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Antioxidant Activity and Trace Elements Profile of leaf extract of *Vetiveria zizanioides*

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Abstract:

Poaceae or Gramineae is a family of monocotyledonous flowering plants and some of the grass species have been proved to show therapeutic effect as they contain bioactive components called antioxidants which delay or prevent the oxidation of cellular substrates. These compounds show a wide spectrum of chemical and biological activities including radical scavenging activity. Trace elements are concern with environment and harmful to humans and animals when present in large amount, for this reason it is very important to investigate trace elements in plant samples in term of environmental pollution and particularly for plants with nutritional requirements. Essential (Fe, Cu, Zn etc) and non-essential elements (Pb, Ni, Cd etc) influence biochemical processes (metabolism) in the human body. This paper will discuss the total content of phenolic compounds present in the sample of *Vetiveria zizanioides* species of Poaceae family by Folin-Ciocalteu method. The paper will also highlight the total flavonoid content with the help of aluminium chloride colorimetric method. The results indicated that *Vetiveria* is a good natural source of antioxidant compounds for use in food and pharmaceutical industry. The mineral analysis showed the presence of Cr, Zn, Cu, Mn, Fe, Ca, K, Mg and Na in the leaves of the plants. Only the micro-minerals were present in traces while the macro-minerals were present high quantities as compared to the micro-minerals.

ABBREVIATIONS:

FW	Fresh weight
GAE	Gallic acid equivalent
RE	Rutin equivalent
TCP	Total phenolic content
TFC	Total flavonoid content
WEC	Water extract of Centipedegrass

INTRODUCTION:

The grasses – Poaceae (Gramineae) is the most important group of useful plants. Species from this family contain bioactive components including flavonoids (e.g. *C*glycosides of apigenin, luteolin, tricin), phenolic acids (e.g. ferulic acid, caffeic acid, *p*-hydroxybenzoic acid) and triterpenes, saponins, sterols. Some of the grass species have been proved to show therapeutic effect (e.g. strong antioxidant properties) and have been effective in the treatment of inflammations and sclerosis (1, 2). Natural antioxidants, particularly in fruits and vegetables have gained increasing interest among consumers and the scientific community because epidemiological studies have indicated that frequent consumption of natural antioxidants is associated with a lower risk of cardiovascular disease and cancer (3). The defensive effects of natural antioxidants in fruits and vegetables are related to three major groups: vitamins, phenolics, and carotenoids. Ascorbic acid and phenolics are known as hydrophilic antioxidants, while carotenoids are known as lipophilic antioxidants.

There is a great interest in macro and trace element composition of medicinal plants. It is believed that great majority of elements act as key components of essential enzymes for vital biochemical functions. Various minerals or inorganic nutrients are also required for maintaining the health of the body and accordingly are consumed as herbal health drinks or in orthodox medicines [6, 7]. The quantitative estimation of trace elements concentration is important for determining the effectiveness of medicinal plants in treating various diseases and also to understand the pharmacological action [5, 8].

Vetiveria zizanioides locally known as *Khus* grass is a perennial grass of Indian origin which is present throughout the plains, lower hills, on riverbanks and rich marshy soil across the country. Khus grass has variety of uses from household to therapeutic. Its roots are used as curtains, dried stems to make brooms and dried plant to make roofs. Mainly the roots are used for medicinal purposes which are both aromatic and have sedative effect on nervous system and is also used to treat intestinal parasites, fever, skin diseases and poisonous stings. Vetiver oil is obtained from roots of plant. It is used for flavouring Khus Sharbat and in making of cosmetics, perfumes, soaps etc. The contents of phenolic compounds and their antioxidant activity in selected grass species have been poorly investigated. Therefore, testing their antiradical properties is of interest, primarily in order to find new sources of natural antioxidants (4).

