REPORT

Comprehensive nutrients analysis of rhizomes of *Polygonatum verticillatum*

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Abstract: The current study was undertaken to estimate the concentration of micronutrients (Zn, Fe, Pb, Cu, Ni, Cd, Cr, Co, Sb and Mn), macronutrients (Na, Ca and K) and essential life nutrients (proteins, fats, carbohydrates and ascorbic acid) along with ash, fiber and moisture contents. Atomic absorption spectrophotometer was employed for the analysis of micronutrients while flame photometry for macronutrients. For proximate analysis (proteins, fats, carbohydrates, ash, fibers and moisture), Association of Official Analytical Chemists methods (AOAC) were used and titration method for ascorbic acid determination. It is evident from the results that the crude extract and its fractions accumulate significant concentrations of both micro and macro nutrients. The significant quantities of essential life nutrients like proteins, fats, carbohydrates and ascorbic acid along with ash, fiber and moisture contents were also found in extracts. It is concluded that the extracts of rhizomes accumulated significant quantities of life indispensible nutrients and validated the ethnobotanical uses of the plant as tonic and energizer.

Keywords: *Polygonatum verticillatum*, Rhizomes, micro and macro nutrients, proximate and ascorbic acid analysis.

INTRODUCTION

Minerals are entailed for the normal physiological functioning of human body. Metals being non-biodegradable have the tendency to persist for longer period in both aquatic and terrestrial environments (Boularbah *et al*., 2006). The minerals essential to human nutrition are accumulated in different parts of plants which is the function of its uptake capacity and intracellular binding states (Clemens *et al*., 2002). In human, trace elements play a pivotal role both as preventive and as curative against various diseases (En *et al*., 2003). Proteins, fats, carbohydrates and vitamins are indispensable life nutrients. They contribute to caloric content of the daily diet.

In global perspective, malnutrition is an international challenge for health concern authorities, devastating in almost all communities of the world. Alarmingly, it has been observed in all ages (Lee and Berthelot, 2010). In Pakistan, large population of the country is confronting challenge of malnutrition (Hall and Kirby, 2010). It is therefore crucial to augment production of nutritious diets by exploiting all the accessible resources. In this regard, an enormous work has been done in developing new chemical and biological methods for the production of high caloric foods and feeds (Khan *et al*., 1994).

P. verticillatum [L.] All. (Nooreallam) has been used as analgesic, antipyretic, anti-inflammatory, diuretic, urinary tract infections, tonic, energizer and sex stimulant etc. (Ballabh *et al*., 2008; Khan *et al*., 2010). In our recent studies, we have provided scientific rationale to some of these uses (Khan *et al*., 2010; Saeed *et al*., 2010a; Saeed 2010b; Khan *et al*., 2011a; Khan *et al*., 2011b; Khan *et al*., 2012a; Khan et al., 2012b). While considering the folk uses of the rhizomes of the plant as tonic and energizer, the present study was aimed to analyze the crude extract of the rhizomes and its subsequent solvent fractions for various micro and macro nutrients, life essential components like proteins, fats, carbohydrates and ascorbic acid using modern sophisticated techniques.

MATERIALS AND METHODS

Plant materials

P. verticillatum [L.] All. was collected from district Swat, KPK, Pakistan, in July-Aug 2007. The botanical identity of the plant material was done by the Taxonomy Department of PCSIR Laboratories, Peshawar and a specimen with catalogue No: 9970 (PES) was deposited in the herbarium of PCSIR Laboratories Peshawar.

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Extraction and fractionation

The air-shade dried rhizomes of the *P. verticillatum* (8 kg) were ground to fine powder. The powdered material was extracted by maceration with methanol at room temperature for 14 days with occasional shaking (Khan *et al*., 2009). The methanol extracted residue was filtered off with a muslin cloth and the filtrate was concentrated under vacuum at low temperature (40°C) using rotary evaporator, yielded a dark greenish semisolid material (2200 g, 27.50% w/w). The crude methanol extract (1.6 kg) was dissolved in distilled water and sequentially fractionated with *n*-hexane, chloroform, ethyl acetate and *n*-butanol, yielding hexane (258 g, 16.13% w/w), chloroform (219 g, 13.69% w/w), ethyl acetate (226 g, 14.13% w/w), *n*-butanol (265 g, 16.56% w/w), and aqueous (501 g, 31.31% w/w).

Chemicals/ reagents

Different reagents exercised in the analysis were of analytical scale include nitric acid, perchloric acid; l-ascorbic acid, potassium iodide, oxalic acid, sulphuric acid, starch standard test metals, Fe, Cu, Zn, Co, Cr, Cd, Sb, Pb, Mn, Ni, Na, K and Ca (Sigma Aldrich). Dilutions were prepared with deionzed water (Deionizer B 114) and or distilled water.

