg L-1).

2.3. Instrumentation

Experiments were conducted using a Thermo iCAP 6300 Duo ICP OES from Thermo Fisher Scientific in Bremen, Germany. Pure Argon (Ar) served as the plasma source (White Martins-Praxair, Campo Grande, MS, Brazil), and sample digestions were carried out in TFM™-PTFE vessels (DAP-60).

2.4. Pre-treatment of Samples

Following sample collection, specimens were promptly transported to testing laboratories at the University of Sindh, Jamshoro for further analysis. Plant samples underwent a three-step washing sequence, with three additional washes in deionized water. Cleaned plant samples were air-dried and then homogenized by grinding in an Agate mortar, followed by sieving through a nylon sieve (100 mm).

2.5. Sample Digestion

A weighing 0.2 g of each plant sample was placed directly into conical flasks. Subsequently, 6.0 ml of concentrated HNO_3 and H_2O_2 in a 2:1 ratio were added, and digestion occurred on a hot plate (80°C). The samples were then diluted up to 10 ml with 0.2 N HNO3, filtrated using filter paper of a specific size, and analyzed by ICP-OES.

2.6. Method Validation

As shown in Table 3, the calibration chart showed the linear range, regression, correlation coefficient (R), limit of detection (LOD), and limit of quantification (LOQ) values for eleven components when it was created under ideal operating conditions. Every coefficient (R) value was higher than 0.999, indicating that the evaluation's linearity was excellent.

3. RESULTS AND DISCUSSION

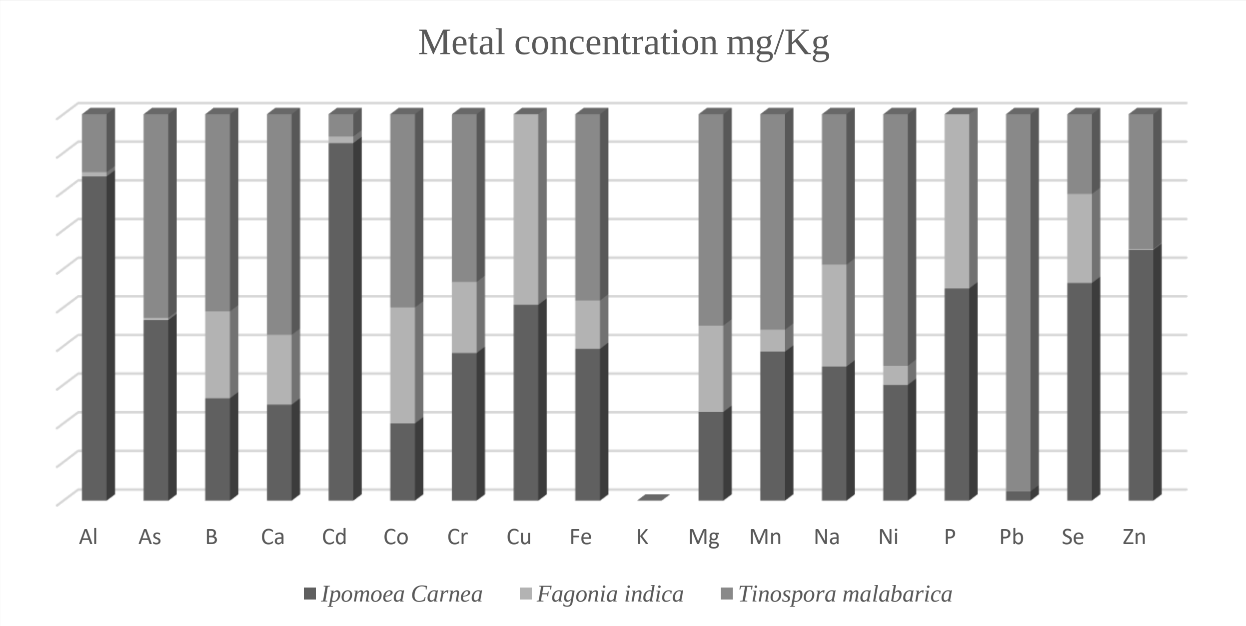

Figure 01. Comparative profile of metals of three medicinal plants selected in the present study.

