Research Article

Evaluation of Proximate, Mineral and Amino Acid Compositions of *Boerhavia procumbens* and Roots for Nutraceutical Applications

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Abstract | *Boerhavia procumbens* is a Pakistani medicinal herb, used in the treatment of various ailments, including asthma, cough and jaundice. The aim of the study was to determine and compare the proximate, mineral, amino acid compositions of the aerial parts and roots of *B. procumbens*. The result of carbohydrate (75.56±0.046%) of aerial parts was found higher as compared to the roots (31.22±0.05%) while protein (37.46±0.02%) and organic matter (21.25±0.03%) was recorded higher in roots than the aerial parts of *B. procumbens*. Calcium (309.73±0.06mg/100g), potassium (274.59±0.08mg/100g) and iron (32.58±0.05mg/100g) were found in highest amounts in aerial parts as compared to the roots. The Cadmium metal was not detected while lead was found in permissible limits in both the aerial parts and roots of *B. procumbens*. The aspartic acid (31.25±0.08g/100g), Glutamic acid (25.27±0.06 g/100g), Alanine (12.74±0.12g/100g) was found in higher concentration in aerial parts than roots while some essential amino acid, Tryptophan (6.23±0.06g/100g), Arginine (3.49±0.08 g/100g), Phenylalanine (1.77±0.08 g/100g) were recorded higher in roots as compared to the aerial parts of *B. procumbens*. It is concluded that aerial parts may serve as a supplement for human diet as well as fodder for livestock. Detailed studies on amino acid of roots are recommended to determine and isolate compounds responsible for the specific therapeutic properties.

Received | January 24, 2017; **Accepted** | March 29, 2017; **Published** | April 13, 2017
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Citation | Khalil, A.W. and Iqbal, Z. 2017. Evaluation of proximate, mineral and amino acid compositions of *boerhavia procumbens* and roots for nutraceutical applications. *Sarhad Journal of Agriculture,* 33(2): 206-211.
DOI | http://dx.doi.org/10.17582/journal.sja/2017/33.2.206.211
Keywords | *B. procumbens*, Aerial parts, Amino acids, Proximate composition, Minerals

Introduction

Boerhavia procumbens (family Nyctagenaceae) commonly known as spreading hogweed/ pig weed in English and locally named as Biskhapra / Jangli itsit. *B. procumbens* is an herbaceous, widespread tropical herb, occurs plentiful as a weed and found in the plains, waste places in clay loam soil. *B. procumbens* is located in south Asia, India and Pakistan, tropical Africa and America. In Pakistan it is found in Sind, Baluchistan, Attock, Rawalpindi, Jhelum, Multan, Dir, Swat, Malakand, Hazara, Abbottabad, Peshawar, Kohat, and Kurram agency (Nasir, 1977). It has been traditionally used for the treatment of jaundice, enlargement of spleen, dyspepsia, anti-stress agent, abdominal pain whereas its roots have been reported for curing of jaundice disease (Sankaranarayanan et al., 2010; Hussain et al., 2010; Shaheen et al., 2012). Some of the other biological activities of *B. procumbens* previously investigated include anti-asthmatic (Bokhari and Khan, 2015), anti-inflammatory and anti-oxidant (Bokhari et al., 2015). Bokhari et al. (2015) has reported a multitude of phytochemical constituents such as alkaloids, phenolics, flavonoids and cardiac glycosides in plant of *B. Procumbens*. Several studies show that *B. procumbens* plant contained vital nutrients such as carbohydrates, protein, lipids, macro and micro minerals. Some of essential and non-essential amino acid has been found in the Genus of *Boerhavia*. (Ujowundu et al., 2008; Miralles et al., 1988). Literature survey shows that the therapeutic efficacy of herbal plant is on the basis of proximate and elemental compositions.

