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Phytochemical analysis and free radical scavenging potential activity of *Vetiveria zizanioides* Linn

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Abstract
The sufficient sources of strong natural fiber from the plant kingdom are called as "*Vetiveria zizanioides*". It had plentiful properties like antioxidant & inherently containing with good aroma. The soxhlet hot extraction method was used for various extracting vetiver using aqueous, methanol, ethanol, hexane, chloroform and acetone. Under the standard qualitative phytochemical screening method, this particular extract was tested. The results were discovered, the presence of phytochemical structures followed by carbohydrates, proteins, steroids, alkaloids, flavanoids, phenols and at last tannins. The quantitative analysis illustrated that the alkaloids, flavonoids, tannin, saponin and phenol. Free radical scavenging activity were analyzed by DPPH method and Ferric reducing antioxidant power (FRAP) assay and ABTS assay.

Keywords: DPPH, FRAP, Free radical scavenging activity, phytochemicals, *Vetiveria Zizanioides*

1. Introduction
Chemical species which are free radicals having one or more unpaired electrons. As a result they are extremely unstable and produce damage to the other molecules by drawn out electrons from them in order to attain stability. Free radicals are continuously produced in the human body, as they are essential for energy supply, detoxification, chemical signaling and immune function [1]. Ultraviolet light, ionizing radiation, chemical reactions and metabolic processes can induce the production of reactive oxygen species (ROS) in the cells. Free radicals can initiate the oxidation of bio molecules, such as protein, lipid, amino acids and DNA which will lead to cell injury and can induce numerous diseases [2]. An imbalance between antioxidants and reactive oxygen species results in oxidative stress, leading to cellular damage and oxidative stress is the main cause of several diseases: cancer, cataracts, age related diseases and Parkinson's disease. This activity is because of the power of antioxidants which helps to reduce oxidative stress by neutralizing or scavenging of reactive species by hydrogen donation [3]. The followings are help the medicinal plants to find their application. They are pharmaceutical, cosmetic, agricultural and food industry [4]. *Vetiveria zizanioides is* commonly called as *Khas Khas*, *Khas* or *Khus* grass in India. Vetiver *is* a member of the family Poaceae. The term "vetiver" is used to call differently in other Indian language it is known as *Vala*,Telugu it is called as *Kuruveeru*, *Vettiveellu*,*Vettiveerum*.In tamil it is described as *"Vettiver"*, Kannad people called it as *Vattiveeru*,*Laamancha*,*Kaddu*, at finally in Malayalam it refer as *Ramaccham, Vettiveru*. Vetiver can grow upto 1.5m tall. The stems are tall and the leaves are long, thin and rather rigid. Vetiver root grows downward upto 2–4 meters in depth [5]. The different parts of vetiver are used in different forms like essential oil, powder and soap and this is used in medicine and perfume ndustries [6, 7]. Vetiver oil is normally light to dark brown in color, viscous and it has a deep smoky and earthy woody odor. The smell and color may vary according to the source of vetiver [8]. In this regard, the roots of *V.zizanioides* was extracted with ethanol and used for the evaluation of various *in vitro* antioxidant activities such as reducing power ability, superoxide anion radical scavenging activity, deoxyribose degradation assay, total antioxidant capacity, total phenolics and total flavonoid composition [9]. Recently antioxidant activity in vetiver oil has been attributed to β-vetinene, β-vetinene and α-vetinone [10]. However, ferric reducing, nfree radical scavenging and antioxidant potential of vettiver root methanol extract have been reported. Therefore, in the present study, the concentration-dependent ferric reducing, free radical scavenging and antioxidant potential of root extract have been investigated.

2. Materials and Methods

2.1 Collection of plant materials

Roots of *Vetiveria zizanioides*, was purchased from local market Vellore district. It was dried in shade and then powdered with a mechanical grinder. The powder was passed through sieve and stored in a labeled air tight container for further studies. The material powder of the plants were exposed to soxhlet extraction by using different solvents such as double distilled water, Methanol, Ethanol, Acetone, Hexane and Chloroform for about 24 hours. Each solvent extract was evaporated to dryness.

2.2 Preliminary Phytochemical Screening

The various solvents extracts of *Vetiveria zizanioides* plant was subjected to different chemical tests for the detection of different phytoconstituents presence using standard procedures [11, 12, 13].