Three plant varieties, *Ipomoea carnea*, *Fagonia indica*, and *Tinospora malabarica*, were collected for ICP analysis (Figure 01). *Ipomoea carnea* exhibited the highest calcium content at 167.4 mg/Kg among essential metals, while *Fagonia indica* had 121.7 mg/Kg of calcium, and *Tinospora malabarica* had the lowest at an undisclosed value. In essential metals, *Ipomoea carnea* showed the lowest iron content at 5.787 mg/Kg, *Fagonia indica* had 11.83 mg/Kg of sodium, and *Tinospora mallabarica* had 7.1 mg/Kg of iron is shown in Table 1.

Table 1. Concentration of essential elements, heavy and trace metals (mg/Kg) in *Ipomoea Carnea*, *Fagonia indica*, *Tinospora malabarica* samples

Elements	Al	As	B	Ca	Cd	Co	Cr	Cu	Fe
Ipomoea Carnea	386.1 (±18.3)	0.0320 (±0.0014)	0.8079 (±0.0304)	167.4 (±6.3)	0.194 (±0.006)	0.0016 (±0.0)	0.376 (±0.0185)	1.932 (±0.086)	5.787 (±0.203)
Fagonia indica	5.127 (±0.216)	0.0004 (±0.0)	0.6850 (±0.0312)	121.7 (±5.085)	0.0036 (±0.0001)	0.0024 (±0.0001)	0.181 (±0.070)	1.879 (±0.073)	1.844 (±0.052)
Tinospora malabarica	69.03 (±3.451)	0.0361 (±0.0018)	1.555 (±0.077)	384.8 (±19.2)	0.0121 (±0.0006)	0.0040 (±0.0002)	0.427 (±0.021)	-	7.100 (±0.355)

Elements	K	Mg	Mn	Na	Ni	P	Pb	Se	Zn
Ipomoea Carnea	-	33.72 (±1.686)	2.242 (±0.112)	15.56 (±0.77)	0.0115 (±0.0005)	0.4936 (±0.0246)	0.2371 (±0.0118)	0.0494 (±0.0024)	4.785 (±0.239)
Fagonia indica	-	32.83 (±1.6415)	0.330 (±0.016)	11.83 (±0.59)	0.0019 (±0.0000)	0.4054 (±0.0202)	0.0054 (±0.0002)	0.0202 (±0.0010)	0.0202 (±0.0010)
Tinospora malabarica	-	80.33 (±3.01)	3.242 (±0.112)	17.45 (±0.77)	0.0250 (±0.0006)	0.4080 (±0.0104)	9.550 (±0.377)	0.0181 (±0.0006)	2.572 (±0.108)

In heavy metals, *Ipomoea carnea* displayed the highest zinc level at 4785 mg/Kg, *Fagonia indica* had 5.127 mg/Kg of aluminum, and *Tinospora malabarica* had 69.06 mg/Kg of aluminum. *Fagonia indica* exhibited the lowest copper content at 1.879 mg/Kg in heavy metals, while arsenic was 0.0004 mg/Kg in *Fagonia indica* and 0.0320 mg/Kg in *Ipomoea carnea*.

(38). A recent report of determination of metal concentration on another species of this genus (*Ipomea aquatica*) is reviewed here for comparison. The said report covers only Mn, Cu, Pb, Zn, and comparatively Mn, Cu and Zn are found higher in *Ipomoea carnea* however, Pb value is bit difficult to compare as the previous report has value either below detectable limit or detected with very high standrard deviation i.e. almost 100% of actual value. However, lead detected in *Ipomoea carnea* is 0.2371 mg/Kg that is in accordance with previous report value and overall within the range as per suggested safe limits of lead in plants i.e. below 85 mg/Kg (39).