Proximate analyses of medicinal plants play a vital role in evaluating their nutritional significance (Pandey et al., 2006). Some studies reported that *B. procumbens* can be used as food along with its medicinal benefits and assessing of nutritional importance can help to understand the value of this plant (Shinwari et al., 2015). World health organization also emphasizes the need and significance of determining proximate and elemental composition of the herbal plants for drug's standardization. (Niranjan and Kanaki, 2008). Moreover, these mineral serve also as structural components of tissues and function in cellular and basal metabolism and water and acid-base balance (Macrae et al., 1993a; Smith, 1988; Nielsen, 1984). On the other hand, some of the essential microelements induce toxic effects in human beings, when their intake concentration is higher in herbal plants. Furthermore, the level of toxic elements is also increasing in medicinal plants due to industrialization and environmental pollution. Therefore, the toxic metal concentrations in both medicinal plant and their finished products should be evaluated and checked with the permissible limits.

The analysis of proximate and minerals composition in herbal plant is not sufficient to know their therapeutic potential as it may also depend on the protein and amino acid compositions. Amino acids are essential in the synthesis of proteins and act as precursors in the formation of secondary metabolism compounds (Pérez-Urria et al., 2009).

Keeping in view the importance of *B. procumbens* as a natural and traditional healer, it was felt necessary to analyze proximate, minerals, amino acids composition of aerial parts and roots, which may provide a scientific data base for the researchers.

Materials and Methods

Collection of plant material

The *B. procumbens* plant was collected during flowering season in the month of June from botanical garden of PCSIR Labs. Complex Peshawar, Pakistan and identified by the taxonomist at the Department of Botany, University of Peshawar, Pakistan. A voucher specimen (Catalogue No. Bot. 20077) has been deposited in the herbarium of University of Peshawar for future reference.

Processing of the plant

The plant was washed with tap-water and dried under shade at room temperature for a period of 7-10 days. The dried plant was separated into aerial parts and roots and pulverized separately in a Willy mill. The pulverized samples were used for the analysis of chemical composition.

Proximate composition

Proximate analysis of aerial parts and roots of *B. procumbens* were carried out in triplicate for measuring moisture, organic matter, lipids, protein, and fiber contents by methods as described by (AOAC, 2005). Ash content (organic matter) was analyzed by dry ashing method, whereas the moisture contents were conducted through an oven at a temperature of 105°C. The crude fat was extracted through Soxhlet apparatus in organic solvent (petroleum ether) at 40-60°C and the crude fiber was investigated by titrimetric method. Auto-Kjeldhal equipment was used for the assessment of nitrogen, while the total protein content was calculated as N × Factor (100/16: 6.25). The amount of nitrogen free extract (NFE) (carbohydrate) was calculated by the difference between the weight of the sample taken and the sum of its moisture content, organic matter, crude lipid, crude protein, and fibre as described by (James, 1995).

Percent digestible carbohydrate was calculated as 100 – (% moisture + % crude fibre + % organic matter, + % crude lipid + % crude protein).

Minerals composition

Dry ashing: This process was carried out according to AOAC (2000) with slight modifications in the procedure as mentioned by (Hussain et al., 2005). Powder of aerial parts and roots were placed in a crucible for charring process. The process was accomplished on a low flame inside a fume hood till all the organic matter decomposed completely. The carbon-free sample was then ashed in a muffle furnace (MTI Corporation, U.S.A) at 600ºC for 5 hours. The grey-white ash was dissolved in 2 mL concentrated HNO_3 (Merck) and heated on a low flame for one minute. Afterward, it was cooled and filtered through Whatman No. 42 filter paper and volume was made up with triple distilled water (witeg, Germany) in a 50 mL volumetric flask. A blank sample was also prepared using similar experimental procedure. The mineral analysis was performed in three replicates in the study.

Instrumentation: The concentration of heavy metals including Fe, Cu, Zn, Cr, Cd, Pb, and Ni was determined through flame Atomic Absorption Spectrometer (AAS) (Hitachi Polarized Z-8000 Japan) and the macro-elements like Na, K and Ca were analyzed by Flame Photometer (Jenway PFP7, UK). The instrumental operations were controlled for each element on both techniques AAS and Flame Photometer, which are summarized in Table 1.

