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In vitro qualitative phytochemical analysis and antioxidant activity of bioactive extracts of *Vetiveria lawsonii* (Hook. f.) Blatt. & McCann

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Abstract
The ancient medical science of Ayurveda, which is experiencing a renaissance at present, it perhaps the most sophisticated and comprehensive approach to healthcare the world has known. The importance of medicinal plants is demonstrated by the fact that even in developing countries, about 35 percent of prescribed drugs are of natural origin and 50 percent of drugs are of plant origin. In the current study, the methanol extracts of leaves and stem *Vetiveria lawsonii*were evaluated for qualitative phytochemical analysis and antioxidant potentail. The qualitative analysis on methanol and chloroform extracts of *V. lawsonii leaf*revealed; alkaloids, flavonoids, saponins, phenols, tannins were present but terpenoids and steroids were absent. Methanol extract of *V. lawsonii* root showed the presence of phenols, tannins, terpenoids, and steroids but absences of alkaloids, flavonoids, and saponins. The phytochemical investigation, and antioxidant profile study of the *Vetiveria lawsonii* exhibits antioxidant potential.

Keywords: *Vetiveria lawsonii*, antioxidant, antifungal, grass

Introduction
Vetiveria lawsonii is a perennial tussock grass belonging to the family Poaceae. It is tall (1-2 m) and fast growing. The massive and complex root system is long (3-4 m) that can penetrate the deeper layers of the soil. Vetiver is an ancient Old-World grass and cultivated in at least 70 countries in the world. The genus Vetiveria is comprised of 11 species. Among these 11 species, five species are endemic to Australia, two are found in Africa, one is found in Southeast Asia and one is endemic to Mauritius and neighboring island of Rodrigues in the Indian Ocean. *Vetiveria lawsonii* is found in southern India and *Vetiveria zizanioides* are found in northeastern India and Bangladesh. *Vetiveria zizanioides* L. Nash is the only species that exists in Bangladesh (Rahman *et al*., 1996) [21]. The generic name, Vetiveria, is derived from a Tamil word; 'vettiver' means to root that is dug up and the species name zizanioides, means by the riverside. That is vetiver is commonly found along with the waterways (NRC, 1993) [17]. Vetiver grass is commonly found all over Bangladesh except littoral forest of Sundarbans, greater Sylhet, Chittagong, and Chittagong Hill Tracts districts as well as under the shades of the Sal (*Shorea robusta*) forest of Tangail, Mymensing and Gazipur near the sandy banks of the river Brahmaputra in the district of Mymensing, Jamalpur and Sherpur. It is cultivated in Nawabganj for thatching material. In Bangladesh, the grass grows quite well on hillocks and undulating lands, fallow lands, dikes of crop fields, marshy habitats, river sides and low-lying areas, where it never grows inside forest under shade. If it is introduced inside open forest patches, vetiver can thrive and establish very well. The root of vetiver grows very fast. It grows downwards without any competition of neighboring crops. Tillers, culm branches and culm cuttings can do its multiplication (Moula and Rahman, 2008) [15]. Vetiver can survive in broad environmental conditions and grow with annual rainfall ranges from 200 to 5,000 mm (Rahman *et al*., 1996) [21]. It can tolerate adverse climatic change. It can survive with temperature ranging from 0 °C to 50 °C. Vetiver is suitable for growing on different types of soil (NRC, 1993) [17]. It is suitable for both highly acidic (pH 4) and alkaline (pH 8) soil (Rahman *et al*., 1996) [21]. It is generally pest and disease resistant. Vetiver bears both xerophytic and hydrophytic characteristics. It can tolerate drought, flood, windstorm, grazing animals, long periods of waterlogging and other forces of nature except freezing. It cannot grow in saline area (Islam, 2010) [8]. It has multiple uses, such as forage, firewood thatch and roofing materials, fencing materials, shedding materials, raw materials in cottage industries and herbal medicine (Kirtikar and Basu, 1986) [11].

