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Research Article

COMPARATIVE STUDY ON HIGH-PERFORMANCE THIN LAYER CHROMATOGRAPHY PROFILE AND ANTIMICROBIAL ACTIVITY OF ETHANOLIC AND HYDROALCOHOLIC EXTRACT OF *VETIVERIA ZIZANIOIDES* L. ROOT

SARAVANA KUMAR SIVAGURUNATHAN, GAYATHRI KRISHNAMOORTHY*

Department of Biochemistry, Vels University, Chennai - 600 117, Tamil Nadu, India. Email: gayathri.sls@velsuniv.ac.in

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ABSTRACT

Objective: Scientific evaluation of traditionally using medicinal herbs for their pharmacological activity is a leading and valuable area of research. The aim of this study is to compare the antimicrobial activity of ethanolic and hydroalcoholic extract of *Vetiveria zizanioides* root and analyze the major bioactive compounds present in those extracts.

Methods: Antimicrobial activity of both ethanolic and hydroalcoholic extracts was carried out against various pathogens such as *Staphylococcus aureus*, methicillin-resistant *S. aureus* (MRSA), *Pseudomonas aeruginosa,* and *Candida albicans*. A number of active compounds present in both extracts were compared by developing different compounds of the sample in high-performance thin layer chromatography (HPTLC) stationary phase using mobile phase petroleum ether:ethyl acetate:toluene:formic acid (5:5:1:1).

Results: Ethanolic extract acts against pathogens such as *S. aureus* and MRSA, significantly ($p<0.05$) potent than that of hydroalcoholic extract. Significant difference has not been observed between ethanolic and hydroalcoholic extract when acts against *P. aeruginosa* and *C. albicans*. HPTLC profile of hydroalcoholic and ethanolic extract shows the presence of 10 and 14 different compounds, respectively, when developed with the same mobile phase. Gallic acid, a phenolic compound, was found to be present with higher % peak area in hydroalcoholic extract (3.25%) against ethanolic extract (2.98%).

Conclusion: The results of this study reveal that zone of inhibition exhibited by both ethanolic and hydroalcoholic extracts was found to be different with dissimilar pathogens. A more number of compounds were eluted from hydroalcoholic extract than ethanolic extract.

Keywords: Pathogens, Ethanolic extract, Hydroalcoholic extract, High-performance thin layer chromatography.

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INTRODUCTION

Global usage of plant and its extracts in treating various diseases is mounting the end. The World Health Organization has reported that more than 80% of people are relying traditional medicine for their activities [1]. Plants are used in different countries for their potential therapeutic efficacies. Antimicrobial activity of rhizome from *Stephania glabra* [2], stem and flower of *Woodfordia fruticosa* [3], bark of *Betula utilis* [4], etc., is reported earlier evaluation of a herb for their antimicrobial activity plays an important role in the past few decades.

Many modern medicines have been used as antibacterial drugs, especially in the treatment of diseases such as patients met with fire accidents. External application of modern medicine does not cause maximum number of adverse effects. Internal administration of modern medicine for antimicrobial activity may cause additional illness such as gastritis, bulging of the stomach, and gastrointestinal irritation. herbal medicine with antimicrobial activity does not cause all these adverse effects. Nowadays, research on evaluating the antimicrobial activity of herbs is foremost.

Vetiveria zizanioides (VZ), belongs to the family, *Poaceae*, commonly known as Khas Khas or Khus grass in India, is a perennial grass with thick fibrous adventitious roots. It is used as a relaxant for the nervous system, lowers heart rate, and normalizes breathing. It exhibits anti-inflammatory property, controls diabetes, and cures skin diseases [5]. The root is well-known traditional herb for their cooling and diuretic activity. The root decoction is used as an analgesic, anthelmintic [6], antipyretic [7], antioxidant [8], and antituberculosis agent [9]. Aqueous extract from whole plant of VZ exhibits antioxidant activity [10] and its root extract exhibits anticancer activity against breast cancer cell lines [11].