Tinospora malabarica had the highest chromium level at 0.427 mg/Kg among trace elements, whereas *Ipomoea carnea* showed 0.0494 mg/Kg of selenium and *Tinospora malabarica* had 0.0181 mg/Kg of chromium. A previous report (40) revealed the presence of 6 metals in different species of Tinospora (*Tinospora cordifolia*) including Ca, P, I, Cu, Zn, and Mn. Comparatively only Ca is higher in malabarica as compared to cordifolia while Cu was not detected in malabrica and 3.733 (ppm) was found in cordifolia. However, present study cover almost three times higher number of metals which are not reported in a single report on malabarica as well as well as other species of genus Tinospora hence further comparison are not possible.

Fagonia indica had the lowest nickel content at 0.0019 mg/Kg among trace elements, and phosphorus was 0.4054 mg/Kg in *Fagonia indica* is described in Table 1. In a previous report (41) detection of 13 Metals is carried out by using flame atomization (FAAS). Different parts of plants werer selected for metal analysis and there was considerable difference was found however, the mostly used part of plant for meidicnal purpose is leaves and steam hence in the present study only those parts were subjected for metal analysis hence here comparison is being given only for those parts. Fe, Cu, Mg, Ca, Ni, were found much higher in previous report as compared to present in same species which is might be due to reason of plant origin region. Previous report is on *Fagonia indica* collected from Balochistan province that is the land of hills and mountain which is full of minerals while the *Fagonia indica* selected in this study is of Sindh province which is normal sandy land region. Surprisingly Cd and Co are not detected in previous report while traces are found in present study results. Arsenic is higher reported in previous report as compared to present work results and Cr is almost similar. It can be concluded that metals vary with region hence it is not only important to report metals of medicinal plants but also a comparative figures are necessary to get about metal concentration of similar species of different origins.

Table 2 shows the single-one way ANOVA (Analysis of Variance) test. ANOVA test to determine if there are significant differences in element concentrations among the different plant species. If the ANOVA test shows a significant result ($p < 0.05$), it indicates that there are significant differences in at least one pair of plant species. There are two sources of variation: Between Groups" and "Within Groups." Variation in the means of the elements across different groups (i.e., between different elements).Variation within each group (i.e., within each element), representing random variability or error. SS (Sum of Squares) is measures the total variability in the data. df (Degrees of Freedom), it indicates the number of independent pieces of information available for estimating statistical parameters. For "Between Groups," df equals the number of groups minus one (k - 1), where k is the number of groups. For "Within Groups," df equals the total number of observations minus the number of groups (N - k). MS (Mean Square), The variance estimate, calculated by dividing the sum of squares (SS) by its corresponding degrees of freedom (df). F (F-ratio), The test statistic calculated by dividing the mean square for between groups by the mean square for within groups. It indicates whether there are significant differences in means among the groups. P-value, the probability of observing the F-ratio (or a more extreme value) if the null hypothesis (i.e., no difference between group means) is true. A p-value less than the chosen significance level (usually 0.05) suggests that the observed differences are statistically significant. F crit (Critical Value), the critical value of F from the F-distribution table, used to determine statistical significance. If the calculated F-value exceeds the critical value, the null hypothesis is rejected. In the present study, the p-value (0.0004538) is less than the chosen significance level (usually 0.05), indicating that there are significant differences in the mean concentrations of elements among the groups. The F-ratio (2.609267) compares the variability between groups to the variability within groups. Since it is greater than 1, it suggests that the variability between groups is greater than expected by chance alone. Based on the significant p-value, we reject the null hypothesis and conclude that there are statistically significant differences in the mean concentrations of elements among the groups (elements). This analysis suggests that the concentrations of elements vary significantly among the different elements examined. Further post-hoc tests may be conducted to identify which specific pairs of elements have significantly different mean concentrations.

