

Full Length Research Paper

Chemical Profiling and Inorganic Element Analysis of *Calendula officinalis* and *Sonchus asper*

Iqbal Hussain^{1*}, Riaz Ullah^{1, 3}, Naeem Khan¹, Sultan Ayaz⁴, Shabir Ahmad¹, Shanzeb¹, Majed Ahmed¹, Pir Tariq Hasan¹ and Farhat Ali Khan²

¹Department of Chemistry, Kohat University of Science and Technology, Kohat, Pakistan.

²Department of Pharmacy, Sarhad University of Science and Information Technology, Peshawar, Khyber Pakhtunkhwa, Pakistan.

³Department of Chemical Engineering, College of Engineering, King Saud University, P. O. Box 800, Riyadh 11421, KSA.

⁴Department of Zoology, Kohat University of Science and Technology, Kohat, Pakistan.

Accepted 19 November, 2025

The present work was carried out to investigate the quantitative determination of crude phytochemicals (alkaloids, flavonoids and saponins), heavy metals and inorganic constituents in *Calendula officinale* and *Sonchus asper*. Heavy metals were determined by atomic absorption spectrophotometer and inorganic constituents by the available methods (AOAC, 2000). The studied plants showed variable amount of phytochemicals, heavy metals and inorganic constituents. In case of heavy metals, Pb, Fe, Cu, Zn, Ni and Ag were recorded. The amount of inorganic constituents was also found in considerably different levels. The study is of particular importance for providing a scientific data base line.

Key words: Phytochemicals, *Calendula officinale*, heavy metals.

INTRODUCTION

Plant based drugs have been in use against different ailments since time immemorial (Hussain et al., 2011). The primal man used herbs as healing agents and medicament (Mushtaq et al., 2009). Medicinal plants are of great importance to the health of individuals and the society. The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body. Some of the most important bioactive constituents are alkaloids, saponins, flavonoids, tannins, terpenoids, phenolic compounds, essential oils, steroids, glycosides, phenolic compounds. The use of plant materials to prevent and treat infectious diseases successfully over the years has attracted the attention of scientist's worldwide (Hackett, 2003). Compounds isolated from plants with antiviral properties used in

traditional medicine includes: Polysaccharides, flavonoids, terpenes, alkaloids, phenols and amino acids (Paredes et al., 2001). Plants are the potential source of natural anti-oxidants. Natural anti-oxidants or phytochemicals anti-oxidants are the secondary metabolites of plants (Phillipson and Wright, 1991). Carotenoids, flavonoids, cinnamic acids, benzoic acids, folic acid, ascorbic acid, tocopherols, tocotrienols etc., are some of the anti-oxidants produced by the plant for their sustenance. Beta-carotene, ascorbic acid and alpha tocopherol are the widely used antioxidants.

In recent years, secondary plant metabolites (phytochemicals), previously with unknown pharmacological activities, have been extensively investigated as a source of medicinal agents. Thus, it is anticipated that phytochemicals with adequate antibacterial efficacy will be used for the treatment of bacterial infections (Chawira et al., 1987).

Calendula Officinale Linn is used medicinally in Europe,

*Corresponding author. E-mail: afridiriaz@yahoo.com.

Table 1. The quantitative determination of alkaloids in *C. officinale* and *S. asper*.

S/N	Plant code	Wt taken in g	Wt of crude alkaloid(g)	%
1	NCO	10	1.26	12.59
2	KCO	10	1.16	11.56
3	PSA	10	1.07	10.68
4	NSA	10	1.33	12.25
5	KSA	10	1.19	11.86

N = Nowshera, K = Kohat, P = Peshawar, CO = *Calendula officinale*; SA = *Sonchus asper*.

Table 2. The quantitative determination of flavonoids in *C. officinale* and *S. asper*.

S/No	Plant code	Plant taken in grams	Weight of the crude sample	%
1	NCO	10	1.573	15.77
2	KCO	10	1.68	16.77
3	PSA	10	1.40	14.03
4	NSA	10	1.39	13.96
5	KSA	10	1-35	13.51

China, US and India. It belongs to the family, Asteraceae, and is commonly known as Zergul (Hindi), African marigold, *Calendula*, Common Marigold (Krishnaraju et al., 2005; Kirtikar and Basu, 1933).

