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EFFECT OF EXTRACTION METHOD ON ANTIOXIDANT ACTIVITY OF WATER EXTRACT OF *VETIVERIA ZIZANIOIDES*

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ABSTRACT
Natural products from medicinal plants, either as standardized extracts or as pure compounds, provide vast opportunities for new drug leads because of the incomparable availability of chemical diversity. Extraction is considered as the crucial first step in the analysis of plants, because it is necessary to extract the desired chemical components from the plant materials for further separation and characterization. Proper care should be taken to assure that potential active components are not lost, distorted or destroyed during the preparation of the extract from plant samples[1, 2]. Present study aimed at comparing different extraction methods with respect to their abilities to extract antioxidant components from *Vetiveria zizanioides*. The extract was prepared in water by two different extraction methods, and was compared with respect to their extraction efficiency, total phenol content, total flavonoid content and antioxidant activity. The sample extracted by method II showed high polyphenolic and flavonoid content as well as strong antioxidant potential when compared with method I of extraction. The *Vetiveria zizanioides* 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 antioxidants 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.

KEYWORDS: *Vetiveria zizanioides*, Phenolics, polyphenolic, nutraceutical.

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

INTRODUCTION
Extraction as the term pharmaceutically used, can be defined as the technique used for separation of therapeutically desired active constituent(s) and elimination of unwanted insoluble material by treatment with selective solvents[3]. Extraction mainly involves the release of complex plant constituents and solubilization of secondary metabolites from the matrix, followed by separation of soluble target compounds from the crude extract through selective use of solvents.[4]

Screening the crude plant extracts for the desired bioactivity is among the most important operations in medicinal plant research, and extraction is the first crucial step of the process. To have a complete idea of bioactivity of crude extracts, it becomes necessary to optimize the extraction methodology, so as to achieve maximum possible extraction efficiency. Obtaining better quality and high efficiency of extraction from herbs being significant, one has to optimize the extraction methods for better extraction efficiency. Efficacy of plant extracts may in some cases be dependent on extraction efficiency. The need for selection of most appropriate extraction methodology is evident from the fact that when different methods are applied on same plant material with same solvent, extraction efficiency can vary significantly. In addition, the method selected as the most appropriate one also needs to be standardized so as to achieve acceptable degree of reproducibility. Standardization of extraction procedures contributes significantly to the final quality of the herbal drug.

Vetiver, commonly known as *Khus* grass is a perennial grass of Indian origin which is found 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[6].

MATERIALS AND METHODS

Sample preparations

Method I: 40 gm of leaves of *Vetiveria zizanioides* were weighed and mascinerated in morter and pestle with the help of water. The mascinerated sample was boiled in 200ml of distilled water for half an hour. The residue was filtered and mixed with 200 ml of distilled water. Sample was kept in the capped bottle in refrigerator till further use.

Method II: Fresh leaves of *Vetiveria zizanioides* were shade dried and coarsely powdered in grinder. 40 gm of this powder was soaked in 200ml distilled water and kept for extraction for 48 hours at room temperature. This residue was filtered and the final volume was made up to 400ml.

Determination of Total Phenolic Content

The determination of TPC of the grass extract was performed by using Folin-Ciocalteu reagent[5]. Briefly, 1ml of extract was prepared 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 RT and the absorbance was measured at 765nm. The concentration of the TPC was determined as mg of Gallic acid equivalents (GAE) per gm FW.

Determination of Total Flavonoid Content

TFC of grass extract was determined by using the aluminium chloride colorimetric method[7]. 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 FW.

Determination of Reducing Power

The reducing power was determined by the method Athukorala et al (2006)[8]. 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 500C for 20 minutes. 1ml of 10% TCA (W/V) was added and then the samples were centrifuged at 2000 rpm for 10 minutes, 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 the readings 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.

RESULTS

Total phenolic content

The variation of the total phenolic content over time for various concentrations of both the samples is presented in Table 1. TPC of the extract was expressed as mg GAE/g FW. Phenolic content is increasing with increasing concentration in both the samples and it is observed that the content is remarkably high in the sample extracted by the Method II. The maximum concentration is observed at 0.1 gm/ml concentration.

Table 1: Total phenolic content of *Vetiveria zizanioides* by both the methods.

S. No.	Sample conc. (gm/ml)	Phenolic content GAE (gm/gm)	
		Method I	Method II
1	0.04	1.2	1.5
2	0.06	1.65	2.04
3	0.08	1.8	2.56
4	0.1	2.1	3