Table 2. Anova: Single Factor

Anova: Two-Factor						
SUMMARY	*Count*	*Sum*	*Average*	*Variance*		
Ipomoea Carnea	17	617.7275	36.33691	9754.997		
Fagonia indica	17	176.8964	10.40567	888.8698		
Tinospora malabarica	17	576.5602	33.91531	8762.555		
Al	3	460.257	153.419	41626.23		
As	3	0.0685	0.022833	0.000382		
B	3	3.0479	1.015967	0.221694		
Ca	3	673.9	224.6333	19762.14		
Cd	3	0.2097	0.0699	0.011569		
Co	3	0.008	0.002667	1.49E-06		
Cr	3	0.984	0.328	0.016857		
Cu	3	3.812	1.270667	1.209742		
Fe	3	14.731	4.910333	7.482792		
Mg	3	146.88	48.96	738.2557		
Mn	3	5.815	1.938333	2.18764		
Na	3	44.84	14.94667	8.178233		
Ni	3	0.057	0.019	5.2E-05		
P	3	1.306	0.435333	0.002496		
Pb	3	9.802	3.267333	29.61625		
Se	3	0.088	0.029333	0.000292		
Zn	3	5.378	1.792667	2.365444		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows	6975.646	2	3487.823	0.950845	0.397062	3.294537
Columns	193122.5	16	12070.16	3.290547	0.002012	1.971683
Error	117380.2	32	3668.131			
Total	317478.4	50				

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Al	17	460	153.3333	41624.33
Ca	17	672	224	19729
Fe	17	13	4.333333	9.333333
B	17	3	1	0
Mg	17	145	48.33333	752.3333
P	17	1.3	0.433333	0.003333

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	135062.2	5	27012.44	2.609267	0.0004538	3.105875
Within Groups	124230	12	10352.5			
Total	259292.2	17				

4. CONCLUSION

This study focused on assessing the accumulation of heavy metals and trace elements in the leaves of commonly used medicinal plants from the Thar Desert in Umerkot, Sindh. The region is abundant in medicinal plant and the study revealed presence of 17 essential elements, heavy metals, and trace metals in plant extracts. Notably, the extracts contain significant amounts of magnesium and high levels of aluminum, both essential trace elements for human consumption.

While certain trace elements like Se (selenium) and Pb (lead) are vital for daily dietary intake within prescribed limits, there is no defined limit for plant intake set by the United States Food and Drug Administration (US-FDA) or the World Health Organization (WHO). In this study, the observed p-value (0.0004538) falls below the typically chosen significance threshold of 0.05. This suggests that notable distinctions exist in the mean concentrations of elements across the various groups under examination. Some elements found in the plants exhibit immunological, anti-inflammatory, and antioxidant properties, offering protective effects against toxic elements and diseases such as cancer and heart problems. This is a first report on all three species selected in which 17 elements are quantified and statistical analysis is also carried out which could be helpful for future work in different direction about these three medicinal plant species.

Acknowledgments
The authors would like to thank Dr. M. A. Kazi Institute of Chemistry, Jamshoro, Sindh Pakistan for providing a pleasant environment for this study.

Author Contributions
All authors conceived of the presented idea. All authors discussed the results and contributed to the final manuscript.

Declarations
Conflict of interests
The authors declared that they have no conflicts of interest.