The plant is an annual, seldom biennial. It grows to between 30 and 50 cm high, and has about 20 cm long tap root and numerous thin, secondary roots. The stem is erect, angular, downy and branched from the base up or higher. The alternate leaves are almost spatulate at the base, oblong to lanceolate above and are all tomentose (TWI, 1992; Editorial board, 2003).

It is used because of the broad area of biological activities like anti-inflammatory, anti-mutagenic, diuretic, antispasmodic activities. It is also used for in gastrointestinal, gynecological, eye diseases, skin injuries and in some cases of burns. The plant is rich in many pharmaceutical active ingredients like flavonoids, carotenoids, glycosides and sterols (Norman and Max, 2001).

Sonchus asper (L) Hill used in various human disorder including wounds and burns, cough, bronchitis, asthma gastrointestinal infection, inflammation, diabetes, cardiac dysfunction, kidney, liver disorders dysfunction in humans, jaundice and cancer. *S. asper* contains flavonoids, glycosides, ascorbic acid and carotenoids, possess antioxidant, anticancer, and anti-inflammatory properties (Rahmat et al., 2010).

Due to the potent biological activities and their wide use in pharmaceutical industries/herbal industries, and by local practitioners for various diseases, the present initiatives have been made to evaluate the crude phytochemicals content, heavy metals and inorganic constituents and to provide a scientific data base which

will play a vital role for the consumers.

MATERIALS AND METHODS

The quantitative determination of crude phytochemicals (alkaloids, flavonoids and saponins), heavy metals and inorganic constituents were determined in *C. officinale* and *S. asper* collected from Peshawar, Nowshera and Kohat area. The phytochemicals were determined quantitatively using the literature methods; heavy metals were determined by atomic absorption spectrophotometer and inorganic constituents by the available methods (AOAC, 2000).

RESULTS AND DISCUSSION

As shown in Table 1, the concentration of alkaloids was found high in *C. officinale* collected from Nowshera area while low concentration was found in *C. officinale* collected from Kohat region (11.56%). The concentration of alkaloids in *S. asper* collected from Peshawar region was low (10.68) and its concentration in other samples were found (11.86 and 12.25%) from Kohat and Nowshera area.

The concentration of flavonoids in Table 2 showed variable amount of flavonoids. For example, a relatively high concentration of flavonoids was found in the *C. officinale* collected from Kohat region (16.77%), followed by its concentration (15.77%) in the samples collected from nowshehra area. The flavonoids contents in *S. asper* sample obtained from peshawar region is (14.03%)

Table 3. The Quantitative determination of the Saponins in *C. officinale* and *S. Asper*.

S/N	Plant code	Plant taken in grams	Weight of the crude sample(g)	%
1	NCO	10	0.6	6
2	KCO	10	0.8	8
3	PSA	10	0.5	5
4	NSA	10	0.7	7
5	KSA	10	0.6	6

Table 4. The concentration in (mg/kg) of inorganic constituents in the *C. officinale* and *S. Asper*.

Parameter	KCO	NCO	KSA	NSA	PSA
Alkalinity	3.2	6	3.2	5.76	3.2
Biocarbonates	160	300	160	288	160
Carbonates	Nil	Nil	Nil	Nil	Nil
Calcium	22	80	22	78	22
Magnesium	40	36.5	42.53	79.95	37.67
Hardness	240	350	230	524	210
Sodium	220	81	230	131	226
Potassium	2	1	2	1	2
Sulphates	270	150	260	418	265
Nitrates	0.63	2.38	0.63	7.3	0.63
Fluorine	0.7	69.978	0.66	0.47	0.7
T D S	887	288	895	942	886

while in other samples from nowshehra and Kohat area, the flavonoids contents were 13.96 and 13.51%, respectively.

Table 3 presents the results of saponins in which high concentration of crude saponins was found in the *C. officinale* obtained from Kohat region (8%). While equal concentration of saponins was recorded in *C. officinale* and *S. asper* collected from Nowshera and Kohat region (6%). *S. asper* collected from Peshawar has 7% of saponins while low concentration of saponins in *S. asper* was obtained from Peshawar region 5%. The yield of the crude phytochemicals obtained in the plant samples collected from different areas of N.W.F.P showed significantly differently amounts depending upon their environmental conditions in which these plants are grown. The result is of particular importance by comparing the effect of different environmental factors on the yield of their crude phytochemicals. Besides this, the alkaloids have analgesic, antispasmodic, antibacterial effects. Saponin has the property of precipitating and colligating red blood cells. Saponin also foams in aqueous solutions, hemolytic activity, cholesterol binding properties and bitterness, flavonoid on the other hand, are potent water soluble antioxidants properties which prevent oxidative cell damage, have strong anti-cancer

activity.