It is also used for soil conservation and in perfume industry. It is also used in the recovery of degraded areas, especially in erosion control due to the presence of small rhizomes, and thin-matted root. The root reaches in deep and allows the plant to remain adhered to the soil aggregates and thus resistance to drought and runoff (Mickovski *et al*., 2005) [14]. *Vetiveria lawsonii* is considered as a renowned folk medicine used against diseases and infections like: arthritis, rheumatic pains, respiratory problems, wounds, urinary infections, dysentery and also aphrodisiac. Stem, leaves, and roots are reported to possess hydrocyanic acid and delphinidin. Several flavonoids such as cyanidins are reported in the leaves (Sowmya, *et al*., 2015) [27]. Infusion of seeds along with extract of tubers is traditionally given orally to diabetic patients to check sugar level of blood. The whole plant is used in diuretics, tumors, neuralgia and splenopathy (Singh, *et al*., 2012) [23]. Widely used in aromatherapy and perfume, Khas - Khas is a cooling agent, tonic and blood purifier. It is used to treat many skin disorders and is known to have a calming effect on the nervous system. Other medicinal uses of Khas - Khas include ringworm treatment, indigestion and loss of appetite. Khas - Khas is used to treat gastrointestinal disorders like flatulence and indigestion. The plant is used as analgesic, antibacterial, antiperspirant/ deodorants, astringent, digestive and skin tonic etc. The present study focused onthe antioxidant potential of leaf and stem's methanol extract of *Vetiveria lawsonii*.

Material and Methods

Sample Collection and identification

*Vetiveria lawsonii*collected from Sawaimadhopur district of Rajasthan, India during the August to October 2017. Healthy plants are collected from fertile land. These collected plant material authenticated by the Department of Botany, the University of Rajasthan.

Drying and Grinding of the Plant

The collected leaf and stems were sliced into tiny pieces by using scissors and knives. They were kept for drying under shade to avoid and protect from surrounding contamination and dust in the environment. Shade drying was done in a room for about two weeks without any exposure to light. After completely drying the plants, grinding was done to obtain powder in uniform size and to enhance the surface area for a better extraction process.

Soxhlet Extraction

20 g of finely ground uniform-size range particle containing powder of the plant sample is put in a porous bag made from cellulose strong filter then kept in a thimble chamber of Soxhlet apparatus. Extraction was accomplished in 200 ml of solvent (methanol and chloroform) fill in the round bottom flask of Soxhlet apparatus. The upper part was assembled with a condenser connect to water inflow and outflow. The solvent was burning at a moderate temperature around 40 °C, and the solvent evaporates slowly and entered in to the thimble chamber contain sample pouch where is condensed, and returned back when the solvent containing extracts reaches the siphon arm of condenser apparatus and empty into round bottom flask and this process repeat again and again. The process was continuously run for 24hrs until clear solvent drop observed. Furthermore, forwarding on the next step, the extract was filtered to get dried extract to analyze biological activities. It was stored in an airtight bottle at 4 °C.

Preliminary phytochemical analysis

The different extracts were evaluated to various phytochemical tests alone for the recognition of different active substances (Harborne, 1999) [6].

Quantitative phytochemical estimation

Determination of total phenols

The amount of total phenolics in extracts was done by the procedure of Folin ciocalteu (Singleton and Rossi, 1965) [24]. The addition of Folin-ciocalteu reagent and sodium carbonate to the plant leaf extract leads to the formation of a strong base sodium hydroxide (NaOH) and aweak acid carbonic acid (H_2CO_3) in an aqueous medium. Amino acids present in plant leaf extract react with strong base in the aqueous medium and get precipitated. Therefore, phenolics in crude leaf extracts of the plant react with Folin's reagent to give blue colour in weak acidic medium. The total phenolic content present in the three crude leaf extracts were done according to the method followed by Folin ciocalteu. Two hundred microlitres of respective samples were taken into test tubes. To this 1 ml of FC reagent and 0.8 ml of 7.5% sodium carbonate were added and mixed. Then the test tubes were kept in incubator for 30 min at 37 °C and absorbance was read at 765 nm. The results were reported as gallic acid equivalents mg per gram tissue. The results were obtained from standard graph of gallic acid.

Quantitation of total flavonoid content

The total flavonoid content (mg/mL) was determined using aluminum chloride ($AlCl_3$) method (Kariyone *et al*., 1953) [9] The assay mixture consisting of 0.5 mL of the plant extract, 0.5 mL distilled water, and 0.3 mL of 5% $NaNO_2$ was incubated for 5 min at 25 °C. This was followed by addition of 0.3 mL of 10% $AlCl_3$ immediately. Two milliliters of 1 M NaOH was then added to the reaction mixture, and the absorbance was measured at 510 nm. Quercetin was used as a standard.