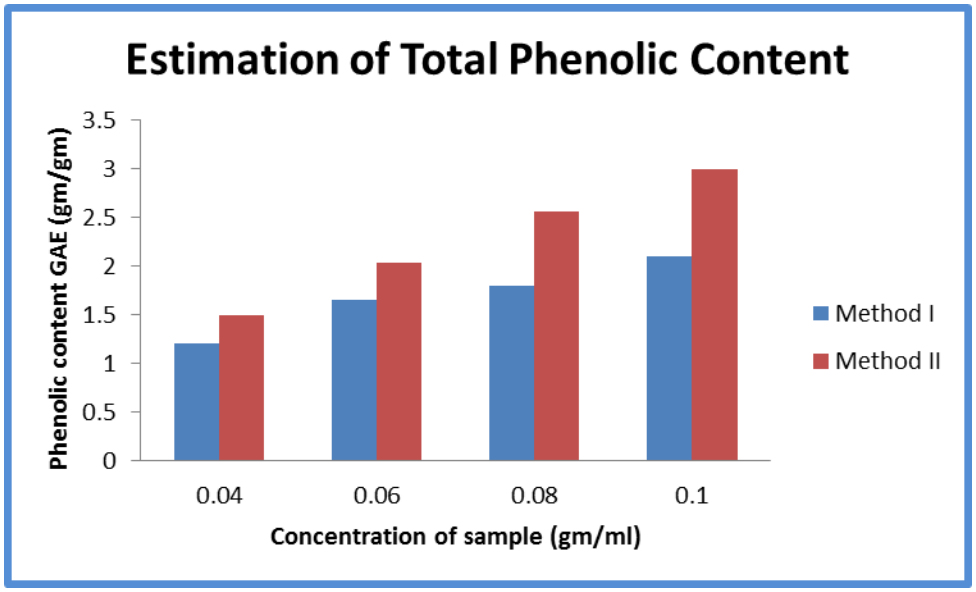

Figure 1: Total phenolic content of *Vetiveria zizanioides* by both the methods.

Total flavonoid content

The variation of the flavonoid content over time for various concentrations of both the samples is presented in Table 2. Flavonoid content of the extract was expressed as gm RE/gm FW. Total Flavonoid content (TFC) is increasing with increasing concentration in both the samples and it is observed that the amount of flavonoid is high in the sample extracted by the Method II. The maximum concentration of 10.8 RE gm/gm of FW is observed at 0.1 gm/ml concentration.

Table 2: Total flavonoid content of *Vetiveria zizanioides* by both the samples

S. No.	Sample conc. (gm/ml)	Flavonoid content RE (gm/gm)	
		Method I	Method II
1	0.04	4.1	4.4
2	0.06	4.8	7.6
3	0.08	7.2	10
4	0.1	8.6	10.8

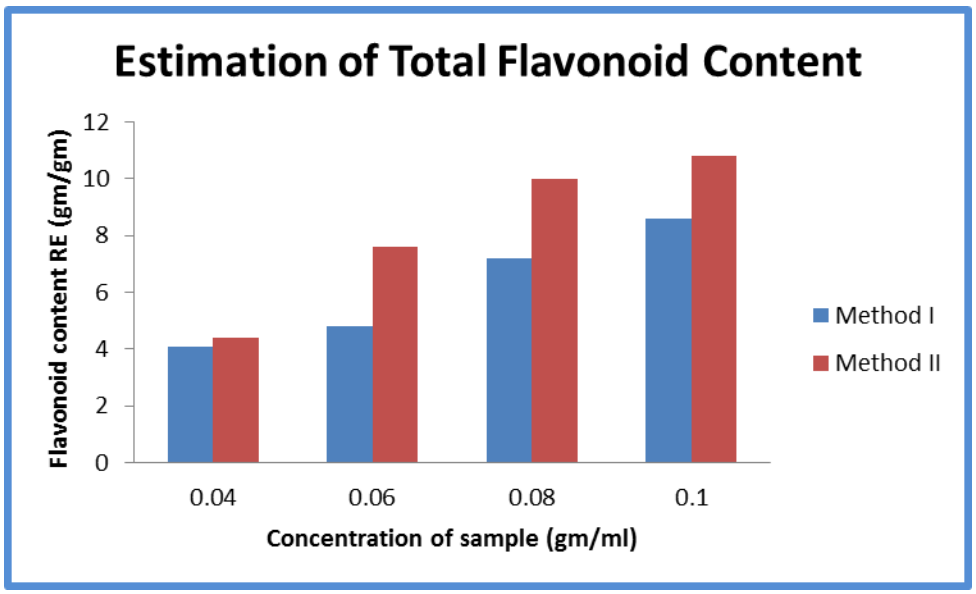

Figure 2: Total flavonoid content of *Vetiveria zizanioides* by both the samples

Reducing power

The variation of the reducing activity over time for various concentrations of both the samples is presented in Table 3. It is observed that the reducing power is concentration dependent in both the samples and it is comparatively high in sample extracted by Method II. The maximum activity is observed at 0.1 gm/ml concentration.