Table 1: *Instrumental condition for working of micro- and macro-minerals*

Micro minerals	Lamp current (mA)	Wavelength (nm)	Silt width (nm)
Cd	7.5	228.8	1.3
Cr	7.5	359.3	1.3
Cu	7.5	324.8	1.3
Fe	10	248.3	0.2
Ni	10	232	0.2
Pb	7.5	283.3	1.3
Zn	10	213.8	1.3
Macro minerals	**Filter used**		
Ca	Ca filter	422.7	0.7
K	K filter	766.5	2.0
Na	Na filter	589	0.2

Amino acid composition

Sample Preparation: Normal hydrolysis were carried out by the method, described in HPLC Amino Acid Analysis system application data book (Shimadzu). Samples of aerial parts and roots were taken separately in digestion tube, with the addition of HCl (6N; 2ml) and kept for 18-24 hours at 110ºC under vacuum. The hydrolyzed samples were then washed with water and evaporated to dryness on a rotary evaporator in vacuo, at 70ºC. Final volume was made up at 10ml with deionized water. Samples were then filtered through syringe filter (0.22 micron) and diluted with buffer A solution in a sample vial, prior to injection (20 µL) into the amino acid analyzer.

Instrumental conditions: Column: Shim-Pack Amino- Na column (4.6 mm, I.D × 100 mm); Ammonia trap: Shim-Pack ISC-30/SO504 Na; Injector volume: 20 µL; Mobile phase: Amino Acids Mobile Phase Kit (Na type), gradient elution; Mobile phase: Flow rate: 0.4ml/min; Column temperature: 60ºC; Reaction reagent flow rate: 0.2 mL/min, each; Reaction temp: 60˚C; Detection: RF-20Axs (Ex. 350 nm, Em. 450 nm); Cell temp: 25˚C.

Statistical analysis

In this study, all the results were expressed as mean values of three replicates ± standard deviation. The significance difference was determined using Statistix 8.1 computer software (Statistix, 2006) by performing one-way (ANOVA). The comparisons among means were carried out using Least Significant Difference (LSD) test at 5% level of significance (Steel et al., 1997).

Results and Discussion

Proximate composition

Proximate composition of aerial parts and roots of *B. procumbens* is presented in Table 2. The level of moisture (9.29±0.05%) of aerial parts was higher as compared to roots (7.36±0.04%). The protein and organic matter levels (37.46±0.02 %, 21.25±0.03 %) of roots were significantly higher ($p<0.05$) than aerial parts of *B. procumbens* (6.78±0.05 %, 2.18±0.04 %). There were no significant differences ($p>0.05$) in carbohydrate and crude fat contents of aerial parts and roots. The high concentration of crude fiber (4.35±0.03 %) was recorded in aerial parts as compared to roots.

Table 2: *Proximate composition (%) of the aerial parts and roots of B. procumbens*

Compositions	Aerial parts	Roots
Moisture	9.29 ± 0.05^a	7.36 ± 0.04^b
Organic matter	2.18 ± 0.04^b	21.25 ± 0.03^a
Crude protein	6.78 ± 0.05^b	37.46 ± 0.02^a
Crude fiber	4.35 ± 0.03^a	0.96 ± 0.02^b
Crude fat	1.84 ± 0.05^a	1.75 ± 0.03^a
Carbohydrate	75.56 ± 0.046^b	31.22 ± 0.05^a

Values are mean ± SD; n = 3. Means followed by different superscript in the same row are significantly different at (p<0.05).

The proximate analysis results indicated that the aerial parts and roots contained an appreciable amount of carbohydrate. It suggests that aerial parts of *B. procumbens* can be used as a source of energy which plays a major role in cellular metabolism (Mensah et al., 2008). The protein and organic matters contents were highest in the roots of *B. procumbens* that can contribute to the daily protein need of 21.44g for adults recommended by the National Research Council (NRC, 1975) whereas organic matters could be a good source of minerals. The high value of crude fiber was found in aerial parts of *B. procumbens* that may help in the prevention of chronic diseases such as cardiovascular diseases, cancer and diabetes mellitus (Bowman and Russell, 2001). In the present study, the concentration of carbohydrate and protein of the aerial parts and roots of *B. procumbens* were recorded higher than the aerial parts of *B. diffusa* as reported by Juna Beegum et al. (2014).

