Essential Oil and Heavy Metals Analysis of *Boerhaavia procumbens* L

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Abstract: Medicinal herbs have wide applications in the pharmaceutical industries and play an important role in the health maintenance of human. In the presence study concentrations were focused on the analysis of essential oil using GC-MS and heavy metals by Atomic Absorption Spectrophotometer in different parts including roots, stem and leaves of the herb *Boerhaavia procumebens.* From the essential oil of the roots of *B. procumebens,* 5 different volatile components including Sabinene, 21.28, 1o-Cymene, 40.61, 3-Thujanone, 10.58, 1-terpinene-4-ol, 5.38, Limonene oxide, cis, 22.14 and 8 heavy metals including Fe, Zn, Ni, Cr, Co, Cu, Pb, and Cd were determined in the roots, stem and leaves with different concentrations, however the level of concentration of all the studied metals were well below the maximum permissible level. For pharmaceutical consumption and for other health purposes the plant must be collected from environmentally pure area

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Introduction

Medicinal plants are of great importance to the health of individual and society. The medicinal value of these plants lies in some of chemical substances that produce a definite physiological action on the human body [1]. The use of plants materials to prevent and treat infectious disease successfully over the years has attracted the attention of scientist's world wide. The selection of crude plant extracts for screening programs is potentially more successful in initial steps than the pure compounds [2]. Even though hundreds of plant species have been tested for antimicrobial properties, the vast majority of them have not yet been evaluated [3]. Essential oils and plants extracts have been screened for their potential uses as alternative remedies for the treatment of many infection diseases [2]. Essential oils have been shown to possess anti-bacterial, anti-fungal, antiviral, insecticidal and anti-oxidant properties [4-5].

Medicinal plants are the raw material (in the form of whole plant, crude extracts or purified constituents) for many herbal formulations and popular supplements. The assimilation of heavy and toxic metals in the plants is evident [6-13] The metals have great tendency to accumulate in the living organisms including human beings if exposed for prolong period of time. The presence of such metals above the permissible levels can cause serious consequences. Thus both the deficiency and excess of essential micro-minerals such as Fe, Zn, and Cu are harmful to the human. Effects of toxic metals (Cd, Cr, Pb, As, Co, Ni etc) on human health and their interaction with essential trace elements may produce serious health problems. WHO recommends that medicinal plants which form the raw material for various medicines should be checked for the presence of different contaminants such as heavy/ toxic metals, pesticides, fungi and micro-organisms [14-16].

Boerhaavia procumbens L belongs to the family Nyctaginaceae, locally called Biskhapra / Jangli Its while in english it is called spreading hogweed. It is found in India, Africa, USA, and in Pakistan, it is found in Peshawar, Hazara, Thal to Kurram, Sind, Baluchistan, Multan, Attock, and Rawalpindi. Its colour is purplish red and grows in the month of January-August. This herb found in India is one of the prized ayurvedic herbs.

The chemical constituents it contains include alkaloids, tricontanol, hentriacontane, sitosterol, ursolic acid, flavone, glucose, fructose [17]. The herb is a diuretic that acts on the glomeruli of the kidney through increasing the heart-beats and strengthening and raising the blood pressure as a result. Medicinal applications include bitter, diaphoretic, diuretic, emetic, expectorant, laxative, rejuvenative, stomachic, root- anthelmintic, febrifuge, purgative

Due to the high demand and wide applications of the *B. procumbens* in the folk medicine pharmaceutical industries and different formulations for health benefits, the present initiative is therefore a part of investigation focusing on the determination of

essential oil composition and heavy metals in *B. procumbens*

Results and Discussion

From the GC-MS analysis of the *B. procumbens* roots essential 5 different volatile components were identified belonging to various groups Sabinene, o-Cymene, 3-Thujanone, 1-terpinene-4-ol, Limonene oxide, cis).

