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ANALYSIS OF PHYTOCHEMICAL AND ANTI-MICROBIAL ACTIVITY ON *VETIVERIA ZIZANIOIDES* ETHANOLIC EXTRACT FOR HEALTHCARE APPLICATIONS

V. KRISHNAVENI [1*] AND S.AMPRITHA[2]

[1*&2] *Department of Fashion Technology, Kumaraguru College of Technology, Coimbatore-641049, India.*

ABSTRACT

One of the abundant sources of strong natural fiber from the plant kingdom is *Vetiveria Zizanioides* and it has numerous properties such as antimicrobial and inherently filled with good aroma. The soxhlet hot extraction method was used for extracting vetiver using ethanol. The extract was tested under standard qualitative phytochemical screening method and the test results revealed the presence of phytochemical constituents such as carbohydrates, proteins, steroids, alkaloids, flavanoids, phenols and tannins. The vetiver extract(100µg/ml) was also assessed for antibacterial activity using agar well diffusion standard test method over gram positive bacterial pathogens such as *Staphylococcus aureus*, *Klebsiella pneumoniae* and gram negative bacterial pathogens *Pseudomonas auruginosa*, *Escherichia coli* and the antifungal activity were assessed against *Aspergillus brasiliensis* and *Aspergillus fumigates*. The measured zone of inhibition results showed good antibacterial activity against gram positive bacteria than gram negative bacteria. The antifungal test also exhibits better results in *Aspergillus brasiliensis* than *Aspergillus fumigates*.

KEYWORDS: ***Vetiveria Zizanioides*, Ethanolic extract, Phytochemical constituents, Antimicrobial, Microorganisms, Agar well diffusion**

***Corresponding author**

DR. V. KRISHNAVENI
Associate Professor, Department of Fashion Technology,
Kumaraguru College of Technology, Coimbatore-641049, India.

INTRODUCTION

Vetiveria zizanioides is commonly called as *Khas Khas*, *Khas* or *Khus* grass in India. Vetiver *is* a member of the family Poaceae. This is known in other Indian languages as Marathi *Vala,* Telugu *Kuruveeru, Vettiveellu, Vettiveerum,* in Tamil *Vattiver,* Kannad *Vattiveeru, Laamancha, Kaddu* and in Malayalam *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[1]. The different parts of vetiver are used in different forms like essential oil, powder and soap and this is used in medicine and perfume industries[2,3] . The morphological parts of the vetiver grass are used for various skin ailments such as boils, fever, scorpion sting, burns, epilepsy, snake bite and sores in the mouth and the root extraction is for headache and toothache, the leaf paste for lumbago, sprain and rheumatism, the leaf juice is used as an anti- helmintic, the stem decoction is used for urinary tract infection, the extract vapor is for malarial fever and the root ash is for curing acidity[3,4]. 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[5]. Vetiver oil also possess several beauty benefits and emotional characteristics. It helps to balance the activity of the sebaceous oil glands which has deodorizing properties and helps to normalize oily skin and clear acne. It nourishes the dry and dehydrated skin and it has a rejuvenation effect on mature skin as well as wounds, inflamed skin and preventing stretch marks.[3] Vetiver plant possess antimicrobial components such as tannins, alkaloids, phenols, essential oils and other aromatic compounds which induces antimicrobial property[6,7]. Vetiver plant consists of various chemical constituents includes vetiverol, vetivone, vetivene, khusimone, khusimol, benzoic acid, vetivenyl vetivenate, khositone, terpenes, iso khusimol , β- vetivone, tripene-4-ol, β-humulene, epizizianal and vetivazulene. The whole parts of the plant plays many functions, namely digestive, constipating, carminative stomachic, antispasmodic, antiasthmatic, antigout, haematinic, expectorant, antimicrobical, anthelmentic, diuretic, anemia, amenorrhea, dysmenorrhea and helmenthiasis. The vetiver root also helps in cooling brain and ulcer treatment. Vetiver oil contains sedative property and it has been commercially used for aromatherapy in order to overcome nervous tension, stress, anxiety and insomnia.