The concentration in mg/kg of inorganic constituents in selected medicinal plants are appended in Table 4, the plants collected from different areas possesses different amount of inorganic constituents. The following inorganic constituents were studied in all the plant samples including HCO_3 , CO_3 Na, Mg, K, F, Cl, Fe and TDS.

All the inorganic constituents are believed to play a vital role both in plants and animals. Some initiates growth while the others activate enzymes. Sodium is major component of our bodies; its small amount in the body cause hypertension. Calcium strengthens the bones. Its importance almost becomes double during pregnancy. Calcium also helps in blood coagulation.

Potassium activates some type of enzymes. The iron is necessary for the hemoglobin. Similarly all the inorganic substances are essential in one or another way. So the importance of the inorganic constituents cannot be neglected at any level.

Bicarbonates

Table 5 revealed that the concentration of bicarbonates were found high in *C. officinale* from Nowshera (300 mg/kg) followed by *S. asper* of Nowshera (288 mg/kg).

Table 5. The Physical and aesthetic parameters of *C. Officinale* and *S. Asper*.

Physical parameter	KCO	NCO	KSA	NSA	PSA
pH	8.5	7.4	8.2	8.4	8.3
EC μ S/cm	1674	1512	1620	1480	1474
Trub.	0	0	0	0	0
Color	C.less	C.less	C.less	C.less	C.less
Taste	T.less	T.less	T.less	T.less	T.less
Odor	O.less	O.less	O.less	O.less	O.less

C.less = Colorless; T.less = tasteless, O.less = odourless.

Bicarbonates concentration was found equal in *S. asper* samples collected from the Peshawar and Kohat region.

Carbonates

The carbonates were found under detection limits in all the plant samples.

Calcium

The relatively higher concentration of calcium was found in *C. officinale* from Nowshera 80 mg/kg followed by the *S. asper* from Peshawar (78 mg/kg). The slightly low concentration of calcium was found in rest of the plant samples for example 22 mg/kg. Calcium is very important constituent used in the synthesis of new cell walls and normal extra cellular calcium concentrations are necessary for blood coagulation. 5 meq/L of calcium is present in the blood plasma of human.

Magnesium

Significantly high concentration (79.974 mg/kg) of magnesium was found in *S. asper* from Nowshera with respect to the other two samples from Peshawar and Kohat region (37.66 and 42.52 mg/kg). Low concentration was found in *C. officinale* from Nowshera region (36.5 mg/kg); *C. officinale* from Kohat yielded 40 mg/kg of magnesium.

Sodium

The relatively high concentration of sodium was found in *S. asper* from Kohat region (about 230 mg/kg) while the concentration in the other two samples were low (PSA, 226 mg/kg and NSA, 131 mg/kg) while the rest of the plant samples of *C. officinale* from Kohat and Nowshera showed different concentrations of sodium for example, 220 and 81 mg/kg.

Potassium

The concentration of potassium was also determined which confirmed that 2 mg/kg of K was found in *S. asper* samples collected from Peshawar and Kohat while 1 mg was found in the rest of the plant samples from other areas.

Sulphates

It can be seen from the Table 3 that high concentration of sulphates were found in *S. asper* from Nowshera region (418 mg/kg). The slightly different results, that is, 260 and 265 mg/kg were observed in *S. asper* from Peshawar and Kohat region. Low concentration of sulphates (150 mg/kg) was found in the *C. officinale* from Nowshera.

Nitrates

In Table 4, nitrates concentration was maximum in *S. asper* obtained from Nowshera region (7.3 mg/kg). The concentration of nitrates was 2.38 mg/kg in *C. officinale* collected from Nowshera. The minimum concentration of nitrates (0.66 mg/kg) was found in the remaining plant samples.

High concentration of nitrate and especially nitrite in drinking-water may cause Methaemo-globinaemia. Groups especially susceptible to methaemoglobin formation are young infants, children and pregnant women.

Fluoride

The concentration of fluoride was 69.078 mg/kg found in the *C. officinale* from Nowshera region. While the concentration of fluoride was recorded ranging in the samples from (0.6 mg/kg to 0.7 mg/kg). The Low concentration of fluorine was observed in the *S. asper* from Peshawar area. Fluoride levels between 0.5 and 1 mg/L provide substantial protection against dental caries. However, for fluoride, the margin between beneficial and

Table 6. The concentration of heavy metals in *C. officinale* and *S. asper*.