The concentration of five main volatile oil chemical components obtained from the roots of *B. procumbens* using GC-MS were Sabinene, 21.28, 1o-Cymene, 40.61, 3-Thujanone, 10.58, 1-terpinene-4-ol, 5.38 , Limonene oxide, cis, 22.14 **Table-1**.

Table-1. Quantitative results of the GC-MS of essential oil from the roots of *B. procumbens*

Peak No	Name	Area	Conc.%	R. Time
2	Sabinene	11031	21.281	10.32
4	o-Cymene	21051	40.611	12.69
5	3-Thujanone	5487	10.585	16.66
10	1-terpinene-4-ol	2789	5.380	19.39
11	Limonene oxide, cis	11478	22.143	26.42

The importance of the above compounds obtained from the roots of *B. procumbens* to the plants and human is yet to known. Some compounds of *B. procumbens* can play role as attractance and pheromones of some insects for example some branched hydrocarbons and aliphatic compounds.

The chemical composition of the essential oil has been noted to depend upon different geo-environmental conditions including climatic conditions, Seasonal, geographical, soil, irrigation, harvesting time and scientific distillation techniques [18], supporting the above results of the essential oil obtained from the hydro-distillation of the roots of *B. procumbens*.

Beside the essential oil analysis, the concentration of heavy metal in different parts including roots, stem and leaves of B. procumbens were analyzed and is depicted in the **Table-2**

Table-2 Concentration of Heavy metal in roots, stem and leaves of *B. procumbens*

Plant parts	Fe	Zn	Ni	Cr	Co	Cu	Pb	Cd
Roots	117.86	45.95	2.17	3.51	2.68	11.2	1.17	nd
Stem	82	23.63	0.53	3.27	1.62	6.25	2.18	nd
Leaves	97	21.80	1.56	3.15	1.28	3.97	2.83	nd

WHO permissible limits for Pb: 10 mg/kg (WHO 1998) FDA permissible limits for Cr, 120ug (RDI); Ni: 0.1 mg/l (FDA 1993. [12], 1999 [13]

Iron

From the **Table-2** it is very clear that the relatively high concentration of iron was found in root (117.82 ppm) followed by leaves 97 ppm, the stem (82 ppm). The iron contents was variable, the decreasing order is root > leaves > stem. Although Fe is an important and enzymatic metal necessary for the maintenance of health (both for pant and human), however its high concentration beyond permissible limit may bring undesirable consequences in human health.

Zinc

From **Table-2** the concentration of zinc was found in roots (45.95 mg/kg) followed by the stem (23.63 mg/kg), and leaves (21.8 mg/kg) of zinc. The zinc contents was variable, the order of concentration of Zn was root > stem > stem >.

Nickel

It can be seen from the **Table-2** that the Ni concentration of 2.17 mg/kg was recorded in the *roots*. The sequence was followed by the *stem and leaves* they showed (0.527 and 1.56 mg/kg, the lowest contents were observed in *stem* 1.56 mg/kg.

Chromium

Nearly same amount of chromium was detected in roots, stems and leaves (3.51, 3.27, 3.15 mg/kg respectively.

Lead

The concentration of Pb recorded in the roots, stem and leaves of *B. procumbens* were 1.17, 2.18, 2.83 mg/kg respectively. The relatively high concentration of Pb was found in the leaves **Table-2** may be due to the exposure of the plant to different environmental factors. Although Pb is highly toxic and has no health benefits, it concentration was found below the maximum permissible limits [16].

Copper

Copper is as an important enzymatic metal and plays vital role in the enzymatic reaction of human health. The **Table-2** shows that prominent amount of copper was detected in the root, stem and

leaves11.2, 6.25, 3.97 mg/kg respectively. High concentration was found in root followed by stem and then leaves.

Cadmium

No cadmium was found in root, stem, and leaves of the plant *B procumbent.*

Cobalt

From the **Table-2** it is clear that roots, stem and leaves of the herb *B. procumbens* contains nearly small amount of Co. The concentration in the roots is 2.68 mg/kg, stem yielded 1.62 mg/kg, and leaves yielded 1.28 mg/kg. Leaves amount of cobalt was found in leaves.