For health care issues, herbal medicines are valuable and readily available resources. The plant kingdom contains many species of plants possessing substances of medicinal value that had to be discovered[8]. Though large numbers of plants are constantly being analyzed for their antimicrobial effects still there is a search for natural antibiotic. Genetic resources have been evaluated in many plants to identify their active constituents holding antimicrobial activities[9]. There are two possibilities that may account for the higher antimicrobial activity of ethanolic extract of vetiver. One is the nature and quantity of active constituents present in the plant (phenols, alkaloids, flavonoids, essential oil, tannins, etc.) and another one is the capacity of ethanol which yields a great number of active constituents responsible for antimicrobial activity[10]. Based on the antimicrobial property, it can be used for medical applications, apart from that the vetiver is used for the development of various home textile and apparel products. Hence the present research work aims at developing antimicrobial medicated apparels for healthcare applications.

MATERIALS AND METHODS

Materials

The vetiver plant has been chosen for screening the phytochemical constituents and analyzing the antimicrobial properties.

Collection of plant material

The roots of *Vetiveria Zizanioides* were selected for the study on the basis of their medicinal and skin disease curing properties and it was procured from Ayurvedic centre, Coimbatore.

Preparation of plant extract

The vetiver roots were shadowed dried for about two days, then it was chopped and converted into powder form by using automatic machines. 100 gms of the fine powder were mixed with 500 ml of ethanol for seven hours using soxhlet apparatus by a hot extraction method in order to obtain the yield of extraction. After the extraction process, the solution was kept open at room temperature for solvent evaporation. The obtained precipitate of the developed extract was stored in a refrigerator with the help of tight containers at 4°C. Based on the requirement, the extract was diluted and utilized for further use[11-13].

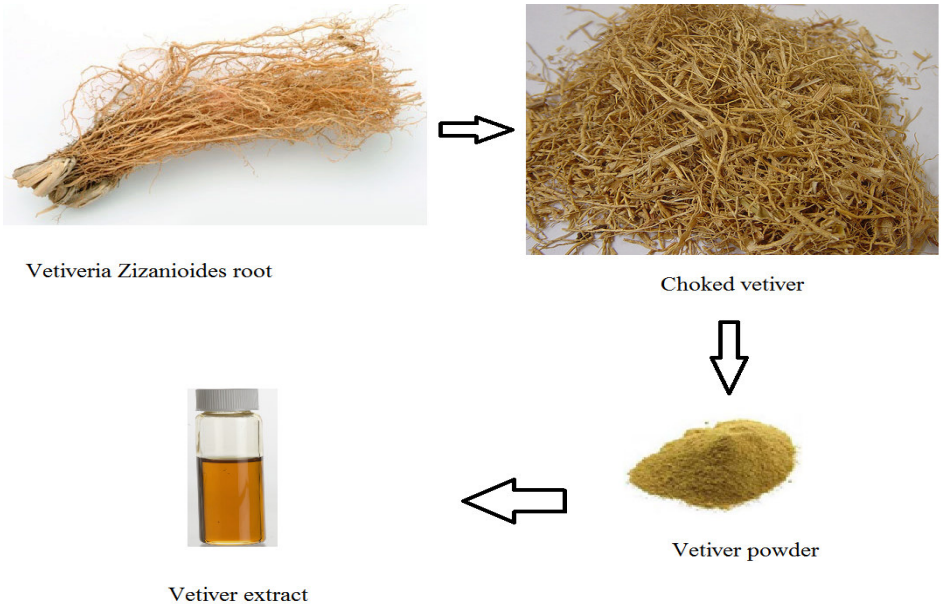


Figure 1
Method of preparing vetiver extract

METHODS

The different methods were used for identifying the phytochemical constituents and antimicrobial activity in the vetiver root extracted solution.

(i) ***Preliminary phyto-chemical screening***
The qualitative phytochemical analysis method is used to identify the presence of active phytoconstituents in the vetiver extract such as carbohydrates, proteins, steroids, alkaloids, flavanoids, phenols, saponins and tannins. The preliminary test was conducted as per standard test procedures.