Ethical approval
Not relevant

Human and Animal participation Not applicable

[1] Petrovska BB. Historical review of medicinal plants usage. *Pharmacogn Rev.* 2012; 6(11): 1–5.
[2] Tolo U, Kahwa C, Nuwagira I, Weisheit UA, Ikiriza H. Medicinal plants used in treatment of various diseases in the Rwenzori Region, Western Uganda. 2023; *Ethnobot. Res. Appl.*, 25:1–16.
[3] Chhavi S, Sharma A, Dhiman A. An update on phytochemistry and therapeutic properties of *Ipomoea carnea*. *J Pharmacogn Phytochem* 2021; 10:01-06.
[4] Aziz MA, Adnan M, Khan AH, Shahat AA, Al-Said MS, Ullah R. Traditional uses of medicinal plants practiced by the indigenous communities at Mohmand Agency, FATA, Pakistan. *J. Ethnobiol. Ethnomed* 2018; 14(2): 1-16
[5] Ali K, Khan H. *Fagonia indica;* A Review on Chemical Constituents, Traditional Uses and Pharmacological Activities. *Curr Pharm Des*. 2021; 27(22):2648-2660.
[6] Sundar M, Lingakumar K. Investigating the efficacy of topical application of *Ipomoea carnea* herbal cream in preventing skin damage induced by UVB radiation in a rat model. *Heliyon*. 2023; 9(9).
[7] Fortune Business Insights (FBI) 2022 Fortune Business Insights (FBI) Herbal medicine market size & covid-19 impact analysis retrieved 30 october 2022. by application (pharmaceutical & nutraceutical, food & beverages and personal care & beauty products), by form (powder, liquid & gel, and tablets & capsule), and regional forecast. Market Res. Report. Food Suppl., Herbal Med. (2022), pp. 2022-2029. https://www.fortunebusinessinsights.com/herbal-medicine-market-106320.
[8] World Health Organization (WHO) 2003. World Health Organization (WHO).Guidelines On Good Agricultural and Collection Practices (GACP) For Medicinal Plants. WHO, Geneva, Switzerland (2003).
[9] Soetan KO, Olaiya CO, Oyewole OE. The importance of mineral elements for humans, domestic animals and plants: A review. *Afr. J. Food Sci.* 2010; 31;4(5):200-22.
[10] White PJ, Brown P. Plant nutrition for sustainable development and global health. *Ann. Bot.* 2010; 1;105(7):1073-80.
[11] Mehri A. Trace elements in human nutrition (II)–an update. *Int. J. Prev. Med.*. 2020 Jan 1;11(1):2.
[12] Mitra S, Chakraborty AJ, Tareq AM, Emran TB, Nainu F, Khusro A, Idris AM, Khandaker MU, Osman H, Alhumaydhi FA, Simal-Gandara J. Impact of heavy metals on the environment and human health: Novel therapeutic insights to counter the toxicity. *J. King Saud Univ. Sci.* 2022; 1;34(3):101865.
[13] Fagbohun OF, Olawoye B, Oriyomi OV, Joseph JS. Multivariate analyses of selected trace elements from Kigelia africana (Lam.) Benth. plant by ICP-OES: a chemometrics approach. JTEMIN. 2023; 1;5:100081.
[14] Barreca S, Orecchio S, Orecchio S, Abbate I, Pellerito C. Macro and micro elements in traditional meals of Mediterranean diet: Determination, estimated intake by population, risk assessment and chemometric analysis. *J. Food Compos. Anal*. 2023;1;123:105541.
[15] Jomova K, Makova M, Alomar SY, Alwasel SH, Nepovimova E, Kuca K, Rhodes CJ, Valko M. Essential metals in health and disease. *Chem Biol Interact*. 2022;1;367:110173.