MATERIAL AND METHODS:

Sample preparations: The leaves of vetiveria were collected from local areas of Greater Noida, U.P. These leaves were shade dried and grinded to fine powder. 5 gm powder was weighed and added to 50 ml distilled water and 50 ml ethanol. These were kept for extraction for 48 hours at room temperature. After 48 hours the extracts were filtered and the final volume was made up to 50ml with respective solvents and was kept under refrigerated conditions till further use.

Determination of total phenolic content: The total content of phenolic compounds in the extracts was determined by using Folin-Ciocalteu reagent (Singleton and Rossi 1965). Briefly, 1ml of extracts were mixed with 1.8 ml of Folin-Ciocalteu reagent (10 fold diluted) and kept for 5 min at 25 degree Celsius. Later 1.2 ml of 15% Sodium Carbonate was added to the reaction mixture and kept for 90 min at room temperature and the absorbance was measured at 765nm. The concentration of the TPC was determined as mg of Gallic acid equivalents (GAE) per gm sample.

Determination of total flavonoid content: Total flavonoid content was determined by using the aluminium chloride colorimetric method (Chang et al. 2002). Briefly, 0.5 ml of the extract, 1.5 ml of methanol, 0.1 ml of 10% aluminium chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water were mixed for 5 min by vortexing. Reaction mixture was kept at RT for 30 min and the absorbance was measured at 415 nm. The results were expressed as mg of rutin equivalents (RE) per gm sample.

Determination of reducing power: The reducing power assay can be determined by the method Athukorala et al (2006). 1 ml sample of different concentration were taken. 1ml 0.2M sodium phosphate buffer pH 6.6 was added to each sample. 1ml of 1% potassium ferricyanide was added and incubated at 50^0C for 20 mins. 1ml of 10% TCA (W/V) was added and then the samples were centrifuged at 2000 rpm for 10 mins, 2.5ml of upper layer was taken and mixed with 2.5 ml DW. 0.5 ml of 0.1% fresh ferric chloride was added and then the readings for their optical density were taken at 700 nm.

Determination of Antioxidant Activity: Different concentration of butylated hydroxyl toluene (25-125µg/ml) in distilled water were prepared as standard. 0.3ml of extracts were taken in test tubes. Reagent was prepared by mixing 10ml of 0.6M sulphuric acid, 10ml of 28mM sodium phosphate, 10ml of 4mm ammonium molybdate into a beaker. 3ml reagent solution was added to the tubes. 0.3 ml reagent of methanol served as blank. All the tubes were incubated at 95ºC for 90 min. Tubes were cooled to room temperature. Optical density was measurement at 695nm using UV Spectrophotometer.

Elemental Analysis: An analytical portion of 5gm is decomposed with nitric acid and hydrogen peroxide in a high-pressure Teflon lined digestion vessel using microwave heating and a feedback program to control temperature and pressure. A 50 mL analytical solution is prepared from the digest. Analytical solutions are nebulized and aerosol is transported to plasma where desolvation and excitation occur. Characteristic atomic emission spectra are produced by radio frequency inductively coupled plasma. Spectra are dispersed by a grating spectrometer, and line intensities are measured with a light sensitive detector, a photomultiplier tube. Photocurrents are processed by a computer system. A background correction technique is used to compensate for variable background emission contribution to analyte signal.

Approximately 0.5g of each sample was acid digested using a mixture of HNO3 and HCl in a closed vessel microwave digestion system. After digestion, the samples were made up to volume (50mL) using ultra pure water. The standard calibration solutions, blank and rinse solution, were prepared in 1% (v/v) HNO3. The major elements (Na, Mg, P, S, K and Ca) were prepared at calibration concentration levels of 25, 50 and 100 mg·L-1 and the minor elements (balance of analytes) at concentrations of 25, 50 and 100 µg·L-1. Internal standard correction was applied, with Ga, Rh, and Ir at 20, 10 and 10µ·gL-1 respectively.