Test for Carbohydrates
a) Fehling's Test
The plant extract of 2.0 ml was mixed with 1.0 ml of Fehling's solution. The mixed solutions were boiled for a few minutes. The formation of red of brick red precipitate indicates the presence of reducing sugar.

b) Benedict's Test
The 0.5 ml of the extract was added to 5.0 ml of Benedict's reagent and boiled in hot water bath for about 5 minutes. The appearance of red, yellow or green color precipitate showed the presence of reducing sugar.

2. Test for Protein
a) Millon's Test:
Millon's reagent 1.0 ml was added drop by drop with 3.0 ml of water and 1.0 ml of the extract and this mixture was heated, the white precipitate turns into the red which indicates the presence of proteins.

b) Biuret Test
The 1.0 ml of the extract was added to 1.0 ml of 10% sodium hydroxide and then heated. To this mixture, a drop of 0.7% copper sulphate solution was added drop by drop. Purplish violet color was formed and it indicates the presence of protein.

c) Ninhydrin Test
0.5 ml of 2.0% ninhydrin reagent was added to 1.0 ml of the extract and this was mixed thoroughly and heated for 2 minutes in the boiling water bath. The appearance of purple color indicates the presence of protein and free amino acid compounds.

3) Test for Steroids
a) Salkowki's Test
Concentrated sulphuric acid 1.0 ml was added to 2.0 ml of the extract carefully along the sides of the test tube, then the red color was formed in the chloroform layer which indicates the presence of steroids.

4) Test for Alkoloids
a) Wagner's Test
1.5% v/v of hydrochloric acid and a few drops of Wagner's reagent were added to acidify the plant extract of 1.0 ml, then the formation of brown or reddish precipitate indicates the presence of alkaloids.

b) Meyer's Test
Few drops of Meyer's reagent were mixed with 1.0 ml of the extract. A yellow creamy precipitate indicates the presence of alkoloids.

5) Test for Flavanoids

a) Alkaline Reagent Test

A few drops of the extract were added with 4 to 5 drops of sodium hydroxide solution. The intense yellow color was formed and turned into colorless on addition of a few drops of dilute hydrochloric acid indicates the presence of flavanoids.

6) Test for Phenols

a) Ferric Chloride Test

The 2.0 ml of distilled water was added to 1.0 ml of the extract followed by a few drops of 10% aqueous ferric chloride solution. Blue, green or violet color precipitate was appeared and it indicates the presence of phenols.

b) Lead Acetate Test

1.0 ml of the plant extract was diluted with 3.0 ml of distilled water to which a few drops of 1% aqueous lead acetate solution were added. The formation of yellow precipitate indicates the presence of phenols.

7) Test for Saponins

a) Foam Test

A few drops of sodium bicarbonate solution were added to 1.0 ml of the vetiver extract and shaken it vigorously and kept this mixture for 3 minutes. A honey comb like froth was formed and it indicates the presence of saponins.

8) Test for Tannins

a) Ferric Chloride Test

Few drops of aqueous 5% Ferric chloride were mixed with 1.0 ml of the extract. A bluish black color was formed and after sometime it gets disappeared in addition of a few drops of dilute sulphuric acid and a yellowish brown precipitate was formed which indicates the presence of tannins.

b) Lead Acetate Test

A few drops of 1% solution of lead acetate were added to 5.0 ml of the extract, the formation of yellow or red precipitate indicates the presence of tannins[7,14-19].