The chemical constituents present in the plant are vetiverol, vetivone, khusimone, khusimol, vetivene, khositone, terpenes, benzoic acid, tripene-4-ol, ß-humulene, epizizianal, vetivenyl vetivenate, iso-khusimol, ß-vetivone, and vetivazulene. In the roots, the main component was valencene (30.36%), while in the shoots and leaves, they were 9-octadecenamide (33.50%), 2,6,10,15,19,23-hexamethyl-2,6,10,14,18,22-tetracosahexaene (27.46%), and 1,2-benzenedicarboxylic acid, diisooctyl ester (18.29%). The results showed that there were many terpenoids in its volatile oil. In shoot volatiles, there existed 3 monoterpenes, 2 sesquiterpenes, and 1 triterpene. Most of the volatiles in roots were sesquiterpenes [12].

The aim of this study is to analyze the number of compounds present in ethanolic extract of VZ (EVZ) and hydroalcoholic extract of VZ (HVZ) root by high-performance thin layer chromatography (HPTLC) fingerprinting and to compare the antimicrobial activity of both extracts against various pathogens.

METHODS

Collection and extraction of VZ root

Root of VZ was collected from Chennai. It was identified and authenticated at Institute of Herbal Botany and Plant Anatomy Research Centre, Chennai. The authentication number was PARC/2015/3159. It was washed with water, dried under shade for 15 days. The dried root was coarsely powdered. About 100 g of coarsely powdered root material was soaked in 1 L ethanol for 72 hrs. Another 100 g of root

was soaked in 1 L hydroalcohol (ethanol:water – 70:30) for 72 hrs. The extract was filtered and concentrated under reduced pressure. The yield of EVZ and HVZ was calculated as 4.2±0.5 and 6.7±0.3 g/100 g. The concentrated sample was stored in desiccator for further studies.

Antimicrobial activity of EVZ and HVZ

The pathogenic bacteria, *Staphylococcus aureus,* methicillin-resistant *S. aureus* (MRSA)*, Pseudomonas aeruginosa*, and two fungal strains *Candida albicans* and *Candida tropicalis* were selected for this study. These selected pathogenic strains were obtained from Microbiology Division (Jayagen Biologics Analytical Laboratory, Chennai). The antimicrobial activity was determined by disc diffusion methods [13]. About 25 ml of molten Mueller-Hinton agar was poured into a sterile petri plate (Himedia, Mumbai, India). The plates were allowed to solidify, and after 18 hrs, optical density was adjusted to 0.6. A volume of 100.0 µl of above-mentioned pathogenic bacteria and fungus was transferred onto plate and made culture lawn using a sterile L-rod spreader. After 5 minutes setting of the pathogenic microbes, a sterile cork borer was used to make 5.0 mm well on the agar. The test samples were dissolved in sterile saline and loaded into wells with various concentrations such as 50.0, 100.0, 150.0, and 200.0 µg/well. The solvent dimethyl sulfoxide loaded well served as negative control and tetracycline amended (20.0 µg/ml) well served as positive control for bacteria and clotrimazole (20.0 µg/ml) served as positive control for fungus. The plates were incubated at 37°C in a 400 nm fluorescent light source for 24 hrs. The antimicrobial activity was determined by measuring the diameter of the zone of inhibition around the well using antibiotic zone scale.

HPTLC fingerprinting of EVZ and HVZ

EVZ and HVZ were dissolved in 70:30 ethanol: water in the stationary phase with 10X10 size, silica gel 60F 254 HPTLC plate. Petroleum ether: ethyl acetate: toluene:formic acid (5:5:1:1) was used as a mobile phase. The thin layer chromatography chamber was initially saturated with the mobile phase at 25°C for 2 h. The sample was applied to the silica gel 60F 254 HPTLC plate at 1 cm distance from bottom and side of the plate. The plate was developed up to 8 cm. The plate was removed, dried in room temperature, and scanned at 254 and 366 nm.

Statistical analysis

Values are mean±standard deviation of triplicate. Significant difference has been served between antimicrobial activity of two extracts using Student's t-test.