2.2.1 Test for Tannins: 1 ml of the sample was taken in a test tube and then 1 ml of 0.008 M Potassium ferricyanide was added. 1 ml of 0.02 M Ferric chloride containing 0.1 N HCl was added and observed for blue-black coloration.

2.2.2 Test for Phlobatannins: When crude extract of each plant sample was boiled with 2 % aqueous HCl. The evidence for this presence of phlobatannins has been taken by the deposition of a red precipitate.

2.2.3 Test for Saponins: Crude extract was mixed with 5 ml of distilled water in a test tube and it was shaken vigorously. Add some drops of olive oil. The formation of stable foam which serves to indicate the presence of saponins.

2.2.4 Test for Flavonoids: 5 ml of dilute ammonia solution were added to a portion of the crude extract followed by addition of concentrated H_2So_4.The presence of flavonoids were observed in each extract as a result of yellow colouration. The yellow colouration disappeared on standing.

2.2.5 Test for Steroids: 2 ml of acetic anhydride was added to 0.5 ml crude extract of plant sample with 2 ml H_2So_4. The colour changed from violet to blue or green in samples indicates the presence of steroids.

2.2.6 Test for Alkaloids: Crude extract was mixed with 2 ml of Wagner's reagent. Reddish brown colored precipitate indicates the presence of alkaloids.

2.2.7 Test for Carbohydrates: Few drops of extract are heated with Fehling's A and B solution. Appearance of orange red precipitate indicates the presence of carbohydrates.

2.2.8. Test for Proteins: Add 2 ml of Biuret reagent to 2 ml of extract. Shake well and warm it on water bath. Appearance of red or violet colour indicates presence of proteins.

2.2.9 Test for Terpenoids (Salkowski test): The 5 ml of extract was mixed well with 2 ml of chloroform and 3 ml of concentrated H_2So_4 was carefully added along the sides of the test tube to form a layer. A reddish brown colouration of the inter face was formed to show positive results for the presence of terpenoids.

2.2.10 Test for Cardiac glycosides (Keller-Kiliani test): The 5 ml of extract was treated with 2 ml of glacial acetic acid mixed well and this containing drop of ferric chloride solution. This was underlayed with 1 ml of concentrated H_2So_4. A brown ring of the interface indicates a deoxy sugar characteristic of cardenolides. The violet ring may come into view below the brown ring, while in the acetic acid layer, a greenish ring may form just steadily during thin layer.

2.3 Quantitative Determination of Phytochemical Constituents Determination of TPC

By using the tannic acid as a standard phenolic compound, the whole phenolic content of the Methanolic extract of a selected plans was characterized by standard method with minor modifications [14].

The extract were diluted with distilled water to a known concentration in order to obtain the readings within the standard curve range of 0.0 to 600 µg of tannic acid/ml. 250 µl of diluted extract which is also called as tannic acid solution combined with 1 ml of distilled water ina a test tube followed by the addition of 250 µl of Folin - Ciocalteu reagent. The samples were mixed well and then allowed to for 5 min at room temperature in order to allow completereaction with the Folin-Ciocalteu reagent. Then, 2.5 ml of 7 % sodium carbonate aqueous solution was added and the final volume was made up to 6 ml with distilled water. The absorbance of the resulting blue colour solution was measured at 760 nm using spectrophotometer after incubating the samples for 90 min.

2.3.1 Determination of Alkaloids

5 g of the dried powder of each sample was weighed into a 250 ml beaker and 200 ml of 10 % acetic acid in ethanol was added. The mixture is covered and allowed to stand for 4 hours. It was purified and the extract was observed on a water bath until it goes to one quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The total solution was grant to determine and the precipitated was accumulated and washed with a dilute ammonium hydroxide and later filtered. The residue was the alkaloid, which was dried, weighed and percentage was calculated [13].

2.3.2 Determination of Saponins

20 g of each sample was placed into a conical flask and 100 ml of 20 % aqueous ethanol was added. The samples were heated over a hot water bath for 4 h with continuous stirring at 55 °C. The mixture was filtered and the deposit re-extracted with another 200 ml 20 % ethanol. The mutual extracts were reduced to 40 ml over water bath at 90°C. The concentrate was transferred into a 250 ml separating funnel and 20 ml of diethyl ether was added and shaken vigorously. When the ether layer was throwing away the aqueous layer was recovered. The purification process was done again and again after that 60 ml of n-butanol was added. Then add the combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. It gives the evaporation and the samples were kept dried in the oven to a constant weight and saponin content was measured as percentage [15].