Determination of total alkaloids

This assay was done according to the procedure followed by Sreevidya and Mehrotra, (2003) [28]. A quick, easy and simple spectrophotometric method was developed to determine the total alkaloids precipitated by Dragendorff's reagent (DR) in plant leaf extracts. It is based on the appearance of bismuth complex (yellow in colour) in nitric acid medium with thiourea. Five millilitres of the leaf extract of plant was taken into the test tube, and the pH of the extract was adjusted to 2-2.5 with dil HCl. Two ml of Drangendroffs reagent was added to it. After centrifugation the precipitate was obtained and the complete precipitation was checked by the addition of DR. Supernatant was discarded. The precipitate was washed with alcohol and the filtrate was decanted. 2 ml of disodium sulfide was added to the precipitate. After adding this the brownish black precipitate was obtained which is again centrifuged. Complete precipitation was checked by adding 2 drops of disodium sulfide to the precipitate. Two millilitres of conc. HNO_3 was added to the precipitate and the solution was made up to 10 ml with distilled water. One millilitre was taken and 5 ml of thiourea was added and the absorbance was taken at 435 nm.

Determination of Cardiac glycosides

This assay was done according to the method followed by Muhammad and Abubakar (2016) [16]. Cardiac glycosides in extracts react with Baljet reagent (acid containing reagent) and forms coloured products which can be measured calorimetrically at 495 nm. Eight millilitre of extracts were transferred into each 100 ml volumetric flask. To this, 60 ml of H_2O and 8 ml of 12.5% lead acetate were mixed and filtered. Fifty ml of this filtrate was transferred into another 100 ml flask, to this 50 ml, 8 ml of 47% Na2HPO4 was added to precipitate the excess Pb2+ ion. This was mixed and made up to volume 100 ml with water. The mixture was filtered twice through filter paper to remove excess lead phosphate. 10 ml of purified filtrate was transferred into a conical flask and titrated with 10 ml Baljet reagent. A blank titration was done using 10 ml distilled water and 10 ml Baljet reagent. This was allowed to incubate at room temperature for complete colour development. The readings were taken calorimetrically at 495 nm.

% of total glycoside (g %) = (Absorbance at 495 nm/77) x 100

Quantitation of total phytosterols content

The dried Samples were separately extracted by following the procedure of Kaul and Staba (1968) [10]. Plant samples were hydrolyzed with 30% (v/v) hydrochloric acid (2 gm/20ml) for 4 hours on water bath. The hydrolyzed test samples were washed separately with distilled water till the filtrate attained pH 7.0. Test samples so obtained were dried at 60 °C for eight hours and soxhlet extracted in benzene (200 ml) for twenty-four hours separately. Benzene extracts of various test samples were dried separately in vacuum and taken up in chloroform for further analysis.

Total steroids content

The powdered samples were extracted with 150 mL of solvent methanol for 8 - 12 h by using the soxhlet apparatus. The samples (5 µL) were spotted in the bands of width 5 mm with a microlitre syringe on pre-coated silica gel glass plate. The plates were pre-washed by methanol and activated at 60 °C for 5 min prior to chromatography. The sample loaded plate was kept in TLC twin trough developing chamber (after saturated with Solvent vapor) with respective mobile phase (steroids) and the plate was developed up to 90 mm in the respective mobile phase and Cholesterol taken as standard. The Chloroform-acetone (8:2) was employed as mobile phase for steroids. The developed plate was dried by hot air to evaporate solvents from the plate. The developed plate was sprayed with anisaldehyde sulphuric acid reagent as spray reagent and dried at 100℃ in hot air oven for 3 min (Harborne, 1999) [6].

Antioxidant activity

Ferric reducing antioxidant power (FRAP)

The FRAP assay was used to estimate the reducing capacity of plant extracts, according to the method of Benzie and Strain (1996) [4]. The FRAP reagent contained 2.5 mL of a 10 mM TPTZ solution in 40 mM HCl, 2.5 mL of 20 mM $FeCl_3.6H_2O$ and 25 mL of 300 mM acetate buffer (pH 3.6). It was freshly prepared and warmed at 37 °C. 900 µl FRAP reagent was mixed with 90 µl water and 30 µl of the extract. The reaction mixture was incubated at 37 °C for 30 minutes and the absorbance was measured at 593 nm. The analysis was performed in triplicates.