RESULTS

Table 1 and Figs. 1-5 show the antimicrobial activity of both ethanolic and hydroalcoholic extracts against five different pathogens. Zone of inhibition exhibited by EVZ was found to be significantly higher ($p<0.05$) than that of HVZ when acts against pathogens such as *S. aureus* and MRSA. Whereas significant difference has not been observed in the zone of inhibition value of EVZ and HVZ when acts against various pathogens such as *P. aeruginosa, C. albicans,* and *C. tropicalis.*

In Tables 2 and 3 and Fig. 6, it shows the HPTLC profile of EVZ and HVZ when the sample was applied in Silica Gel 60 GF 254 plate and developed with petroleum ether:ethyl acetate:toluene:formic acid (5:5:1:1), EVZ shows the presence of 14 different compounds, and HVZ gave some 10 compounds. Although the number of compounds eluted from EVZ was found to be higher than that of HVZ, % peak area of gallic acid, the phenolic compound, present in HVZ was found to be higher (3.25%, Table 3 and Fig. 6b) than that of EVZ (2.9%, Table 2 and Fig. 6a).

DISCUSSION

Traditional practitioners are using root of VZ as a scrubber to clean the body and mixed with drinking water to kill various microbes. Various disorders are caused by *S. aureus* including food poisoning and skin disorders [14]. *S. aureus* is recognized in human beings, and some of them are carriers of the same pathogen which increased the risk of infection. *S. aureus* infections affect the bloodstream, skin, soft tissues, and lower respiratory tracts. It also causes central venous catheter-associated bacteremia, ventilator-assisted pneumonia, serious deep-seated infections, endocarditis, osteomyelitis, toxin-mediated diseases, such as toxic shock syndrome, scalded skin syndrome, and staphylococcal foodborne diseases [15]. In this study, the zone of

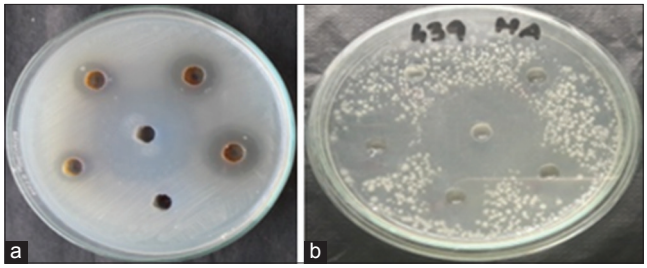

Fig. 1: Antimicrobial activity of ethanolic (a) and hydroalcoholic extract of *Vetiveria zizanioides* (b) against *Staphylococcus aureus*

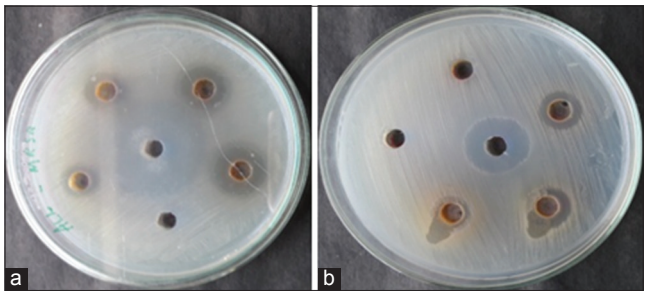

Fig. 2: Antimicrobial activity of ethanolic (a) and hydroalcoholic extract of *Vetiveria zizanioides* (b) against methicillin-resistant *Staphylococcus aureus*

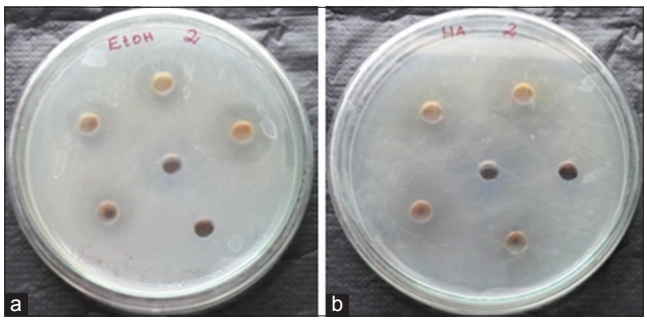

Fig. 3: Antimicrobial activity of ethanolic (a) and hydroalcoholic extract of *Vetiveria zizanioides* (b) against *Pseudomonas aeruginosa*

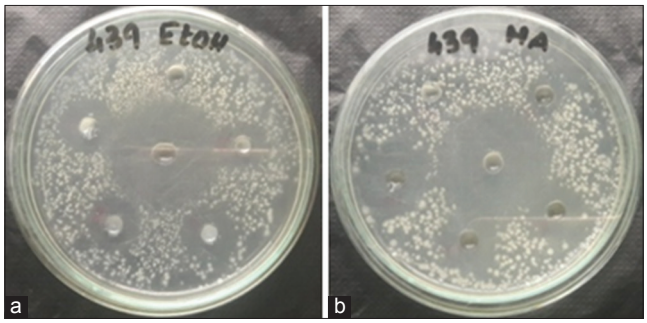

Fig. 4: Antimicrobial activity of ethanolic (a) and hydroalcoholic extract of *Vetiveria zizanioides* (b) against *Candida albicans*