(ii) Assessment method of antimicrobial activity of Vetiveria Zizanioides ethanolic extracts (AATCC-Test Method)

The antibacterial and antifungal activity of the vetiver extracts was analyzed using AATCC standard qualitative test of the agar well diffusion method.

a) Preparation of bacterial and fungal cultures

The four different bacterial cultures and two different fungal cultures were developed from Microbial Type Culture Collection (MTCC), Department of biotechnology Laboratory, Kumaraguru College of Technology, Coimbatore, India. The developed bacterial and fungal cultures for the study were namely gram positive bacterial pathogens such as *Staphylococcus aureus*(MTCC–727) and *Klebsiella pneumoniae(*MTCC-109), gram negative bacterial pathogens namely *Pseudomonas auruginosa (*MTCC-424) and *Escherichia coli*(MTCC-443) and the fungal culture includes *Aspergillus brasiliensis(*MTCC-1344) and *Aspergillus fumigates(*MTCC-343). The developed bacterial cultures were maintained on a nutrient agar slant and the fungal cultures were also isolated and maintained on potato dextrose agar slant and stored separately in a refrigerator at 4°C.

b) Antibacterial activity assessment by agar well diffusion method

The antibacterial activity of *Vetiveria Zizanioides* extract was evaluated using the agar well diffusion method[20]. 25ml of nutrient agar was prepared and allowed for sterilization at 121ºC for about 15 minutes. The petri plates were autoclaved in hot air oven at 121^{o}C for 45 minutes. The ethanolic extract has been made into 100 µg/ml concentration. The nutrient agar of 20 ml was dropped into the petri plates and was allowed to solidify. Then the plant extract was poured in the developed well and the plates were incubated for 24 hours at 37^{o}C. After 24 hours, the antibacterial activity was assessed against the developed test organisms, namely *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas auruginosa* and *Escherichia coli* by measuring the zone of inhibition.

c) Antifungal activity assessment by agar well diffusion method:

Vetiveria Zizanioides extract was evaluated for antifungal activity using the agar well diffusion method. 25ml of potato dextrose agar was prepared and allowed for sterilization at 121ºC for about 15 minutes. The petri plates were autoclaved in hot air oven at 120^{o}C for 45 minutes. The ethanolic extract has been made into 100 µg/ml concentration. 20ml of potato dextrose agar was poured into the petri plates and were allowed to solidify. Then the plant extract was poured in the developed well and the plates were incubated for 72 hours at 37^{o}C[21]. After 72 hours, the antifungal activity was identified by measuring the zone of inhibition against the developed fungal test organisms like *Aspergillus brasiliensis* and *Aspergillus fumigates*.

RESULTS AND DISCUSSION

The preliminary phytochemical screening and antimicrobial activity test results were discussed.

(i) Preliminary phytochemical screening:

The qualitative phytochemical screening of *Vetiveria zizanioides* extract test results is shown in the table 1.

Table 1
Qualitative Phytochemical analysis of the ethanolic extract of Vetiveria zizanioides

S.No	Plant constituents	Ethanolic extract
1	Carbohydrates	
	A) Fehling's test	+
	B) Benedict's test	+
2	Protein	
	A) Millon's test	+
	B) Biuret test	+
	C) Ninhydrin test	+
3	Steroids	
	A) Salkowki's test	+
4	Alkaloids	
	A) Wagner's test	+
	B) Meyer's test	+
5	Flavanoids	
	A) Alkaline reagent test	+
6	Phenol	
	A) Ferric chloride test	+
	B) Lead acetate test	+
7	Saponins	
	A) Foam test	-
8	Tannins	
	A) Ferric chloride test	+
	B) Lead acetate test	+

The test results revealed the presence of phytochemical constituents in the extract such as carbohydrates, flavanoids, phenols, proteins, steroids, tannins and alkaloids, whereas saponins were absent in the vetiver extract. The different color precipitate represents each compounds present in the vetiver plant extract. The presence of these components induces either individually or in combination to posses antimicrobial activity. Flavonoids are found to be very active antimicrobial component against a wide range of micro organisms due to their ability to combine with extra cellular, soluble proteins and bacterial cell wall. The presence of tannins in the roots of *Vetiveria zizanioides* implied that the tannin also an active compound which was responsible for antimicrobial activity in this study. The component tannin expresses better antibacterial activity than other phytochemical components in the vetiver extract[22].

