African Journal of Pharmacy and Pharmacology Vol. 6(3), pp. 144-147, 22 January, 2012
Available online at http://www.academicjournals.org/AJPP
DOI: 10.5897/AJPP11.410
ISSN 1996-0816 ©2012 Academic Journals

Full Length Research Paper

Evaluation of inorganic profile and anti-nutritional values of *Cocculus hirsutus*

Farhat Ali Khan[1], Asfandyar Khan[4], Muneeb Ur Rehman Khattak[2]*, Naseem ullah[1], Muhammad Zahoor[3] and Syed Muhammad Mukaram Shah[3]

[1]Department of Pharmacy, Sarhad University of Science and Information Technology, Peshawar, Pakistan.
[2]Department of Chemistry, Kohat University of Science and Technology, Kohat, Pakistan.
[3]Department of Chemistry, Malakand University, Malakand, Pakistan.
[4]Department of Chemistry, Hazara University, Mansehra, Pakistan.

Accepted 21 December, 2011

As the plants are directly in contact with air, water and soil, the constituents of these sources might contaminate the plants. Useful elements, such as Na, K, Ca, Mg, P, Fe, Cu and Co are also usually present in plants which help in good health. *Cocculus hirsutus,* belongs to the family Menispermaceae and it is a perennial climber mainly used for the treatment of diabetes, laxative, demulcent, anti inflammatory, analgesic and also used as tonic and diuretic. Looking at the medicinal values of *C. hirsutus*, the present study was aimed to estimate the inorganic constituents and anti-nutritional value for safe use. Essential bulk metal ions were observed to be dominant where potassium was highest while phosphorus was lowest. The decreasing order of essential trace metals ions was Fe>Cu>Zn>Ni>Co>Cr. Among the antinutritional components, oxalic acid was 25.32 mg/100 g, while that of phytic acid was 0.280 mg/100 g, on fresh weight basis.

Key words: *Cocculus hirsutus*, elements, oxalic acid, phytic acid.

INTRODUCTION

A mineral is solid or liquid homogenous inorganic substance, which is a direct product of nature. The number of elements believed to be importance in biological material has been increased and at the present time there are about 60 elements identified to be useful for both plants and animals, these elements are largely of low atomic weights (Mark et al., 2000). Those elements that our body needs greater in quantity than 100 mg/day are known as principal or macronutrients. There are seven essential elements: Calcium, Magnesium, Sodium, Potassium, Phosphorus, Sulfur and Chlorine. They constitute 60 to 80% of all the inorganic material in the body. Those elements that we need in amount less than 100 mg/day are known as trace elements. These elements occur in living tissues in small amounts. They may be subdivided into three groups essential, possibly essential and nonessential, according to their dietary requirements in higher animals (SChumacher et al., 1991). Oxalic acid is the chemical compound with the formula $H_2C_2O_4$. This dicarboxylic acid is better described with the formula HOOCCOOH. It is relatively strong organic acid, being about 10,000 times stronger than acetic acid. The dianion known as oxalate is also a reducing agent as well as a ligand in coordination chemistry. Many metal ions form insoluble precipitates with oxalate, a prominent example being Ca oxalate, which is the primary constituent of the most common kind of kidney stone. Oxalic acid and oxalate are abundantly present in many plants, most notably fat hen, sour grass and sorrel. The affinity of divalent metal ions is sometimes reflected in their tendency to form insoluble precipitates. Thus, in the body, oxalic acid also combines with metals ions, such as Ca^{2+}, Fe^{2+} and Mg^{2+} to deposit crystals of the corresponding oxalates, which irritate the gut and kidney. Because, it binds vital nutrients, such as

*Corresponding author. E-mail: muneeb_chem@yahoo.com. Tel: +923009031731.