Table 1: Zone of inhibition exhibited by EVZ and HVZ root against various pathogens

Pathogens	Concentration (µg/ml)								
	Zone of inhibition (mm)								
	50		100		150		200		20
	EVZ	HVZ	EVZ	HVZ	EVZ	HVZ	EVZ	HVZ	
Staphylococcus aureus	16.1±0.45*	8.3±0.56	14.7±0.23*	12.4±0.12	20.7±0.23*	16.5±0.26	24.2±0.12*	19.3±0.11	28.3±0.23
MRSA	14.2±0.23* 14.33333	0.12±0.01	16.1±0.23	8.7±0.67	18.3±0.16*	11.4±0.21	21.3±0.12*	13.6±0.12	20.4±0.32
Pseudomonas aeruginosa	11.1±0.12	15.2±0.21	14.5±0.21	27.3±0.12*	18.3±0.17	22.6±0.20	22.3±0.12	24.5±0.12	22.2±0.12
Candida albicans	12.3±0.12	8.3±0.48	15.3±0.14	12.2±0.21	19.3±0.13	16.4±0.26	21.4±0.22	19.3±0.18	29.3±0.14
Candida tropicalis	15.1±0.12 14.33333	11±0.23	17.7±0.27	14.2±0.43	21.5±0.32	16.4±0.26	22.1±0.12	20.6±0.12	20.3±0.29

Values are mean±SD. *Zone of inhibition exhibited by EVZ and HVZ is statistically differed at p<0.05. Statistics is calculated using Student's t-test.
MRSA: Methicillin-resistant *Staphylococcus aureus,* EVZ: Ethanolic extract of *Vetiveria zizanioides*, HVZ: Hydroalcoholic extract of *Vetiveria zizanioides*

Table 2: HPTLC peak table of ethanolic extract of VZ

S.No.	Max position (Rf)	Max height AU	Area %	Assigned substance
1	0.09	16.9	0.59	Unknown
2	0.12	23.0	0.97	Unknown
3	0.19	39.7	2.98	Gallic acid
4	0.26	22.8	1.03	Unknown
5	0.29	53.0	3.95	Unknown
6	0.37	42.5	3.23	Unknown
7	0.43	25.8	6.86	Unknown
8	0.49	54.4	0.28	Unknown
9	0.53	91.2	0.07	Unknown
10	0.64	41.3	4.51	Unknown
11	0.71	11.7	0.57	Unknown
12	0.81	5.2	6.20	Unknown
13	0.83	36.1	7.84	Unknown
14	0.88	26.9	0.91	Unknown

HPTLC: High-performance thin layer chromatography, VZ: *Vetiveria zizanioides*

Table 3: HPTLC peak table of HVZ

S.No.	Maximum position (Rf)	Max height (AU)	Area %	Assigned substance
1	0.12	21.7	0.85	Unknown
2	0.16	25.9	1.02	Unknown
3	0.20	56.0	3.25	Gallic acid
4	0.30	96.4	7.36	Unknown
5	0.38	00.6	5.95	Unknown
6	0.44	13.0	1.24	Unknown
7	0.51	05.7	1.92	Unknown
8	0.55	33.9	9.81	Unknown
9	0.72	14.9	0.57	Unknown
10	0.84	88.5	8.04	Unknown

HPTLC: High-performance thin layer chromatography, HVZ: Hydroalcoholic extract of *Vetiveria zizanioides*

inhibition exhibited by EVZ and HVZ was found to be significantly differ (p<0.05) when acts against *S. aureus* (Table 1).

Gallic acid was found to be present in both HVZ (3.25%) and EVZ (2.98%). The activity of gallic acid against *S. aureus* is reported earlier [16]. Since the activity of EVZ against *S. aureus* is significantly higher, a compound other than gallic acid is synergistically involved in acting against *S. aureus*.