Catalase

Catalase activity measured by a spectrophotometric procedure. It was a direct assay with pseudo-first order kinetics and measured by the method of Sinha (1972) [25]. The enzyme extract (0.5 ml) was added to the reaction mixture containing 1ml of 0.01 M phosphate buffer (pH 7.0), 0.5 ml of 0.2 M H_2O_2, 0.4 ml H_2O and incubated for different time period. The reaction was terminated by the addition of 2 ml of acid reagent (dichromate/acetic acid mixture) which was prepared by mixing 5% potassium dichromate with glacial acetic acid (1:3 by volume). To the control, the enzyme was added after the addition of acid reagent. All the tubes were heated for 10 minutes and the absorbance was read at 610 nm. Catalase activity was expressed in terms of µmoles of H_2O_2 consumed/min/mg protein.

Lipid peroxidase (LPO)

Homogenize 0.1 gm of leaf tissue added in 0.5 ml 0.1% (w/v) TCA. The homogenate centrifuged for 10 min (15000 rpm, 4.0 °C). Collect supernatant and mix 0.5 ml of supernatant with 1.5 ml 0.5% TBA diluted in 20% TCA. This solution was incubated in water bath at 95 °C for 25 min. End reaction by incubated on ice. In case the solution is not clear, centrifuge for a further 5 min (15000 rpm, 4.0 °C). Measure the absorbance at 532 and 600 nm (Health & Packer, 1968) [7].

Peroxidase

The peroxidase assay was carried out by the method of Addy and Goodman (1972) [2]. The reaction mixture consisted of 3 ml of buffered pyrogallol (0.05 M pyrogallol in 0.1 M phosphate buffer (pH 7.0) and 0.5 ml of 1% H_2O_2. To this added 0.1 ml plant extract and O.D. change was measured at 430 nm for every 30 seconds for 2 minutes. The peroxidase activity was calculated using an extinction coefficient of oxidized pyrogallol (4.5 litres/mol).

Results and Discussion

Qualitative Analysis on Phytochemicals

The qualitative analysis on methanol and chloroform extracts of *V. lawsonii leaf*revealed; alkaloids, flavonoids, saponins, phenols, tannins were present but terpenoids and steroids were absent. Methano extract of *V. lawsonii* root showed the presence of phenols, tannins, terpenoids, and steroids but absences of alkaloids, flavonoids, and saponins. Chloroform extracts of *V. lawsonii* root revealed the presence of phenols, terpenoids, and steroids but absences of alkaloids, flavonoids, saponins, and tannins (Table 1).

Table 1: Qualitative Analysis on Phytochemicals Present in *V. lawsonii* Extract

S. No.	Phytochemicals	Leaf		Root	
		Methanol	Chloroform	Methanol	Chloroform
1	Alkaloids	+	+	-	-
2	Flavonoids	+	+	-	-
3	Saponins	+	+	-	-
4	Phenols	+	+	+	+
5	Tannins	+	+	+	-
6	Terpenoids	-	-	+	+
7	Steroids	-	-	-	+

+ [Presence]; - [Absence]

The quantifiedAnalysis on Phytochemicals

The quantified phytochemical content of Phenols, Flavonoids, Alkaloids, Cardiac glycosides, Phytosterols and Steroids was high in leaves of *V. lawsonii* i.e. 0.21±0.02, 0.25±0.03, 1.61±0.05, 2.01±0.03, 1.1±0.02, and 1.5±0.05 mg/g dry weight respectively as compared to root i.e. 1.12±0.03, 0.19±0.04, 1.31±0.02, 1.85±0.04, 0.95±0.03 and 1.10±0.05 mg/g dry weight respectively (Table 2).

Table 2: Quantitative analysis phytochemicals from leaf and root of *V. lawsonii*

Phytochemicals in *V. lawsonii*	Leaves	Root
	mg/g dry weight (Mean ± SD)	mg/g dry weight (Mean ± SD)
Phenols	0.21±0.02	1.12±0.03
Flavonoids	0.25±0.03	0.19±0.04
Alkaloids	1.61±0.05	1.31±0.02
Cardiac glycosides	2.01±0.03	1.85±0.04
Phytosterols	1.1±0.02	0.95±0.03
Steroids	1.5±0.05	1.10±0.05

The antioxidant activity of *V. lawsonii*

The antioxidant activity of *V. lawsonii* measured by FRAP method was observed in extracts (Table 3, Figure 1). In general, values of antioxidant activity of extracts of leaf samples were higher with respect to the stem samples. The highest values were 0.38±0.06 µM/l/gram in leaf sample. The Antioxidant enzymes catalase (CAT) activity found higher in the leaf (0.79±0.12 µM/l/gram) as compared to root (0.76±0.05 µM/l/gram). Lipid peroxidase (LPO) found higher in leaf (5.70±0.46 µM/l/gram) as compared to stem (4.76±0.51 µM/l/gram). The peroxidase activity found higher in root (0.58±0.08 µM/l/gram) as compared to stem (0.50±0.15 µM/l/gram).