[16] Briffa J, Sinagra E, Blundell R. Heavy metal pollution in the environment and their toxicological effects on humans. *Heliyon*. 2020; 1;6(9).
[17] Genchi G, Lauria G, Catalano A, Sinicropi MS, Carocci A. Biological activity of selenium and its impact on human health. *Int. J. Mol. Sci.*. 2023;30;24(3):2633.
[18] Liu L, Luo P, Wen P, Xu P. The role of magnesium in the pathogenesis of osteoporosis. *Front. Endocrinol.* 2024; 6;15:1406248.
[19] Austin DF. *Ipomoea carnea* jacq. vs. *Ipomoea fistulosa* Mart. ex Choisy. 1977; *Taxon*.26:235-238.
[20] Fatima N, Rahman MM, Khan MA, Fu J. A review on Ipomoea carnea: pharmacology, toxicology and phytochemistry. *J Altern Complement Med.* 2014;11:55-62.
[21] Wadnerwar N, Deogade M. Future perspectives of Therapeutic Claims of an Ethnopharmacological Drug Ipomoea carnea Jacq.-A Critical Review. International Journal of Ayurvedic Medicine. 2021;12:177-184.
[22] Haraguchi M, Gorniak SL, Ikeda K, Minami Y, Kato A, Watson AA, Nash RJ, Molyneux RJ, Asano N. Alkaloidal components in the poisonous plant, *Ipomoea carnea* (Convolvulaceae). *J. Agric. Food Chem.* 2003; 51:4995-5000.
[23] Lekshmipriya S, Pandya M, Prajapati B. Analytical Profile of an Unparalleled Drug Tinospora crispa WRT Guduchi. *J. Pharm. Res. Int.* 2021; 33:195-203.
[24] Mainasara MM. Documentation and evaluation of medicinal plants with potential for breast cancer therapy as used by Jakun Community of Kampung Peta, Mersing, Johor Universiti Tun Hussein Onn Malaysia. 2019.
[25] Perry LM, Metzger J. Medicinal plants of East and Southeast Asia: attributed properties and uses. 1980.
[26] Rizvi MA, Ali SA. Medicinal flowers of Pakistan. *Int J Adv Res*. 2016;4:1313-1341.
[27] Nidhi P, Swati P, Krishnamurthy R. Indian Tinospora species: natural immunomodulators and therapeutic agents. *Int J Pharm Biol Chem* Sci. 201; 2:01-09.
[28] Nutham N, Sakulmettatham S, Klongthalay S, Chutoam P, Somsak V. Protective effects of *Tinospora crispa* stem extract on renal damage and hemolysis during Plasmodium berghei infection in mice. Journal of pathogens. 2015; 738608.
[29] Ahmad VU, Basha A. Spectroscopic Data of Saponins: The Triterpenoid Glycosides, 3-Volume Set: CRC press. 2000.
[30] Soomro A, Jafarey N. 2003. Effect of *Fagonia indica* on experimentally produced tumours in rats. JPMA-Journal of Pakistan Medical Association.53:224-225.
[31] Hammiche V, Maiza K. Traditional medicine in Central Sahara: pharmacopoeia of Tassili N'ajjer. J. Ethnopharmacol. 2006; 105:358-367.
[32] Khattak KF. Microbiological quality assessment of commercially available medicinal plants in Peshawar city, Pakistan. *Pak J Bot.* 2012;44:1203-1208.
[33] Abirami V, Khosa R, Dhar S, Sahai M. Investigation on Fagonia cretica–its effect on hormonal profile and immunomodulation in rats. Anc. Sci. Life. 1996; 15:259-263.
[34] Mahmood A, Qureshi RA, Mahmood A, Sangi Y, Shaheen H, Ahmad I, Nawaz Z. Ethnobotanical survey of common medicinal plants used by people of district Mirpur, AJK, Pakistan. *J Med Plants Res.* 2011. 5:4493-4498.
[35] Nagaraj DS, Venkateswarlu B. Antipyretic effect of aqueous extract of Fagonia cretica L. whole plant on brewer's yeast induced pyrexia in wistar rats. *Int J Pharmacol Toxicol*. 2013; 3:39-42.
[36] Hussain A, Zia M, Mirza B. Cytotoxic and antitumor potential of *Fagonia cretica* L. *Turk J Biol.* 2007; 31:19-24.
[37] Seyal RA, Awan H, Tareen S. Can we really treat thalassemia major. *Proceedings of the Pakistan Academy of Sciences*. 2013; 50:315-325.
[38] Ab Manan WN, Zulkurnain N. Determination of selected heavy metal concentrations in water spinach (Ipomea aquatica). In Materials Science Forum 2021 Apr 29 (Vol. 1025, pp. 279-283).
[39] Amaechi M, Onwuka SU. Determination of the Presence and Level of Heavy Metals in Soils of Automobile Workshops in Awka, Anambra State. *Int. J. Environ. Pollut. Res.* 2021; 3;9(3):18-32.
[40] Rahal A, Prakash A, Verma AK, Kumar V, Roy D. Proximate and elemental analyses of Tinospora cordifolia stem. *Pak J Biol Sci*: PJBS. 2014; 1;17(5):744-7.
[41] Bakhsh W, Memon N, Maria G, Ullah I, Lanjwani AH, Memon AN. Metal composition of *Fagonia indica* of Kech, Balochistan, Pakistan. *Rawal Med J.* 2019; 22;44(2):398.