RESULTS:

Total phenolic content:

The variation of the total phenolic content over time for the leaf extract for various concentrations is presented in Table 1. Phenolic content is increasing with increasing concentration and it is observed that maximum concentration is observed at 0.1 gm/ml concentration. The Gallic acid standard was used and the results are expressed as Gallic Acid Equivalents (GAE).

S. No.	Sample conc. (gm/ml)	Phenolic content GAE (gm/gm)
1	0.04	1.5
2	0.06	2.04
3	0.08	2.56
4	0.1	3

Table 1: Total phenolic content in leaves of *Vetiveria zizanioides*

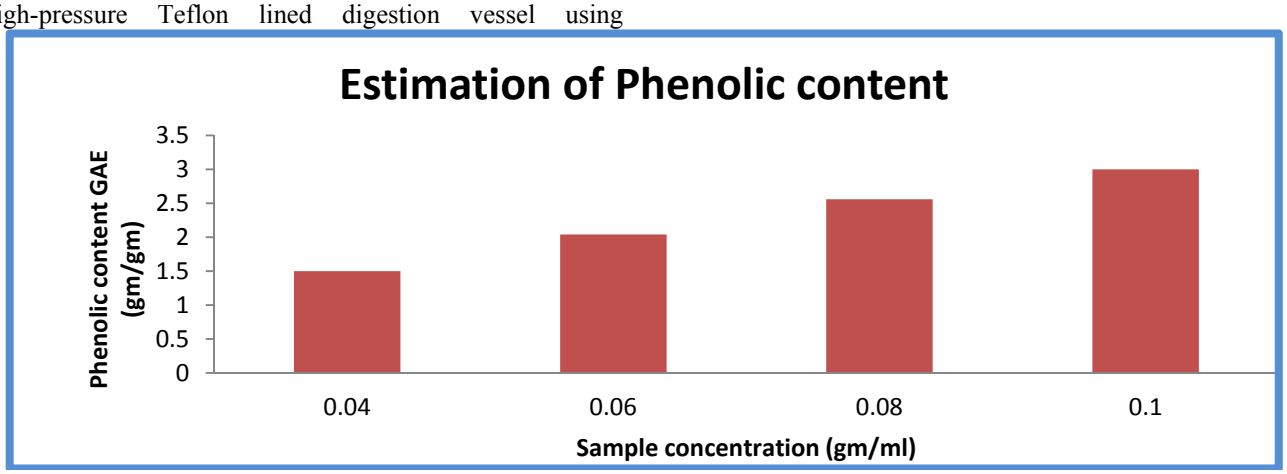

Fig 1: Total phenolic content in leaves of *Vetiveria zizanioides*

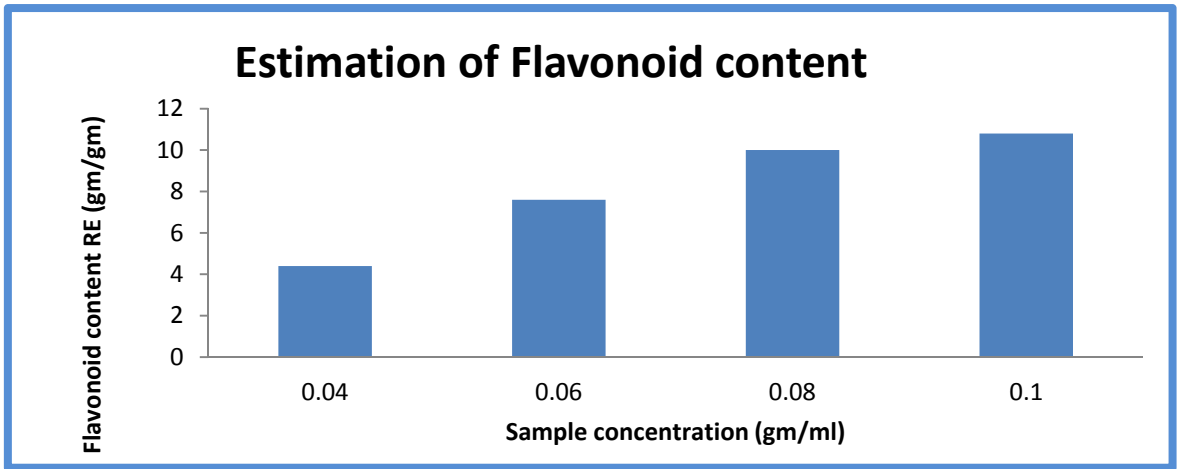

Fig 2: Total flavonoid content in leaves of *Vetiveria zizanioides*

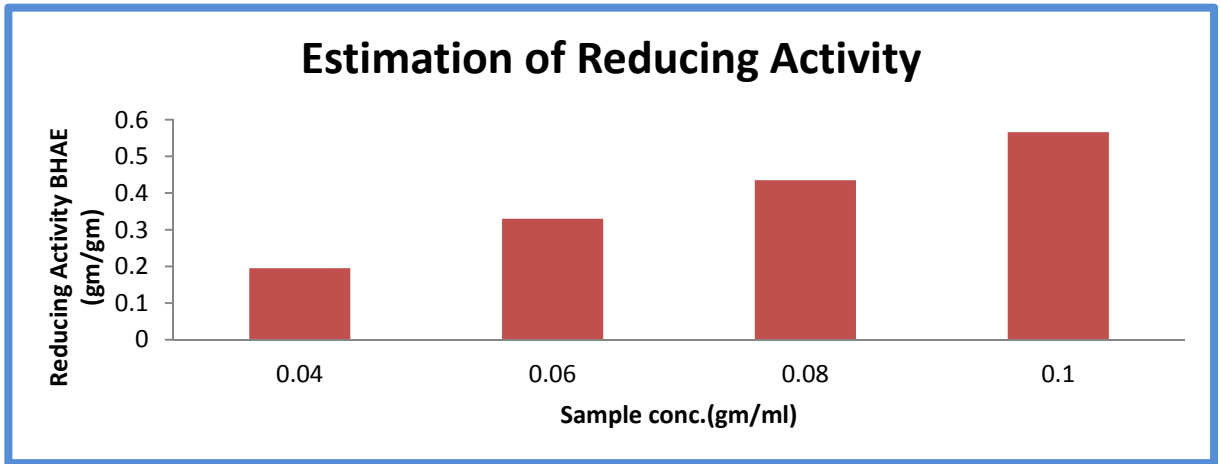

Fig 3: Reducing activity in leaves of *Vetiveria zizanioides*

Total flavonoid content:

The variation of the flavonoid content over time for Vetiveria leaf extract for various concentrations the samples is presented in Table 2. Rutin was used as standard and the Flavonoid content of the extract was expressed as Rutin Equivalent (RE) gm/gm FW. It is observed that the Total Flavonoid content (TFC) is concentration dependent and the maximum concentration of 10.8 RE gm/gm of FW is observed at 0.1 gm/ml concentration.

S. No.	Sample conc. (gm/ml)	Flavonoid content RE (gm/gm)
1	0.04	4.4
2	0.06	7.6
3	0.08	10
4	0.1	10.8

Table 2: Total flavonoid content in leaves of *Vetiveria zizanioides*

Reducing power:

The variation of the reducing activity over time for various concentrations of leaf extract is presented in Table 3. Reducing power is increasing with increasing concentration and it is observed that activity is seen at 0.1 gm/ml concentration. The BHA standard was used and the results are expressed as BHA Equivalents (BHAE).