Table 3: Reducing activity of *Vetiveria zizanioides* by both the methods

S. No.	Sample conc. (mg/ml)	Reducing activity	
		Method I	Method II
1	0.04	0.195	0.061
2	0.06	0.33	0.092
3	0.08	0.435	0.184
4	0.1	0.566	0.215

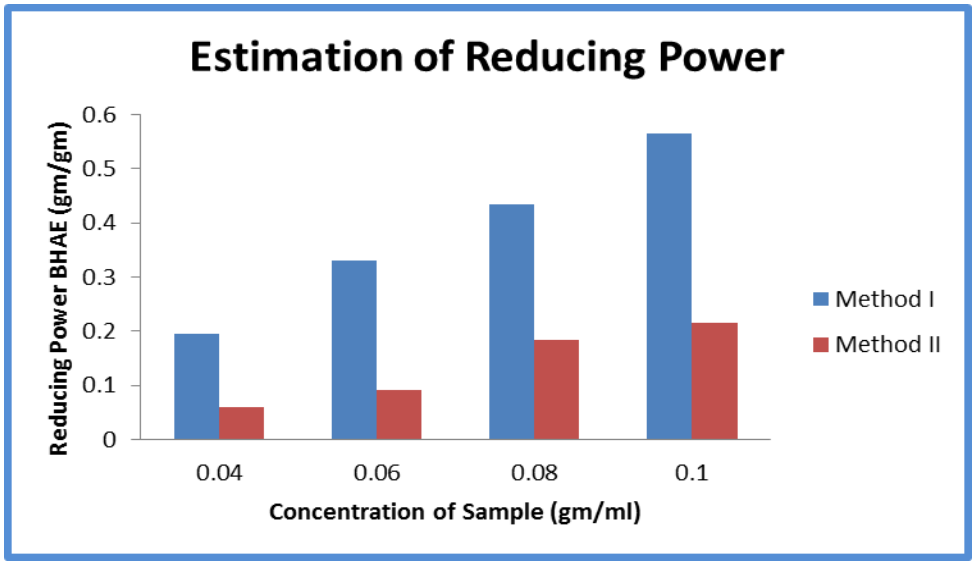

Figure 3: Reducing power of *Vetiveria zizanioides* by both the methods

Total antioxidant activity

The variation of the antioxidant activity over time for various concentrations of both the samples is presented in Table 4. Total antioxidant activity of the extract was expressed as Ascorbic acid equivalents (AAE). Total antioxidant activity is concentration dependent and is high in sample extracted by Method II. The maximum concentration is observed at 0.1 gm/ml concentration.

Table 4: Total antioxidant activity of *Vetiveria zizanioides* by both the methods

S. No.	Sample conc.(mg/ml)	Antioxidant Capacity	
		Method I	Method II
1	0.04	4.4	50
2	0.06	16.4	97.8
3	0.08	29.7	133.4
4	0.1	30	161.2

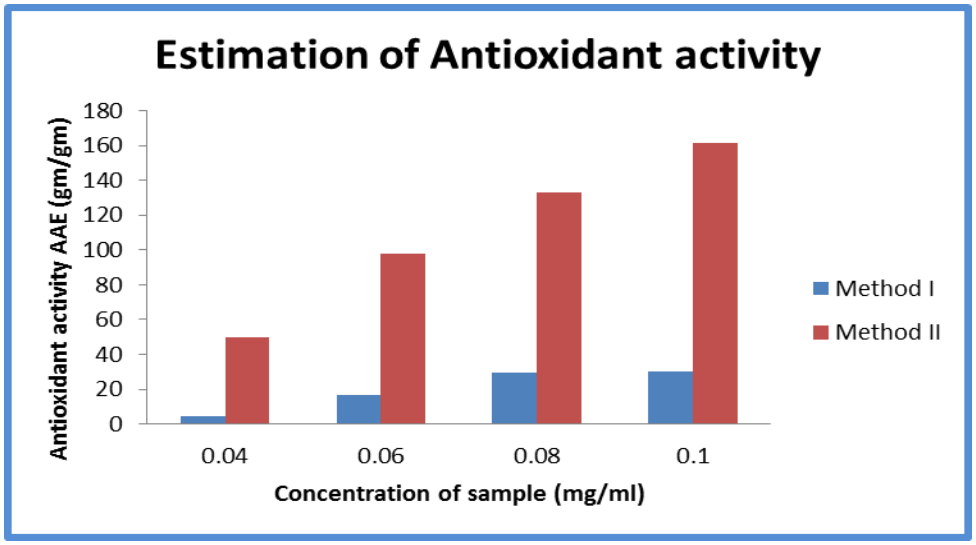

Figure 4: Total antioxidant activity of *Vetiveria zizanioides* by both the methods

DISCUSSION

Selective separation of the desired components from the sample at maximum amount and elimination of interferences are the main objectives of the extraction processes. To have a complete idea of the bioactivity of crude extracts, it becomes necessary to optimize the extraction methodology to achieve the broadest possible range of phytochemicals. The purposes of standardizing extraction procedures for production of crude drugs are to obtain the therapeutically desired portion and to eliminate the inert material by treatment with selective solvents and methods. With the increasing demand for herbal medicinal products, and natural products for health care all over the world, herbal manufacturers aim at using the most appropriate extraction technologies to produce extracts of defined quality with least batch to batch variation, which can also help in scale-up of extraction. Quality of an extract is influenced by several factors such as, plant parts used as starting material, solvent used for extraction, extraction procedure, and plant material: solvent ratio, etc.

CONCLUSION

The sample extracted by method II showed high polyphenolic and flavonoid content as well as strong antioxidant potential when compared with method I of extraction. The Vetiveria 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 antioxidants 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.

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