Table 3: *Minerals composition (mg/100g) of the aerial parts and roots of B. procumbens*

Minerals	Aerial parts	Roots
Na	30.29±0.06[a]	30.29±0.06[a]
Ca	309.73±0.06[a]	102.27±0.02[b]
K	274.59±0.08[a]	92.08±0.04[b]
Fe	32.58±0.05[b]	80.25±0.04[a]
Zn	1.75±0.07[b]	2.66±0.02[a]
Cu	0.63±0.05[b]	6.88±0.04[a]
Ni	0.19±0.07[a]	0.11±0.04[a]
Cr	0.17±0.06[b]	0.29±0.03[a]
Pb	0.023±0.05[a]	0.013±0.03[b]
Cd	ND	ND

ND: Not detected. Values are mean ± SD; n = 3. Means followed by different superscript in the same row are significantly different at (p<0.05).

Mineral composition

Mineral composition of aerial parts of *B. procumbens* and its roots are presented in Table 3. The value of sodium in aerial parts of *B. procumbens* and its roots were not significantly different (p>0.05). Calcium (309.73±0.06 mg/100g) and potassium contents (274.59±0.08 mg/100) were significantly higher (p<0.05) in aerial parts than its roots (102.27±0.02 mg/100g and 92.080±0.04mg/100g). The Iron content (80.247±0.04 mg/100g) of roots was higher as compared to the aerial parts (32.577±0.05 mg/100g). The cadmium metal was not detected while lead contents were found in permissible limits in aerial parts as well as in roots of *B. procumbens.*

The presence of these minerals may account for the ethnomedicinal use of aerial parts and roots of *B. procumbens* in the treatment of many infectious diseases. The highest calcium and potassium level in aerial parts suggest that this plant can be used for growth and maintenance of strong bones, muscular function, synthesis of enzymes, and normal physiological function of the body (Aliyu et al., 2008). The presence of the highest amount of iron in roots indicates that it can be used to improve the anaemic condition of a patient. The value of Na/K ratio of aerial parts and roots of *B. procumbens* were 0.11 and 0.32 which is less than the recommended value of 0.6 for hypertensive patients (Nieman et al., 1992). Therefore, the aerial parts and roots of *B. Procumbens* may have beneficial effects on hypertensive patients. In toxic metals, cadmium was not detected, whereas the concentration of lead was lower than the maximum permissible limit such as 0.3ppm and 10ppm respectively (WHO, 1998). The data of the toxic metals show that this plant is safe for human consumption and can be used in the preparation of herbal medicine. Abd EI-Salam et al. (2013) reported the value of iron and zinc in the different parts including roots, stem and leaves of *B. procumbens* was found higher than the results of our study. In the present study, the leaves of *B. procumbens* contained lower concentrations of sodium (18.37±0.046 mg/100g) and calcium (60.24±0.056 mg/100g) while potassium and iron were found higher as compared to the previous study reported by (Puranik et al., 2012).

Amino acid composition

The Essential and non-essential amino acid compositions of aerial parts and roots of *B. procumbens* are presented in Table 4. The values of Arginine (8.89±0.08 g/100g) and tryptophan (8.82±0.07 g/100g) were found almost similar in roots, while tryptophan contents were found higher than Arginin in aerial parts of *B. procumbens* (3.49±0.08 g/100g and 6.23±0.06). The histidine and leucine amino acids composition of aerial parts and roots were not found significantly different at value (p>0.05). The concentration of Lysine (4.77±0.09 g/100g) was higher in roots as compared to the aerial parts of *B. procumbens* (0.29±0.06 g/100g). On the other hand, the aspartic acid (30.87±0.050 g/100g), glutamic acid (25.23±0.040 g/100g) and alanine (12.89±0.040 g/100g) were found the maximum concentrated amino acids in aerial parts as compared to the roots of *B. procumbens* (26.18±0.07 g/100g, 17.66±0.08 g/100g and 7.83±0.10 g/100g). The proline amino acid was not detected in aerial parts while 0.77±0.06 g/100g was noted in the roots.