S. No.	Sample conc. (gm/ml)	Reducing activity BHAE (gm/gm)
1	0.04	0.195
2	0.06	0.33
3	0.08	0.435
4	0.1	0.566

Table 3: Reducing activity in leaves of *Vetiveria zizanioides*

Total antioxidant activity:

The variation of the antioxidant activity over time for various concentrations of Vetiveria leaf extract is presented in Table 4. Total antioxidant activity of the extract was expressed as Ascorbic acid equivalents (AAE) because Ascorbic acid was used as standard. Total antioxidant activity is concentration dependent and the maximum concentration of 161.2 is observed at 0.1 gm/ml concentration.

S. No.	Sample conc. (gm/ml)	Antioxidant Activity AAE (gm/gm)
1	0.04	50
2	0.06	97.8
3	0.08	133.4
4	0.1	161.2

Table 4: Total antioxidant activity in leaves of *Vetiveria zizanioides*

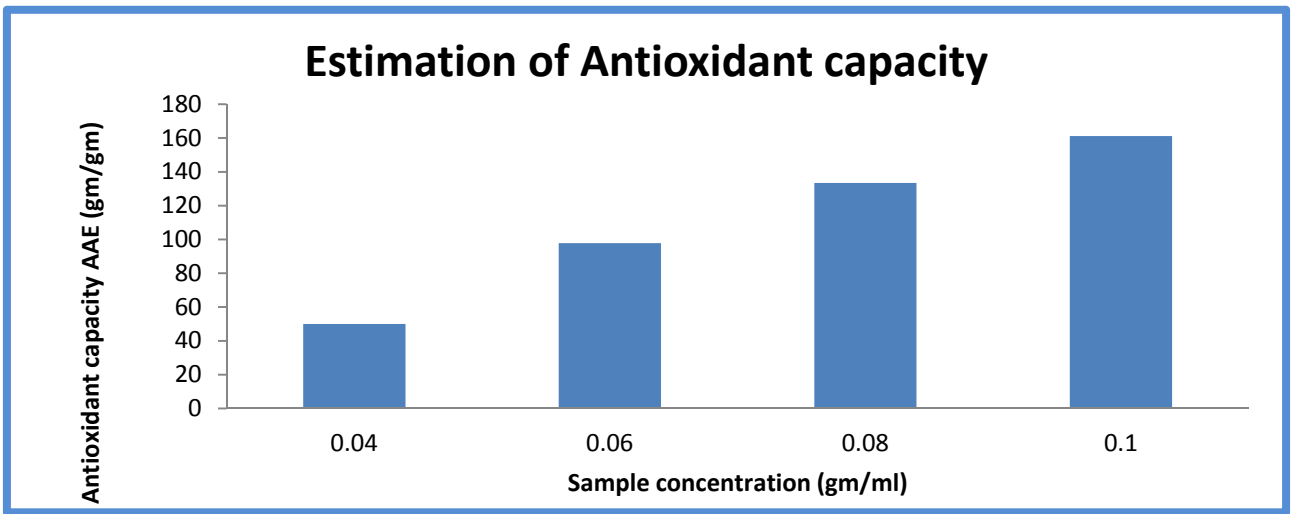

Fig 4: Table 4: Total antioxidant activity in leaves of *Vetiveria zizanioides*

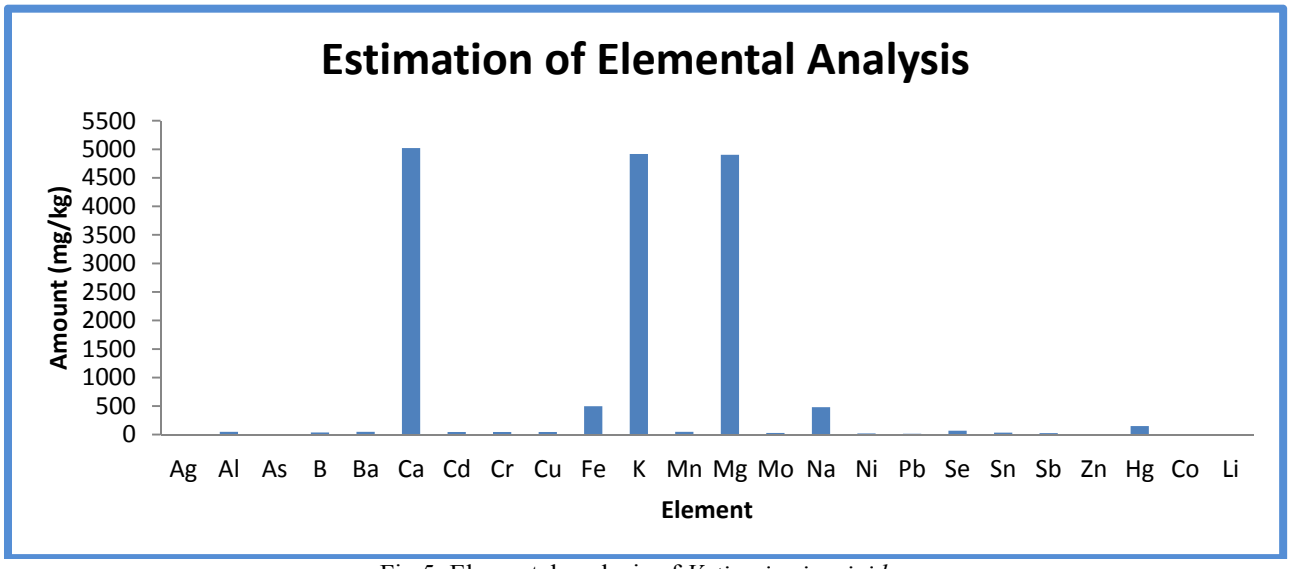

Fig 5: Elemental analysis of *Vetiveria zizanioides*

Elemental Analysis

The elemental concentrations obtained for the leaf extract of *Vetiveria zizanioides* is presented in Table 2. As can be seen in this table, Ca, K and Mg are the most abundant elements in the extracts presenting concentrations at the mg/g levels. The concentrations for Ba, Fe, Mn, Na, Cu, Cr, Cd, Al and Mo were found at the µg/g level and for Co, Cr, Cs, La, Sc and Se at the µg/kg level. These elements are also known to play vital role in human metabolism. The toxic elements As was absent in the sample and Zn, Pb and Ni were found in very low concentrations in the analyzed sample.