2.3.3 Determination of Flavonoids

10 g of plant sample was extracted frequently with 100 ml of 80 % aqueous methanol at room temperature. The entire solution was filtered through Whatman filter paper No 41. The filtrate was allowed to be evaporated into dryness over a water bath and weighed to a constant weight [16].

2.4 *In vitro* Antioxidant activity

2.4.1 1, 1-Diphenyl-2-Picrylhydrazyl (DPPH) radical scavenging assay

The free radical scavenging activity of the extracts, based on the scavenging activity of the stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical was determined by the method [17]. Plant extract (0.1 ml) was added to 3ml of a 0.004% methanol solution of DPPH. Absorbance at 517nm was resolute after 30 min, and the percentage inhibition activity was calculated from [(A0–A1)/A0] x100, where A0 is the absorbance of the control, and A1 is the absorbance of the extract/ standard. The inhibition curves were prepared and IC_{50} values were obtained.

2.5.1 Determination of FRAP radical scavenging activity:

A modified method of Benzie and Strain [18] was adopted for the ferric reducing antioxidant power (FRAP) assay. It depends on the ability of the sample to reduce the ferric tripyridyltriazine (Fe (III)-TPTZ) complex to ferrous tripyridyltriazine (Fe (II)-TPTZ) at low pH. Fe (II)-TPTZ has resolute blue colour which can be read at 593nm. 1.5mL of freshly prepared FRAP solution, containing 25µmL of 300mM acetate buffer pH 3.6, 2.5µmL of 10mM 2,4,6-tripyridyl-s-triazine (TPTZ) in 40µmMµHCl, and 2.5µmL of 20mM ferric chloride {FeCl3·6H2O} solution, was mixed with 1µmL of the extracts, and the absorbance was read at 593nm. The standard curve was linear between 100 and 500μM FeSO4·7H2O. Results are expressed in μM Fe (II)/g dry plant material and compared with that of ascorbic acid.

2.6.1 ABTS radical cation decolorization assay

ABTS stock solution was prepared by mixing 7mM ABTS [19] was modified stock solution with 2.45 mM potassium persulfate (1/1, v/v) and allowing the mixture to stand in the dark at room temperature for 12–16 h until the reaction was complete and the absorbance was stable. The ABTS solution was watery with ethanol (about 1:89 v/v) to an absorbance of 0.700±0.05 at 734nm. The photometric assay was conducted by adding 0.9ml of ABTS solution to different concentration of samples and incubated for 15 min, measurement were taken immediately at 734 nm. The antioxidant activity of the tested samples was calculated by formative the decrease in absorbance at diverse concentrations by using the following equation:

$$E= ((Ac-At)/Ac) \times 100$$

Where,

At and Ac are the respective absorbance of tested samples and ABTS was expressed as µmol.