(ii) Assessment method of antimicrobial activity on Vetiveria Zizanioides ethanolic extract (AATCC-Test Method)

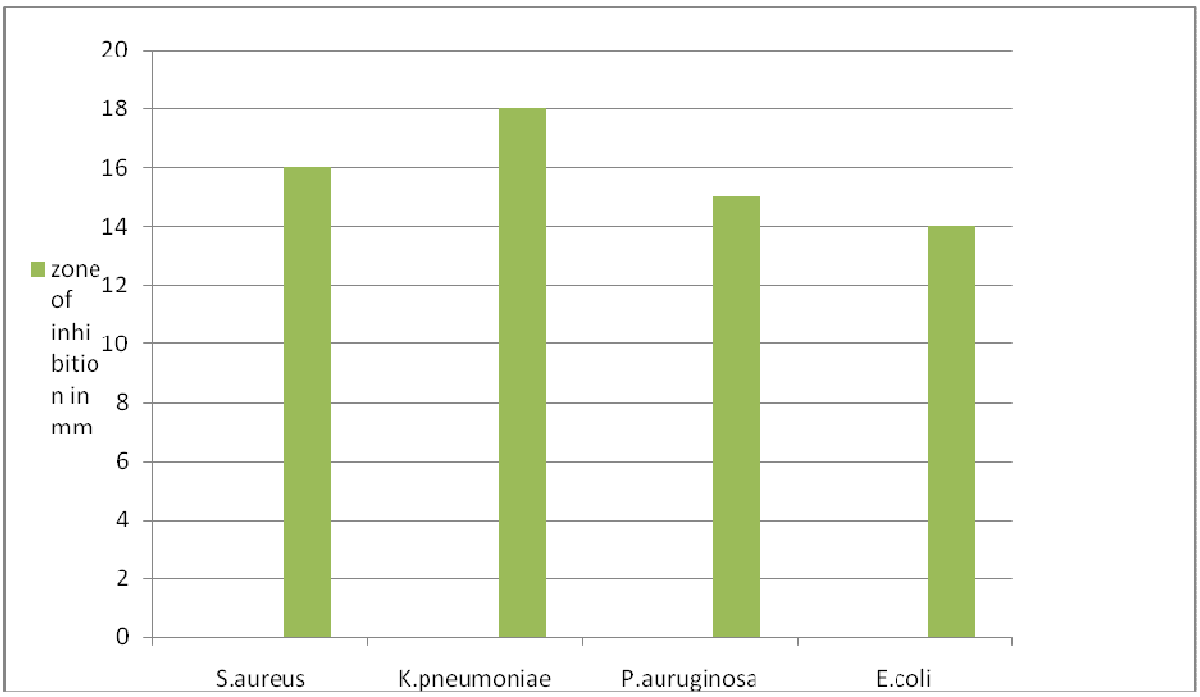
a) Antibacterial activity assessment by agar well diffusion method

The antibacterial activity test results of vetiver extract against gram positive bacterial pathogens namely *Klebsiella pneumoniae*, *Staphylococcus aureus* and gram negative bacterial pathogens namely *Pseudomonas auruginosa* and *Escherichia coli* by agar well diffusion method were shown in the table 2. The zone of inhibition against these bacterial pathogens were also shown in the graph 1.

Table 2
Antibacterial zone of inhibition in (mm) against gram positive and gram negative bacterial pathogens on ethanolic extract of vetiver

Test organisms	Zone of inhibition (in mm)
Staphylococcus aureus- MTCC 727	16
Klebsiella pneumoniae- MTCC 109	18
Pseudomonas auruginosa - MTCC 424	15
Escherichia coli-MTCC 443	14

Graph 1
Assessment of antibacterial activity (zone of inhibition in mm)



The zone of inhibition test results of vetiver ethanolic extract showed good antibacterial activity against gram positive pathogens, namely *Staphylococcus aureus*(16 mm) and *Klebsiella pneumoniae* (18 mm) than gram negative pathogens namely *Pseudomonas auruginosa* (15 mm) and *Escherichia coli* (14 mm). The extract proved that it has better control over the positive pathogens when compared to the negative pathogens.