S. No.	Element	Amount(mg/kg)
1	Ag	NIL
2	Al	49.7
3	As	NIL
4	B	37.5
5	Ba	50.0
6	Ca	5022.6
7	Cd	45
8	Cr	45
9	Cu	47.29
10	Fe	496.875
11	K	4916.54
12	Mn	49.837
13	Mg	4903.10
14	Mo	27.77
15	Na	481.366
16	Ni	20
17	Pb	14.5
18	Se	69.04
19	Sn	35.71
20	Sb	25
21	Zn	4.732
22	Hg	150
23	Co	NIL
24	Li	7.692

Table 5: Elemental analysis of leaves of *Vetiveria zizanioides*

DISCUSSION:

Leaves of *Vetiveria zizanioides* showed high polyphenolic and flavonoid content as well as strong antioxidant potential. The grass extract contains lower amount of phenolics as compared to the flavonoids. The raw material being inexpensive and easily available should be regarded as potential nutraceutical resource, capable of offering significant nutritional dietary supplements. Also the natural antibiotics in the form of phenols and flavonoids can be easily extracted and thus it offers opportunities to formulate value added products in nutraceutical and food applications to enhance health benefits. Ca is known to be an important constituent of bones and teeth, to be participated in the biochemical blood clotting process and to be responsible for proper nerve and muscle function. K is responsible for regulating osmotic pressure of body fluids and for maintaining cardiac rhythm. Mg is known to catalyze important reactions related to muscle contraction and its deficiency in human metabolism can cause neuromuscular dysfunctions.

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