calcium, long-term consumption of foods high in oxalic acid can be problematic (WHO, 1992). Phytic acid known as inositol hexaphosphate or phytate when in salt form is the principal storage form of phosphorous in many plant tissues, especially bran, nuts and seeds. Phosphorus in this form is generally not bioavailable to non-ruminant animals because they lack the digestive enzyme, phytase, required to separate phosphorus from the phytate molecule. Phytic acid is a strong chelator of important minerals, such as calcium, magnesium, iron and can therefore contribute to mineral deficiencies in people whose diets rely on these foods for their minerals intake such as those in developing countries (Agarwal and Paridhavi, 2007). *Cocculus hirsutus* commonly known as broom creeper belongs to the family Menispermaceae and it is a perennial climber (2 to 3 m). Its leaves are 3 to 5 veined from the base and are variable in shape. The older leaves are often distinctly 3 to 5 lobed, while the younger leaves are oblong ovate to somewhat obovate. Its leaves are covered in yellowish velvety hairs, and its apex is found with a small sharp mucro. Flowers are in axillary clusters and they are unisexual, while its sepals are densely hairy. Its fruit is somewhat ellipsoid (4 mm in diameter), fleshy and purple blue when ripe (Kirtikar and Basu, 1981). *C. hirsutus* is wildly distributed in Africa and Asia tropical, particularly Indian subcontinent (Indian-Bihar, Gujrat, Orissa, Rajasthan, Tamil Nadu and Pakistan), Western Asia **(**Iran) and Asia temperate (Saudi Arabia, Yemen). The aqueous extract of leaves of *C. hirsutus* show better antidiabetic activity, while the roots have been mentioned to be used as laxative, demulcent, antiinflammatory, analgesic and also as tonic and diuretic (Glanze, 1996).

MATERIALS AND METHODS

Plant species, *C. hirsutus,* was collected from Bara Khyber Agency (FATA) of Pakistan. Standard botanical field collection methodology was used for the collection of plant sample. Specific sample was obtained with the aid of interpreters and field guides. Multiple specimens of the plant species were collected. Genus and species were confirmed by comparison with herbarium reference materials at Department of Botany, University of Peshawar, Pakistan. Before analysis, sample was inspected for non-plant materials and any visible dirt and insect parts were removed. A sample composite (leaves) was analysed for minerals and antinutrients (oxalic acid and phytic acid).

Wet digestion method

The samples were prepared for determination of trace elements on atomic absorption spectrophotometer. After the pretreatment to remove organic matter, dry digestion method was adopted. The analytical grade reagents of high purity nitric acid (HNO_3), perchloric acid ($HClO_4$) and double deionized water were used. For the dry digestion, nitric acid and perchloric acid were used in the ratio of 2:1

Finely ground plant material of 0.5 g was taken in to a Kjeldhal flask. To this, two volumes of concentrated nitric acid was added and digested on low heat in a digesting chamber, until nitric acid fumes ceased. After adding one volume of perchloric acid, it was heated gently and then vigorously. Thereafter, the content was evaporated until the volume was reduced to 1 to 2 ml, but not to dryness. After cooling the flask, it was diluted up to 100 ml with distilled water and filtered through Whatman No. 1 filter paper. The solution was analyzed for the determination of trace elements on atomic absorption spectrometer (Salrito et al., 2001; Bassett et al., 1978; Okwu and Morah, 2004).

Determination of phosphorous

The sample solution should not contain more than 0.1 mg of phosphorous as the orthophosphate in 25 ml and should be neutral. 25 ml of digested material was transferred to a 50 ml pyrex volumetric flask. 50 ml of the molybdate solution was added, followed by 2.0 ml of the hydrazine sulphate solution, and was diluted to mark with distilled water, and mixed well. The flask was immersed in a boiling water bath for 10 min, removed and was cooled rapidly. The flask was shaked, the volume was adjusted, and the optical density was measured at 830 µm against either deionized water or a reagent blank. The calibration curve was constructed, using the standard phosphate solution, in the usual manner (Lucas and Markakas, 1975).

Determination of oxalic acid

0.5 g of sample consisting of oxalic acid was dissolved in a measured amount (25 ml) of 1 N sulfuric acid. A 2 ml aliquot of the sample containing from 0.100 to 1.00 mg/ml of oxalic acid were placed in a test tube. 2 ml of each of the pure oxalic acid standard solutions were placed in test tubes. Blank was prepared by placing 2 ml of 1 N sulfuric acid in a test tube. 2 ml of indole reagent was added to each test tube, the reagent was allowed to run down the side of the tube to minimize heat development. It was then left for 60 s. Each test tube was mixed thoroughly and was placed in a water bath at 80 to 90°C for 45 min. These were cooled and absorbance was measured in a photometer with wavelength at 525 mp, setting the blank, consisting of indole reagent and 1N sulfuric acid, at zero. After complete color development, the colored solutions may be quantitatively diluted with distilled water or 1 N sulfuric acid for photometric comparison without loss of accuracy (Anita et al., 2006; Okwu and Morah, 2004).