The results obtained from the analysis of the heavy metals in the different parts including roots, stem and leaves of *B. procumbens* are found well below the maximum permissible limits [14,16].

Experimental

The roots of *B. procumbens* were collected from Peshawar area of Khyber Pakhtunkhwa. The identity was checked by plant taxonomist Mr. Shahid Farooq, Senior Scientific Officer, at Pakistan Council for Scientific and Industrial Research Laboratories Peshawar. A voucher specimen was deposited at the Herbarium of Medicinal Botanic Centre PCSIR Peshawar.

Isolation of essential oil

The essential oil from the roots of *B. procumbens* was obtained by hydro-distillation for 2 h. The oil obtained was dried over anhydrous Na_2CO_3, filtered and stored at +4 °C until analysis.

Gas Chromatography-Mass Spectrometry

Reagents: Dichloromethane, HPLC grade

GC/MS analysis was performed using a Shimadzu Model QP 2010 plus, Injector temperature: 240 °C, Ion source temperature (EI): 240 °C, Interface Temperature: 240 °C, Pressure: 80 KPa, Carrier gas: helium, Split ratio: 1:50.

Column oven programming:

Rate (°C/min)	Temperature	Hold (minutes)
-	40	0
3	90	0
10	240	15

GC program time: 46.67 minutes total, Solvent cut time: 2.5 minutes, MS start time: 3 minutes, MS end time: 46 minutes, Acquisition mode: Scan M/Z: 40 – 500, Volume injected: 1 µl

Column Specifications

Length: 30 m, id: 0.25 mm, thickness: 0.25 µm, (95% Dimethyl-5% diphenyl polysilphenylene, DB-5MS, Agilent technologies, USA)

Sample preparation:

Dilute approximately 40 mg of oil samples, weighed accurately up to 0.1 mg, with 2 mL of dichloromethane and filtered through 0.45µm - membrane filter and injected 1µl to GC-MS using auto injection system.

The compounds were identified by comparison of their retention time with their retention indices (RI) (Martindale 2009), retention times (RT) and mass spectra with those of authentic samples and /or the NIST/NBS, NIST02, Wiley 575 spectra library and published literature.

For heavy meal determination, the following materials, chemicals and deionized double distilled water was used through out the experiment.

- Distilled Water
- Closed Bottles
- Powdered Plant Materials
- Crucibles
- Furnace and Desiccators
- Plastic Bottles
- Whatmann Filter Paper 42
- HNO_3 (65%, extra pure) from Rd H Laborchemikalien, GmbH &Co., Germany

Instrumentation

A Perkin Elmer Atomic Absorption Spectroscopy (AAS) (Model 3100) was used for measurement under the standard operation condition.

Determination of heavy metals

For heavy metals determinations, 1gm of each plant sample was taken in separate crucible for charring process (4 - 6 minute). After charring, ashing was performed.

Ashing

The plant samples in the crucibles were kept in furnace for 5 h at 600 ^oC. After ashing, the samples were cooled in the desiccator. Then 2.5 ml of 6M HNO_3 was added to each sample in order to dissolve the contents of the samples in the crucibles [8].

The samples were filtered and diluted each to 20 mL. The filtrates of each sample was secured in the plastic bottles. The samples were analyzed for

heavy metals using flameless Atomic Absorption Spectroscopy.

The analysis for heavy metals was done by flame atomic absorption spectrophotometer (Polarized Zeeman Hitachi-2000).

Cr-0.5 and 3.0 mgkg-1
Pb-0.2 and 1.0 mgkg-1
Cu 0.5 and 3.0
Cd -0.2 and 1.0
Fe 0.5 and 5.0
Ni -o.5and 4.0
Mn-0.5 and 2.50

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