The most studied amino acids in the literature survey are glutamic and aspartic acid. Glutamic and aspartic acids are used in higher concentration for the stimulation of brain activities and maintain the cognitive functions of the brain (Leon, 1986; McEntee and Crook, 1993). In this study the aspartic acid and glutamic acid were recorded in higher concentration in aerial parts of *B. procumbens*. It is suggested that this plant can be used as neurotransmitters and stabilizing agent for brain. Other interesting amino acids such as tryptophan and arginine found in aerial parts of *B. procumbens are* involved in modulating neurological and immunological functions through multiple metabolites, including serotonin and melatonin. According to our knowledge and from the literature survey, there are no published data available regarding the analysis of amino acid profile in aerial parts and roots of *B. Procumbens.*

Table 4: *Amino acid composition (g/100g) of the aerial parts and roots of B. procumbens*

Amino acids		
Essential amino acid	**Aerial parts**	**Roots**
Arginine	3.49±0.08[b]	8.89±0.08[a]
Histidine	0.65±0.07[a]	0.65±0.07[a]
Leucine	0.75±0.08[a]	0.66±0.08[a]
Lysine	0.29±0.06[b]	4.77±0.09[a]
Methionine	1.23±0.04[b]	3.73±0.09[a]
Phenylalanine	1.77±0.08[b]	3.36±0.10[a]
Threonine	0.48±0.06[b]	3.52±0.07[a]
Tryptophan	6.23±0.06[b]	8.82±0.07[a]
Valine	3.60±0.08[a]	2.83±0.09[b]
Non-essential amino acid		
Aspartic acid	31.25±0.08[a]	26.18±0.07[b]
Glutamic acid	25.27±0.06[a]	17.66±0.08[b]
Alanine	12.74±0.12[a]	7.83±0.10[b]
Glycine	7.69±0.06[a]	5.65±0.07[b]
Proline	0.00±0.00[b]	0.77±0.06[a]
Tyrosine	0.81±0.06[a]	0.48±0.06[b]
Cysteine	0.31±0.07[b]	1.78±0.05[a]

Values are mean ± SD; n = 3. Means followed by different superscript in the same row are significantly different at (p<0.05).

Conclusion

It is concluded that aerial parts and roots of *B. procumbens* contains vital nutrients suggesting its use as a supplement for human diet and can be added in fodder for livestock. The aerial parts and roots may also be used for the treatment of many infectious diseases such as jaundice and hepatitis.

Authors Contribution

Dr. Zafar Iqbal has supervised Mr. Abdul Wajid Khalil in his PhD program and this article is a portion of his research project.

References

Abd EI-Salam, N.M., I. Hussain, R. Ullah, S. Ahmad and S. Khan. 2013. Essential oil and heavy metals analysis of *Boerhaavia procumbens*. Life Sci. J. 10 (7): 955-958.

Aliyu, A.B., A.M. Musa, J.A. Oshanimi, H.A. Ibrahim and A.O. Oyewale. 2008. Phytochemical analyses and mineral elements composition of some medicinal plant of north Nigeria. Niger. J. Pharm. Sci. (7): 119-25.

AOAC. 2005. Official methods of analysis (Association of official analytical chemists) 15th Ed. Washington D.C: 12-13.

AOAC. 2000. Official methods of Analysis International, 18th Ed. AOAC, Washington, D.C.

Bokhari, J. and M.R. Khan. 2015. Evaluation of anti-asthmatic and antioxidant potential of *Boerhavia procumbens* in toluene diisocyanate (TDI) treated rats. J. Ethnopharmacol. 172: 377-385. https://doi.org/10.1016/j.jep.2015.06.049

Bokhari, J., M. Rashid and K. Ihsan. 2015. Assessment of phytochemicals, antioxidant, and anti-inflammatory potential of *Boerhavia procumbens* Banks ex Roxb. Toxicol. Ind. Health.

Bowman, B. and M. Russell. 2001. Present knowledge in nutrition. Washington DC: International Life Sciences Institute.

Gupta, A. and N. Yadav. 2013. Nutritional and antinutritional factors of underutilized *Boerhavia diffusa* leaves grown in Northern India. Asian J. Dairy Food Res. 32 (3): 246-248.

Hussain, I., L. Khan, T. Mehmood, I. Khan, W. Ullah and H. Khan. 2005. Effect of heavy metals on the growth and development of *Silybum marianum*, in various polluted areas of Peshawar, Pakistan. J. Chem. Soc. Pak. 27: 367-373.

James, C.S. 1995. Analytical chemistry of food.1st ed. Springer, New York. Page, 178. https://doi.org/10.1007/978-1-4615-2165-5

Juna, B.G.R., S. Suhara Beevy and V.S. Sugunan. 2014. Qualitative phytochemical screening and GC-MS analysis of *B. diffusa*. Int. J. Emerg. Technol. Adv. Eng. 4 (7): 318-324.