Heavy metal	NCA	NSA	KSA	PSA	KCO
Iron	34.15	11.25	12.00	36.25	21.25
Zinc	3.10	1.05	1.60	1.75	1.85
Nickel	n.d	n.d	n.d	n.d	n.d
Lead	n.d	n.d	n.d	n.d	n.d
Copper	0.65	0.20	0.30	0.40	0.25
Cadmium	n.d	n.d	n.d	n.d	n.d
Silver	0.55	0.55	0.55	0.55	0.55

n.d = Not detected.

toxic effects is rather small. Excessive exposure may lead to adverse health effects varying from mottling of teeth to crippling skeletal fluorosis.

Total dissolved solids

So far as the samples of *C. officinale* are concerned, high TDS (887) was observed in samples obtained from Kohat region while in other samples, the TDS was low that is, 288. In *S.asper* samples the trend was NSA>KSA>PSA. Total dissolved solids (TDS) in drinking-water consist mainly of chloride, sulphates, carbonates, sodium, magnesium and calcium. Excessive dissolved solids in drinking-water may lead to objectionable taste, and corrosion or encrustation in water distribution system. At concentrations greater than approximately 1000 mg/L, the taste of water becomes increasingly unpalatable. As far as health aspects are concerned, there is no evidence of adverse physiological reactions at TDS levels greater than 1000 mg/L, on the contrary, there are vague indications from epidemiological studies that high levels of certain salts (calcium and magnesium) may have beneficial health effects. It should be emphasized that the factor of acclimatization to TDS is particularly important. Many people enjoy highly mineralized waters containing more than 2000 mg/L of TDS.

The results of heavy metals in the studied plants are given in Table 6. In the present study, heavy metals like Pb, Cr, Zn, Cd, Cu and Ni were determined. The level of heavy metals in plants is conditionally depending upon geochemical characteristics of the soil and the ability to selectively accumulate some of these elements. Plants easily assimilate through the roots such elements, which dissolve in water and occur in ionic forms. It is believed that the great majority of heavy metals act as key components of essential enzyme systems or other proteins, e.g. the haemoprotein.

Arsenic, Cd, Hg, Pb, etc are highly toxic for the human bio-system even at very low levels of intake and they are

usually present in plants because of the increasing industrialization and associated pollution of the biosphere, taken up from the soil, water, fertilizer, pesticide treatment and other industrial and anthropogenic operations.

Iron

The plant samples collected from the study areas of NWFP have different concentrations of the iron. The high concentration of iron was found in the *S. asper* collected from peshawar region (36.25 mg/kg) while in other two samples from Nowshera and Kohat, the iron concentration was 11.25 and 12 mg/kg. In case of *C.officinale* samples, the order was NCO > KCO.

Although Fe is an essential element for plants, animals and humans, its deficiency can cause problems in metabolism. For e.g. iron is constituents of active site of various enzymes reductases and hydrogenases, most frequently being associated with sulphur containing ligands . Fe together with hemoglobin and ferodoxin play a vital role in metabolism. Deficiency of Fe in plants produces chlorosis.

Zinc

From Table 6, it can be seen that high concentration of zinc was found in *C. officinale* collected from Nowshera (3.10 mg/kg) and Kohat region (1.85 mg/kg) .The slightly different amount of zinc was observed in the rest of the samples of *S. asper*. They were in the order PSA>KSA.>NSA. Zn is another essential and enzymatic metal for both plants and animals growth. The dietary limit of zinc is 100 ppm.

Nickel

The concentration of nickel in plant samples were found

under detection limits. EPA has recommended daily intake of Ni should be less than 1 mg while the permissible range by FAO/WHO is 0.17 to 0.7 mg per day. It is a necessary trace element for some microbes, plants and invertebrates.

Lead

From the Table 6, it can be seen that the concentration of lead was found in traces or its concentration was beyond the detection limits. The maximum acceptable concentration of lead in foodstuffs is around 1 mg/kg. FDA's "tolerable" level for lead in children's daily diet load is 6 mcg.