Determination of phytic acid

N/2 HCl was used for the extraction of phytic acid from plant material. 25 ml of N/2 HCl was used for each gram. 25 ml of the solution for analysis (neutral or just acid to litmus) was pipetted into a dry pyrex boiling tube and 5 ml of standard $FeCl_3$.HCl solution was added. The tube was covered by a glass bulb and was heated in a rack in a boiling water bath for 15 min with the level of the water above that of the contents of the tube. The ferric phytate was separated as an ivory colored flocculent precipitate. After cooling for 15 min in a bath of cold water, the contents of the tube were made up to 50 ml with 6 N HCl. The contents of the flask were filtered into a dry boiling tube through a dry 90 cm no. 31 Whatman filter. 20 ml of the filtrate was transferred into a 90 ml flask (in tubes where the amount of phytic acid appears on the upper limit determinable by the method of small excess of iron that makes it preferable to use 30 ml, instead of 20 ml of this filtrate). 5 ml of N/2 HCl and 10 ml of 10% KCNS solutions were added, the solution was made up to 50 ml with N/6 HCl, mixed and compared without Delay in a colorimeter with a standard (Lucas and Markakas, 1975).

Table 1. Minerals composition of *C. hirsutus* on dry weight basis.

S/N	Mineral	Quantity (mg/g)	Wave length (nm)
	Macro mineral		
1	Potassium	54.7	766.50
2	Sodium	51.3	589.0
3	Calcium	39.0	422.7
4	Magnesium	4.8	285.2
5	Phosphorus	0.19	660.0
6	K/(Ca + Mg)	12.5	-
7	Ca/Oxalic acid	6.5	-
	Micro mineral		
8	Iron	4.5	248.3
9	Copper	3.4	213.9
10	Zinc	0.3	324.8
11	Nickel	0.08	232.0
12	Cobalt	0.08	357.9
13	Chromium	0.08	357.9

RESULTS AND DISCUSSION

Mineral composition

Unlike other nutrients, mineral elements cannot be synthesized by living organism. Different types of mineral elements are required as structural of body organs, body fluids and tissues as electrolytes, and also required for normal functioning of enzyme and hormone systems (Mark et al., 2000).

The mineral content of *C. hirsutus* is given in mg/g in Table 1. This plant is a good source of both micro and macro minerals. Though, this plant cannot be considered as a sole source of macro and micro elements, but this can be considered as a potential source for providing a reasonable amount of the elements in the diet and otherwise.

Among macro minerals, concentration of potassium was found to be higher than other macro minerals (5.47 mg/g). Gupta et al., (1989) reported 343 mg of potassium. This plant has the high concentration of potassium as compared to sodium. Gupta et al. (1989) reported 126 mg of sodium and 9.4 mg of calcium. The concentration of magnesium and phosphorus was found to be 4.8 g/100 g and 0.19 g/100 g and Gupta et al. (1989) found 35 mg of magnesium and 18 mg of phosphorus in this plant. The ratio of K/Ca + Mg was found to be 1.25 g/100 g and the ratio of Ca/oxalic acid was found to be 0.65 g/100 g, respectively. The absorption of minerals other than calcium would also benefit from lower concentration or absence of phytates, oxalates and fiber. K content and K/(Ca + Mg) in mulberry fruits were markedly lower than in grass. Grass tetany is one of the important diseases during grazing season. The disease has generally been related to low Mg, high K and high K/(Ca + Mg) equivalent ratios in the forage (Golden, 1982; Anderson and Garry, 1981). Gross (1973) reported that generally accepted values of less than 0.2% Mg, more than 2.5% K and K/(Ca + Mg) equivalent ratios greater than 2.2 could cause forage to be tetany prone. It is apparent that mulberry fruits have good mineral component to prevent grass tetany (Karlen et al., 1978). The increasing order of micro minerals in *C. hirsutus* was Fe>Cu>Ni>Co>Cr. The values of Fe, Zn, Cu and Cr reported by Gupta et al. (1989) are 9.86, 0.55, 0.22 and 0.059 mg, respectively.