Leon, C. 1986. Amino acid in therapy. Thorson

Publisher Ltd., New York.
Macrae, R., R.K. Robinson and M.J. Sadler. 1993 a. Encyclopedia of food science, food technol. nutr. Academic Press Inc, San Diego, California (5).
McEntee, W.J. and T.H. Crook. 1993. Glutamate: Its role in learning, memory, and the aging brain. Psychopharmacol. 111: 391-401. https://doi.org/10.1007/BF02253527
Mensah, J.K., R.I. Okoli, J.O. Ohaju-Obodo, K. Eifediyi. 2008. Phytochemical, nutritional and medicinal properties of some leafy vegetables consumed by Edo people of Nigeria. Afr. J. Biotechnol. 7: 2304-9.
Miralles, J., K. Noba, A.T. Ba, E.M. Gaydou and J.M. Kornprobst. 1988. Chemotaxonomy in nyctagynaceae family: sterols and fatty acids from the leaves of three *Boerhaavia* species. Bio. Syst. Eco. 16: 475-478. https://doi.org/10.1016/0305-1978(88)90047-6
Nasir, Y.J. 1974. Nyctaginaceae. In: Nasir E, Ali SI (eds.) Flora of Pakistan 115:116.
Nielsen, F.H. 1984. New essential trace elements in life science. Annu. Rev. Nutr. 4: 21-41. https://doi.org/10.1146/annurev.nu.04.070184.000321
Nieman, D.C., D.E. Butterworth and C.N. Nieman. 1992. Nutrition W.M.C. Brown Publishers, Dubuque.
Niranjan, R.M. and S. Kanaki. 2008. Bioactive molecules and medicinal plant. 349-369.
NRC. 1975. National research council recommended dietary allowance, USA. Nut. Rev. 374-395.
Pandey, M., A.B. Abidi, S. Singh and R.P. Singh. 2006. Nutritional evaluation of leafy vegetable paratha. J. Hum. Ecol. 19 (2): 155-156.
Pérez-Urria, C.E. and A.A. García. 2009. Metabolismo secundario de plantas. REDUCA. 2: 119-145.
Puranik, V., D.K. Tripathi, D. Kaur and D.K. Chauhan. 2012. Nutritional evaluation of leaves of *B. diffusa* and *A. paniculata*: implications for nutraceautical applications. Int. J. Pharm. Bio Sci. 3(4): 315-321.
Sankaranarayanan, S., P. Bama, J. Ramachandran, P.T. Kalaichelvan, M. Deccaraman, M. Vijayalakshimi, R. Dhamotharan, B. Dananjeyan and S.S. Bama. 2010. Ethnobotanical study of medicinal plant used by traditional users in Villupuram district of Tamil Nadu, India. J. Medi. Plant Res. 4: 1089-110.
Shaheen, H., R. Qureshi, A. Akram and M. Gulfraz. 2012. Some important medicinal flora of Noorpur Thal, Khushab, Pakistan. Arch. Sci. 65:1661-1664.
Shinwari, Z.K., S. Malik, A.M. Karim, R. Faisal and M. Qaiser. 2015. Biological activities of commonly used medicinal plant from ghazi brotha, attock district. Pak. J. Bot. 47(1): 113-120.
Smith, K.T. 1988.Trace minerals in food, Marcel Dekker, New York .
Statistix. 2006. Statistix 8 user guide, version 1.0. Analytical Software, PO Box 12185, Tallahassee FL 32317 USA. Copyright © 2006 by Analytical Software.
Steel, R.G.D., J.H. Torrie and D.A. Dickey. 1997. Principles and procedures of statistics. A biometrical approach, 3rd Ed. McGraw Hill Book Co. Inc. New York USA. 172-177.
Ujowundu, C.O., C.U. Igwe, V.H.A. Enemor, L.A. Nwaogu and O.E Okafor. 2008. Nutritive and anti-nutritive properties of *B. diffusa* and *C. nudiflora* leaves. Pak. J. Nut. 7: 90-92. https://doi.org/10.3923/pjn.2008.90.92
WHO. 1998. Quality control methods for medicinal plant materials, WHO Geneva Switzerland.