MRSA is a most harmful bacteria present in hospital, prisons, and affects people with open injuries/wounds. HVZ acts against MRSA significantly better than that of EVZ (p<0.05). *P. aeruginosa*, a bacteria able to cause various diseases in plants, animals including human by producing exotoxin A, which is able to inhibit elongation factor 2. Although dose-dependent potency has not been observed in both extracts, 100 µg of HVZ inhibits *P. aeruginosa* significantly (p<0.05) better than that of EVZ.

C. albicans is a filamentous fungus able to cause diseases such as candidiasis in humans [17]. Both EVZ and HVZ were found to act against the *C. albicans*. However, significant difference has not been observed in the activity between them when acts against *C. albicans*. Both EVZ and HVZ act against *C. tropicalis.* Significant difference has not been observed between the activity of both EVZ and HVZ when acts against *C. tropicalis*.

Antimicrobial activity of VZ is reported earlier [18]. Hexane, chloroform, and methanolic extract from roots and leaves of VZ were evaluated for antimicrobial activity against various bacteria and fungus. Methanolic extract from whole plant of VZ exhibits potent antimicrobial activity against various pathogens.

HPTLC plate containing EVZ and HVZ was developed with same mobile phase. Five compounds with Rf value 0.09, 0.26, 0.64, 0.81, and 0.88 have been observed in EVZ sample and not in HVZ. Likewise, a compound with Rf value 0.16 has not been observed in EVZ. The chemical nature of those compounds should be further evaluated.

The antimicrobial activity of EVZ and HVZ might be due to the presence of gallic acid (Tables 2 and 3; Fig. 6). The antimicrobial activity of gallic acid is reported earlier [16,19]. Zone of inhibition exhibited by EVZ was found to be higher than that of HVZ when acts against *S. aureus* and MRSA. In HPTLC, four more compounds have been observed when developing the EVZ sample than that of HVZ sample. Either any one of these four compounds or their synergistic effect might be responsible for the potent activity of EVZ. Further research work should be carried out by isolating those compounds and evaluated for their antimicrobial activity against *S. aureus* and MBSA.

CONCLUSION

EVZ and HVZ extract of VZ root are a rich source of various phytoconstituents. EVZ contains 4 more compounds which could not observed in HVZ. Likewise, HVZ contains 1 compound which cannot be observed in EVZ. Both samples exhibit antimicrobial activity. EVZ exhibits potent antimicrobial activity against pathogens such as *S. aureus* and MRSA than that of HVZ.

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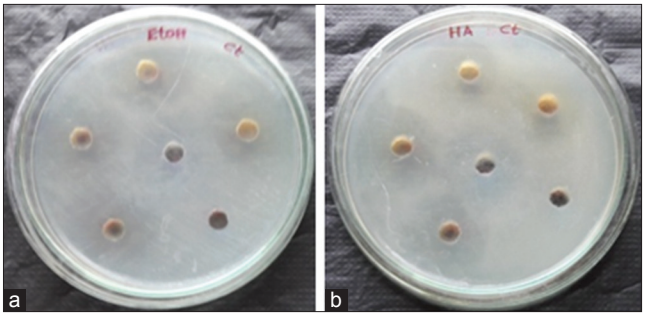

Fig. 5: Antimicrobial activity of ethanolic (a) and hydroalcoholic extract of *Vetiveria zizanioides* (b) against *Candida tropicalis*

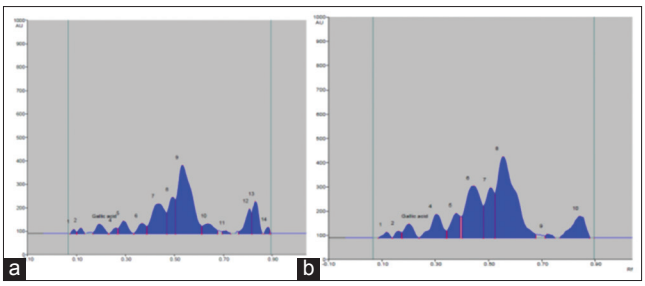

Fig. 6: High-performance thin layer chromatography profile of ethanolic (a) and hydroalcoholic extract of *Vetiveria zizanioides* (b)

wished to acknowledge their thanks to SGS Laboratory, to pursue gas chromatography-mass spectrometry analysis of extract.

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