3. Results & Discussion

Plants are well supplied with a multiple phytochemical molecules (i.e) Vitamins,Terpenoids, Phenolics, Lignins, Tannnins, Flavonoids, Quinines, oils and resins and other metabolites which are high antioxidant activity [20]. Studies have shown that many of the phytocompounds possess anti-inflammatory, anti-diabetic and antimicrobia activities [21]. From the recent years, secondary plant metabolites (phytochemicals), previously with unknown pharmacological activities,which has been largely looked into as a source of medicinal agents [22]. Plant derived substances have recently become a great interest owing to the versatile applications. Medicinal plants and herbs are containing the high quality of bio-resource of drugs of traditional systems of medicine, modern medicine, pharmaceutical intermediates and chemical entities for synthetic drugs. Phytochemical screening of *Vetiveria zizanioides* using various extracts like aqueous, methanol, ethanol, acetone, hexane and chloroform. Phytochemical qualitative analysis of *Vetiveria zizanioides* presented in the Table 1. The screening analysis was performed in order to identify various secondary metabolites which is present in *Vetiveria zizanioides* using a wide range of solvents namely aqueous, methanol, ethnol, acetone, hexane and chloroform. The screening analysis of *Vetiveria zizanioides* using various solvents revealed the presence of carbohydrate, protein, alkaloids, and tannins, phenols, in the methanolic and aqueous extracts. Despite the fact that the occurrence of saponins was noted in chloroform extract. The qualitative phytochemical analysis results explored the presence of a wide range of phytochemical constituents which signifies the *Vetiveria zizanioides* as a valuable therapeutic natural source which will serve as an effective herbal option to compact dreadful infectious diseases. Medicinal plants constitute the group of plants mainly used for health care. Use of them as ntraditional medicine is known since time immemorial. Chemicals present universally in all the plants can be classified as primary and secondary metabolites. Primary metabolites this includes proteins, amino acids, sugars, purines and pyrimidines of nucleic acids and chlorophylls etc., while secondary metabolites phytochemicals are alkaloids to terpenoids and acetogenins to different phenols. These are chemically and taxonomically extremely diverse compounds with obscure function. They are widely used in the human therapy, veterinary, agriculture, scientific research and countless other areas [23]. Understanding of the chemical constituents present in the plants is pleasing because such information will be worth for synthesis of complex chemical substances [24, 25, 26]. Quantitative analysis of *Vetiveria zizanioides* presented in the Table 2. In our present study revealed that Total phenolic content (14.75 w/w) shows higher amount compared to other phytochemicals Alkaloids (1.92 w/w), Saponins (0.30 w/w) and Flavonoids (8.74 w/w). In the DPPH assay, the antioxidant was able to reduce the stable radical DPPH to the yellow colored 1, 1-diphenyl-1, 2-picryl hydrazine.The molecule of 1, 1-diphenyl-1, 2-picryl hydrazine is characterized as a constant free radical by asset of the delocalization of the additional electron over the molecules a complete. The delocalisation also gives rise to the deepviolet colour, characterized by an absorption band in methanol solution centered at 517 nm. The quantity reaction curve of DPPH radical scavenging activity of crude extracts of plant was experimental, when compared with standard ascorbic acid and shown in figure 1. More to the point that, the result is reproducible and linearly related to molar attentiveness of the antioxidants. However, some disadvantage was found in this method, as FRAP assay does not react fast with some antioxidants such as gluthathione [27]. This investigation revealed given concentration, the extract of *Vetiveria zizanioides* showed higher antioxidant potential by FRAP method ABTS radical cation scavenging activity is relatively recent one, which is oftenly used for screening of complex antioxidant mixtures such as plant extract beverages and biological fluids. ABTS assay is a first-rate tool for seminal the antioxidant activity of hydrogen-donating antioxidants and sequence-breaking antioxidants [28]. FRAP assay had been used to determine antioxidant activity as it is simple and quick [29] in figure 2. FRAP assay still can be used for assessment of antioxidant activity in plant materials as humans only absorb limited amount of gluthathione [30]. ABTS is an excellent

substrate for peroxidases and is frequently used to study the antioxidant properties of natural compounds [31]. ABTS scavenging radical scavenging activity examined at different concentrations of ethanol extracts of *Vetiveria zizanioides* (1.0, 2.0, 3.0, 4.0 and 5.0mg) was depicted. In the present study of methanolic extract of *Vetiveria zizanioides* in the concentration range from (200,400,600,800& 1000) showed higher antioxidant potential by ABTS radical scavenging method in figure 3.

Phytochemical analysis and free radical scavenging potential activity of *vetiveria zizanioides linn*

Table 1: Qualitative phytochemical analysis of *Vettiver zizaninoides* extracts

Test	Aqueous	Ethanol	Acetone	Chloroform	Methnol	Hexane
Phlobatannins	-	-	-	-	-	-
Saponins	+	+	-	+	+	-
Flavonoids	+	+	-	+	+	-
Steroids	+	+	-	+	+	-
Alkaloids	+	+	-	-	+	-
Carbohydrates	+	+	+	+	+	+
Proteins	+	+	-	+	+	+
Anthroquinonine	-	-	-	-	-	-
Oil & Resin	-	+	-	-	+	-
Terpenoids	+	+	-	+	+	-
Glycosides	+	+	-	-	+	-

+:Presence; - :Absence

Table 2: *Vettiver zizaninoides* Methanolic extracts of Total Phenolics, Alkaloids, Saponins and Flavonoids

S.no	Phytochemicals	Results (w/w)
1.	Total Phenolics	15.75
2.	Alkaloids	1.92
3.	Saponins	0.30
4.	Flavonoids	8.74