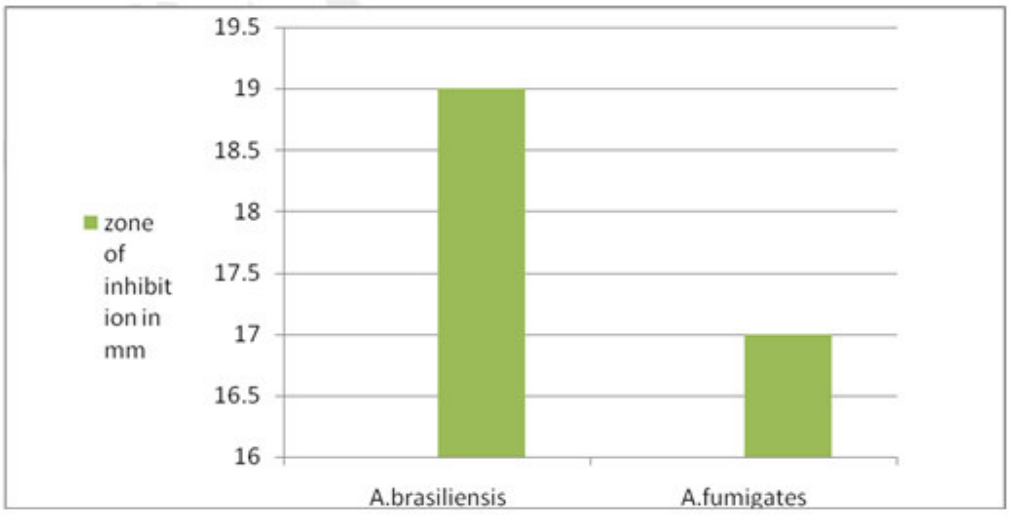
b) *Antifungal activity assessment by agar well diffusion method*

The antifungal activity of the vetiver extract by agar well diffusion method was shown in table 3. The zone of inhibition was measured on the plates against the two fungal pathogen,s namely *Aspergillus brasiliensis and Aspergillus fumigates* were also shown in the graph 2.

Table 3
Antifungal zone of inhibition in (mm) on ethanolic extract of vetiver

Test organisms	Zone of inhibition (in mm)
Aspergillus brasiliensis - MTCC 1344	19
Aspergillus fumigates -MTCC 343	17

Graph 2
Assessment of antifungal activity (zone of inhibition in mm)



The zone of inhibition test results of vetiver ethanolic extract showed better antifungal activity against Aspergillus *brasiliensis* (19 mm) than *Aspergillus fumigates* (17 mm). The test results showed good control over the *Aspergillus brasiliensis* than compared to the *Aspergillus fumigates*. The vetiver extract controls the maximum fungal growth than the bacterial growth.

CONCLUSION

The study results reveal the antimicrobial potential of *Vetiveria zizanioides* root extract. The phytochemical test results exhibits the phytochemical constituents present in the extract includes carbohydrates, proteins, flavanoids, phenols, steroids, alkaloids and tannins. The presence of tannin and flavanoid components in the extract proved that these two components induces better antimicrobial activity. The antimicrobial activity test results also showed a maximum level of antibacterial activity against gram positive bacterial pathogens, namely *Staphylococcus aureus* and *Klebsiella pneumoniae* than gram negative bacterial pathogens *Pseudomonas auruginosa* and *Escherichia coli* and the test results of antifungal activity also exhibits better results over *Aspergillus brasiliensis* than *Aspergillus fumigates*. Based on the qualitative phytochemical screening and antimicrobial activity assessment results, it can be concluded that the vetiver extract controls the spreading of disease through micro organisms and it is more suitable for medical and healthcare applications. Hence, this research work will give an idea for developing nature friendly, antimicrobial medicated apparels for medical practitioner and hospital workers in the healthcare field and it also has major benefits such as 100 % natural resources, renewable, ecofriendly, economic, social and environmental pollution free factors.

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