Copper

In case of copper, high contents of Cu were found in *C. officinale* from Nowshera (0.65 mg/kg). While other *C. officinale* sample revealed (0.25 mg/kg). The *S. asper* sample obtained from Peshawar, Kohat and Nowshera showed results as 0.40, 0.30 and 0.20, respectively. According to the World Health Organization (WHO) recommended acceptable oral intake of Cu was 20 µg /kg body weight per day. The difference in plants samples may be due to the environmental conditions in which the plants were grown and the available soil and water.

Cadmium

Table 6 showed that cadmium content was found beyond detection limits. Typical daily national intake of Cd through diet is 20 to 40 mcg Cd/day. The critical level of Cd in soil is 3 to 5 mg/kg. EPA recommended 5 parts per billion (ppb) or 0.005 parts per million (ppm) of cadmium in drinking water. WHO sets a daily intake limit of 70 mcg/day.

Silver

So far as Ag is concerned, the concentration of silver was found equal in all the plant samples (0.55 mg/kg). EPA recommends concentration in drinking water not to exceed 0.10 parts per billion (ppb).

Chromium

The concentration of chromium was also found under detection limits. According to EPA, the acceptable concentration of Cr is 0.1 ppm while FDA recommends a

concentration not to exceed 1 milligram per liter (1 ppm) in bottled water.

Conclusion

Keeping in view the importance of both the medicinal plants and their use by local practitioners for different types of health diseases, the recent study is therefore an initiative for the scientific evaluation of the crude phytochemical contents including alkaloids, flavonoids, saponins, macro and micronutrients and for the awareness of the local peoples using such types of either crude phytochemicals or the whole plant. This study is also providing a scientific database for the study of compounds.

ACKNOWLEDGEMENT

The authors wish to thank Higher Education Commission, Government of Pakistan for providing financial support for the current study under the National Research Program for Universities (NRPU).

REFERENCES

- Chawira AN, Warhurst DC, Robinson BL, Peters W (1987). The effect of combinations of qinghaosu (artemisinin) with standard antimalarial drugs in the suppressive treatment of malaria in mice. *Trans. R. Soc. Trop. Med. Hyg.*, 81: 554-558.
- Editorial Boards (2003). *PDR for Herbal Medicines*. 2nd edn, Montvale, Thomson-Medical Economics, pp. 497-500.
- Hackett CJ (2003). Innate immune activation as a broad-spectrum biodefense strategy. *Prospects and research challenges. J Allergy. Clin. Immunol.*, 112: 686-694.
- Hussain J, Khan FU, Riazullah, Muhammad Z, Rehman N, Shinwari ZK, Khan IH, Zohaib M, Din I, Hussain SM (2011). Nutrient evaluation and elemental analysis of four selected medicinal plants of KPK Pakistan. *Pak. J. Bot.*, 43(1): 427-434.
- Krishnaraju AV, Rao TVN, Sundararaju D (2005). Assessment of bioactivity of Indian medicinal plants using Brine shrimp (*Artemia salina*) lethality assay. *Int. J. Appl. Sci. Eng.*, 2: 125-134.
- Kirtikar KR, Major Basu BD (1993). *Indian Medicinal Plants*. Vol. II, Deharadun, India, International Book Distributor, pp. 1413-1414.
- Mushtaq A, Rahmatullah Q, Muhammad A, Mir AK, Muhammad Z (2009). Traditional herbal remedies used for the treatment of diabetes from district Attock (Pakistan). *Pak. J. Bot.*, 41(6): 2777-2782.
- Norman GB, Max W (2001). *Herbal Drugs & Phytopharmaceuticals*. 2nd edn, Germany, MedPharm GmbH Scientific Publishers. pp. 118-120.
- Paredes A, Hasegawa M, Prieto F, Mendez J, Rodríguez-Ortega M, Rodríguez-Ortega M (2001). Biological activity of *Guatteria cardoniana* fractions. *J. Ethnol.*, 78: 129-132.
- Phillipson JD, Wright CW (1991). Can ethnopharmacology contribute to the development of antimalarial agents? *J. Ethnol.*, 32: 155-165.
- Rahmat AK, Muhammad RK, Sumaira S, Jasia B (2010). Antimicrobial and phytotoxic screening of various fractions of *S. asper* Afr. *J. Biol.*, 9 (25): 3883-3887.
- The Wealth of India, Raw Materials (1992). *A Dictionary of Indian Raw Material and Industrial Products*. New Delhi, Publications and Information Directorate CSIR, (3): 55-58.