Antinutrients composition

Phytic acid known as inositol hexakis phosphate or phytate when in salt form is the principal storage form of phosphorous in many plant tissues, especially bran and seeds. Phytic acid is a strong chelator of important minerals, such as calcium, magnesium and iron, and can therefore contribute to mineral deficiencies. The dianion known as oxalate is a reducing agent as well as a ligand in coordination chemistry. Many metal ions form insoluble precipitates with oxalate, a prominent example being Ca oxalate, which is the primary constituent of the most common kind of kidney stone (Anita et al., 2006).

Oxalic acid and phytic acid content of *C. hirsutus* was also determined on both fresh and dry weight basis as reported in Tables 2 and 3. On fresh weight basis, oxalic and phytic acid content was found to be 0.60 and 0.006%, respectively. The ratio of Ca/oxalic acid was 0.65 mg/100, respectively. On dry weight basis, oxalic

Table 2. Oxalic acid and phytic acid contents of *C. hirsutus* on fresh weight basis.

Oxalic acid (mg/g)	Oxalic acid (%)	Ca/oxalic acid ratio	Phytic acid (mg/g)	Phytic acid (%)
6.0	0.60	0.65	0.06	0.006

Table 3. Oxalic acid and phytic acid content of *C. hirsutus* on dry weight basis.

Oxalic acid (mg/g)	Oxalic acid (%)	Ca/Oxalic acid ratio	Phytic acid (mg/100 g)	Phytic acid (%)
25.32	2.53	0.65	0.28	0.028

and phytic acid content was 2.53 and 0.028%, while the ratio of the Ca/oxalic acid was 0.65 mg/100.

REFERENCES

Agarwal SS, Paridhavi M (2007). Herbal drug technology. 1st ed. Universities Press (India) Private Limited Hyderabad, pp. 1-3.

Anderson JW, Garry PJ (1981). Human Nutrition, Clinical and Biochemical Aspects. Washington DC: American Association for Clinical Chemistry pp.132-137.

Anita BS, Akpan EJ, Okon AA, Umoren IU (2006). Nutritive and anti-nutritive evaluation of sweet potato (*Ipomoea batatas*) leaves. Pakistani J. Nutr., 5 (2): 166-168.

Bassett J, Denney RC, Jeffery MJ (1978). Vogals text book of quantitative inorganic analysis, The English Language book society, New York, pp. 325-326, 335-338.

Glanze WD (1996). Mosby Medical Encyclopedia. Revised Edition St. Louis MO: C.V. Mosby.

Golden MH (1982). Trace elements in human nutrition, Hum. Clin. Nutr., 6: 448-455.

Gupta RP, Pandey SP, Tripathi BP (1989). Soil properties and availability of nutrient elements in mandarin growing areas of Dhankuta District. PAC technical paper no. 113. Pakhribas Agricultural Centre, Dhankuta. p. 23.

Karlen DL, Ellis RJR, Whitney DA, Grunes DL (1978). Influences of soil moisture by wheat with respect to grass tetany. J. Agron., 70: 919-921.

Kirtikar KR, Basu BD (1981). Indian Medicinal Plants. Reprinted ed. Vol. 1. L. M. Basu, Allahabad, 80- 82: 86-90.

Lucas GM, Markakas P (1975). Phytic acid and other phosphorus compounds of bean (*phase olus vulgaris*). J. Agric. Educ. Chem., 23: 13-15.

Mark PE, Michael JB, Jianwei WH, Christopher DG (2000). Plants as a natural source concentrated mineral nutritional supplements. Food Chem., 71: 181-188.

Okwu DE, Morah FN (2004). Mineral and nutritive value of Dennettia tripetala fruits. Fruits, 59(6): 437-442.

Salrito SR, Kazi TG, Kazi GH (2001). Trace elements in two varieties of indigenous Medicinal plants *Caratharanthus roseus*. Sciences, 1(2): 74-77.

Schumacher MM, Bosque A. Domingo JL, Corbella J (1991). Dietary intake of lead and cadmium from foods in Tarragona Province, Spain. B Environ. Contam Tox., 46: 320-328.

WHO (1992). Expert committee on specification for pharmaceuticals preparation. WHO technical report series 823, Report Geneva WHO, 32: 44-52, 75-76.