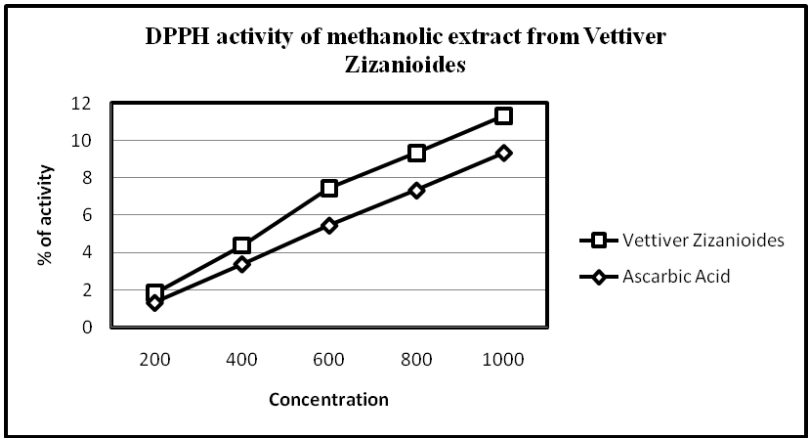

Fig 1: Shows that DPPH activity of *Vettiver zizanioides* from methanolic extract

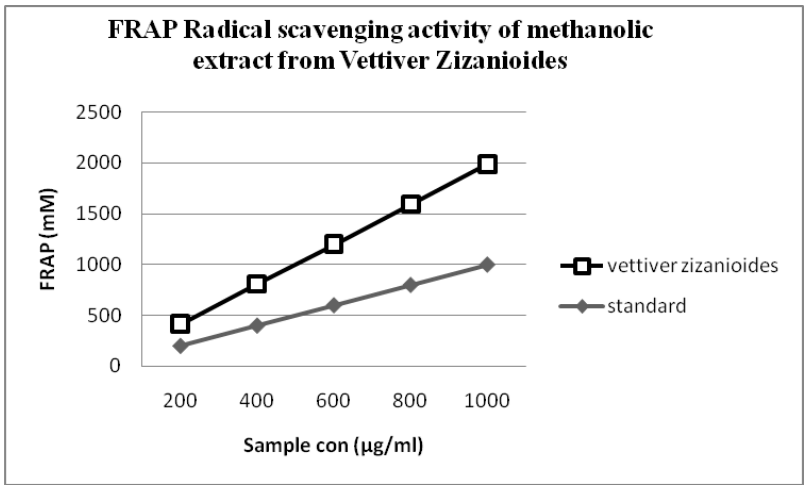

Fig 2: Shows that FRAP activity of *Vettiver zizanioides* from methanolic extract

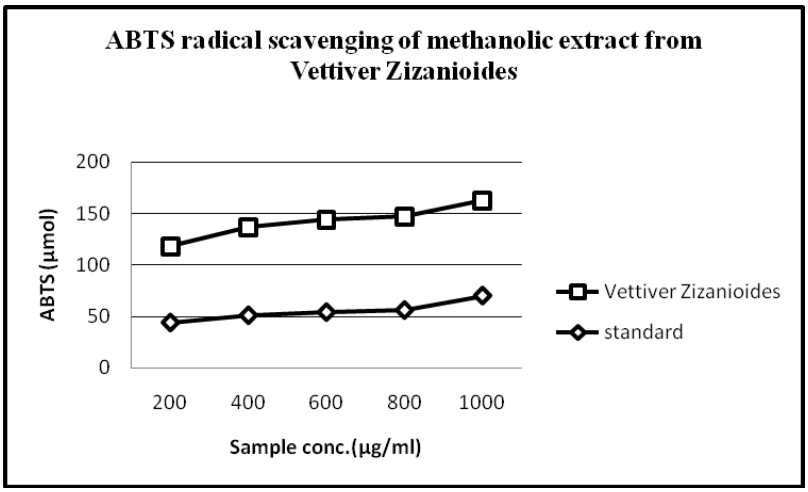

Fig 3: Shows that ABTS activity of *Vettiver zizanioides* from methanolic extract

4. Conclusion

Phytochemical screening of aqueous, methanolic and ethanol extract had exposed the presence Alkaloids, flavonoids, saponin, protein and oils and then resins by positive reaction with the relevant reagent. It was observed that the root extract of *Vetiveria Zizanioides* contained high levels of phenolic content that strength have accounted for the resolute activity observed against free raical scavenging antioxidant activity. The finding of this present investigation suggests that this plant root could be a stalwart source of natural antioxidant that could have enormous importance as restorative agents in preventing or slowing the steps forward of ageing and age related oxidative stress related degenerative diseases. Further investigation on the isolation and characterization of the antioxidant constituents is however required.

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