Table 3: Antioxidant profile of *V. lawsonii* leaf and root (µM/l/gram fresh weight)

Antioxidant enzymes	Leaf (µM/l/gram fresh weight) Mean ±SD	Root (µM/l/gram fresh weight) Mean ±SD
LPO	5.70±0.46	4.76±0.51
FRAP	0.38±0.06	0.17±0.06
Catalase	0.79±0.12	0.76±0.05
Peroxidase	0.50±0.15	0.58±0.08

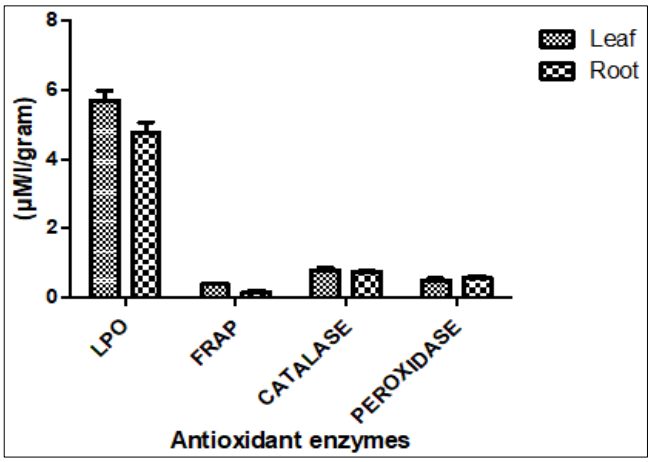

Fig 1: Total level of antioxidant (µM/l/gram fresh weight) in *V. lawsonii*

Antioxidants are widely utilized as food additives in industries to avoid food degradation due to the production of free radicals, which in turn might lead to progression of ailments and illness in humans (Aversa *et al*., 2016) [3]. Novel methods are proposed to measure the antioxidant activity of phytocompounds but the standard scavenging assays followed in this study is based on their free radical scavenging capacities (Lü JM *et al*., 2010) [12]. It is a well-known procedure to determine the antioxidative properties of phytocompounds. The free radicals produced are scavenged by an antioxidant which donates an electron or hydrogen ion to a radical and consequently, a constant molecule is produced (Meganathan *et al*., 2021) [13]. One of the major ROS, hydroxyl radicals can increase the progression lipid peroxidation and in turn could cause overall biological damage in the body (Phaniendra *et al*., 2015) [20]. The results obtained also suggest that extract of *C. trifolia* might be capable of eliminating malondialdehyde, the most mutagenic product of lipid peroxidation. Mostly, it can be used to assess the capability of an antioxidant as an electron donor (Bora and Sharma 2011) [5].

The recent abundant evidence suggesting the involvement of oxidative stress in the pathogenesis of various disorders and diseases has attracted much attention of the scientists and public to the role of natural antioxidants in the maintenance of human health and prevention and treatment of diseases (Niki, 2010) [18], (Abhinav *et al*., 2023) [1]. Thus, antioxidants with free radicle scavenging activities may have enormous significance in the prevention and therapeutics of diseases in which oxidants or free radicals are implicated (Saha *et al*., 2008) [22]. Most of the phytocontituents were observed in solvent extracts from both grasses whereas, very few phytocompounds were present in aqueous extract. Similarly, various researchers revealed the presence of alkaloids, flavonoids, saponins, terpenoids, tannins and phenolics in Vetiveria zizanoides extracts (Soni and Dahiya, 2015) [26], Gahlot *et al*., 2022) [19].

Conclusion

The qualitative analysis on methanol extracts of *V. lawsonii leaf*revealed; alkaloids, flavonoids, saponins, phenols, tannins were present but terpenoids and steroids were absent. Methanol extract of *V. lawsonii* root showed the presence of phenols, tannins, terpenoids, and steroids but absences of alkaloids, flavonoids, and saponins. The qualitative analysis showed that *Vetiveria lawsonii* has higher content of secondary metabolites. The phytochemical investigation, and antioxidant profile study shows very good antioxidant activity

found in *Vetiveria lawsonii*.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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