Micro and macro nutrients analysis

Test sample (1 g) was taken in conical flask and 10 ml of concentrated HNO_3 (67%) was added and kept overnight (12 h) at room temperature followed by 4 ml of $HClO_4$ (67%). The resulting solution was concentrated on hotplate at 60 ^{0}C until a clear solution of approximately 1 ml was left. After cooling, the solution was supplemented with deionized/double distilled water, filtered through Whatman (# 42) filter paper. Latter on final volume (100 ml) was made with deionized water served as stock solution (Saeed *et al*., 2010c; Saeed *et al*., 2011). The sample was then analyzed by flame atomic absorption spectrophotometer (Polarized Zeeman Hitachi 2000) and flame photometer (Jenway PFP7, UK). The materials of all the reference metals were obtained from Merck (Darmstadt, Germany). Stock solutions of standards test metals (Fe, Cu, Zn, Co, Cr, Cd, Sb, Pb, Mn, Ni, Na, K and Ca) containing 1000 ppm of each metal, were used. Appropriate dilution of each metal from stock solutions were prepared for the construction of standard calibration curve.

Proximate analysis

For proximate analysis, standard official protocol was followed (AOAC, 1990). Moisture and ash were quantified using weight difference method. Fiber contents were determined from the loss in weight of the crucible on ignition. Proteins were determined by micro Kjeldahl method. It included sample digestion, distillation followed by titration in order to calculate the nitrogen value, the precursor for proteins. The nitrogen value was converted to protein by multiplying a factor of 6.25. Carbohydrates were obtained when the sum of the percentages of moisture, ash, proteins and fibers were subtracted from 100.

Ascorbic acid analysis

For ascorbic acid (vitamin C) determination, standard method was adopted (Okeri and Alonge, 2006). Briefly, 10 g of fresh and air dried rhizomes were taken in a mortar and 30 ml of 0.03 M H_2SO_4, 20 ml CO_2-free distilled water and 0.5 g of oxalic acid were added. For approximately 20 min, the mixture was stirred rapidly and then filtered. 10 ml of the filtrate was quickly titrated to the end-point with the standardized 0.05 M iodine solution. Starch mucilage (5%) was used as indicator. The titrations were repeated thrice and blank determinations were also performed followed the above procedure but using 10 ml of CO_2-free distilled water instead of the filtrate.

Contamination control

Standard experimental conditions were followed throughout the analysis. In order to prevent contamination, all the glassware were soaked in chromic acid for 24 h and thoroughly washed with deionized water and or distilled water. They were dried in oven and stored in dust free environment without touching their inside.

STATISTICAL ANALYSIS

Data are shown as Mean ± SEM (*n*=3). Statistical software, GraphPad program (GraphPAD, San Diego, CA, USA) was used for this purpose.

RESULTS

The results on the estimation of micronutrients such as Zn, Fe, Cu, Ni, Cd, Cr, Co, Pb, Sb and Mn, using atomic absorption spectrophotometer are listed in table 1. The extracts accumulated significant quantities of Zn, Fe, Cu, Cr, Mn and Ni. However, Pb, Sb, Cd and Co were not detected. The outcomes of our analysis of extracts on macronutrients are illustrated in table 2. Results demonstrated that marked concentrations of macro-nutrients were exhibited by the extracts of rhizomes of the plant. i.e. Ca (90-190 ppm) K (1250-1600 ppm) and Na (60-450 ppm).

Results on the proximate analysis of rhizomes of the plant are presented in table 3. Prominent concentrations of proteins (19.7%), fats (14.7%) and carbohydrates (17.5%) were found. Similarly, prominent quantities of fibers (33.1%) were also observed. Regarding results of ascorbic acid (table 3), profound concentration of ascorbic acid (170 mg/100 g) was accumulated in rhizomes of plant.

Table 1: Micronutrients status of the crude extract and subsequent solvent fractions of *Polygonatum verticillatum*

Micronutrients						
Minerals	Crude	Hexane	Chloroform	Ethyl acetate	Butanol	Aqueous
Zn	50.11±0.58	48.80±0.03	47.10±0.03	40.40±0.02	49.20±0.06	37.70±0.03
Cu	48.80±0.06	21.51±0.29	40.40±0.02	44.00±0.57	41.40±0.03	48.20±0.03
Cr	1.64±0.02	01±00	01±00	1.40±0.01	02±0.06	0.60±0.01
Fe	191.65±0.58	144±2.31	137.2±0.05	124.80±0.03	89.20±0.03	141.60±1.55
Mn	143.55±0.58	08±0.29	10.80±0.01	7.40±0.02	05±0.17	7.60±0.03
Ni	5.02±0.01	0.6±0.01	1.10±0.03	0.30±00	1.40±0.01	0.3±0.01
Pb	ND	ND	ND	ND	ND	ND
Sb	ND	ND	ND	ND	ND	ND
Cd	ND	ND	ND	ND	ND	ND
Co	ND	ND	ND	ND	ND	ND

ND = Not detected. Data are expressed as the Mean ± SEM (n = 3)

Table 2: Micronutrients status of the crude extract and subsequent solvent fractions of *Polygonatum verticillatum*

Macronutrients						
Ca	133.32±0.18	90±1.15	180±1.15	170±0.56	190±1.16	120±1.16
Na	234.83±0.02	280±1.73	450±1.73	450±1.15	280±5.77	60±1.15
K	1267.96±0.02	1320±5.77	1250±2.29	1250±2.31	1570±5.77	1600±5.77

Data are expressed as the Mean ± SEM (n = 3)

Table 3: Proximate values of the rhizomes of the plant

Entry	Component	Percentage composition
1	Ash	9.8 ± 0.04
2	Moisture	5.2 ± 0.02
3	Fibers	33.1 ± 0.06
4	Fats	14.7 ± 0.15
5	Proteins	19.7 ± 0.06
6	Carbohydrates	17.5 ± 0.35
7	Ascorbic (Vit- C)	170 ± 0.20 mg/100g

Data are expressed as Mean ± SEM of three findings.

DISCUSSION

Institute of Medicine (IOM) has recommended the daily intake for all the essential nutrients in order to maintain normal physiological functioning. (IOM, 2001). Profound concentrations in extracts of the plant reflect its importance as iron supplement. Zinc is found in all body tissues mostly in muscle and bone (85%), 11% in the skin and the liver (Tapiero and Tew, 2003). Zinc deficiency in human mostly occurs in pregnancy (Moser-Veillon, 1990) and is characterized by growth failure, impaired parturition (dystocia), neuropathy, decreased cyclic food intake, diarrhoea, dermatitis, hair loss, bleeding tendency, hypotension, seizers and hypothermia. Acute Zinc toxicity causes abdominal pain, nausea, vomiting and diarrhoea. Chronic exposure of Zinc elicits copper deficiency (Saeed *et al*., 2010a). Rhizomes of the plants exhibited significant concentration of Zn. The various fractions of the plant exhibited notable amount of Cu. Systemic decrease in Cu levels causes cellular iron deficiency (Saeed *et al*., 2010a). The recommended dietary allowance (RDA) for Cu is 340-900 µg/day (IOM, 2001).

Mn intoxication is responsible for Parkinsonism which usually becomes progressive and irreversible, may led permanent damage of neurologic structures (Wang and Du, 2008). Plant extracts accumulated significant concentrations of Mn within recommended values. Cr is provisionally considered to be a nutrient because of its metabolic role (Emsley, 2001). It plays important role in the synthesis of fatty acids and cholesterols, metabolism of carbohydrates, proteins, lipids and has also been proved that it facilitates the action of insulin (Saeed *et al*., 2010a). Therefore, Cr based supplements are used for weight loss (Lukaski *et al*., 2007). The results obtained in the present analysis showed prominent accumulation of Cr in extracts.

Nickel is mostly present in the pancreas and plays an important role in the production of insulin. Nickel deficiency is responsible for liver disorders (Saeed *et al*., 2010a). Our data revealed that the plant accumulated reasonable concentration of Ni and could be a potential therapeutic agent for the management diabetic agents.

The results of macronutrients in our study of the crude extract and subsequent solvent fractions of rhizomes of *P. verticillatum* are presented in table 2. The most common dietary source of sodium is common table salt (NaCl). It has got the prime role in the maintenance of normal physiology in all living organisms (Morris *et al*., 2008).

We observed marked concentration of Na in various fractions of the plant. The concentration of K ions is most frequently associated with regulation of action potentials and intercellular signaling in electrically active cells. There is no international limit which reflects the concentration of potassium in plants. However, the average intake of Potassium is 2300 mg/day for adult women and 3100 mg/day for adult men (Saeed *et al*., 2010a).

Ca is one of the most important macronutrients mostly obtained from various dietary sources. Ca intoxication is rarer but when occur is characterized by hypercalcemia, which causes constipation, kidney stones, appetite loss, nausea, vomiting, abdominal pain, confusion, seizures, and even coma (Saeed *et al*., 2010a). Based on our results, extract of rhizomes appeared as a rich source of dietary Ca.

Nutrients and disease are counterparts. Proteins, fats and carbohydrates are the integral parts of daily diet. Variety of life threatening complications has been reported from their deficiencies especially protein (Kate and Meer, 2008). Vitamins supplements exhibited outstanding antioxidant potential and prevent or delay the commencement of various degenerative disorders such as cancer, rheumatoid arthritis, drug-associated toxicity, and postischemic reoxygenation injury, as well as in the degenerative processes associated with aging (Jacob and Sotoudeh, 2002). Rhizomes of the plant exhibited profound concentrations of proteins, fats, carbohydrates and Vitamin C therefore could be used as a potential natural source of essential nutrients.

CONCLUSION

It can be concluded on the base of our results that the rhizomes of *P. verticillatum* is an excellent natural source of micro and macro nutrients as well as primary life related nutrients such as proteins, fats, carbohydrates and ascorbic acid. Using modern technologies, our study validated the ethnobotanical use of the rhizomes of the plant as tonic and energizer.

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