



SCREENING OF PHYTOCHEMICALS AND ANTIMICROBIAL POTENTIAL OF EXTRACTS OF *VETIVER ZIZANOIDES* AND *PHRAGMITES KARKA* AGAINST CLINICAL ISOLATES

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ABSTRACT

Objective: To screen the phytochemicals present in extracts of *Vetiver zizanoides* and *Phragmites karka*. The antimicrobial potential was also determined against selected clinical isolates.

Methods: The antimicrobial potential of extracts from *Vetiver zizanoides* and *Phragmites karka* was evaluated by agar well diffusion method against selected clinical isolates. Preliminary phytochemical analysis and TLC-bioautographic method was also performed.

Results: Antimicrobial activity of *Vetiver zizanoides* and *Phragmites karka* extracts was assessed on nine bacterial and four fungal clinical isolates. The solvent extracts from both the grasses showed maximum activity against *S. aureus* 1, producing the maximum zone of inhibition 15 ± 0.12 mm in *Vetiveria zizanoides* ethanol extract and 13 ± 0.09 mm in diethyl ether extract of *Phragmites karka*. Preliminary phytochemical analysis demonstrated the presence of most of the phytochemicals including flavonoids, saponins, cardiac glycosides, tannins, alkaloids and steroids in both methanol and diethyl ether extracts or in any of them. Thin layer chromatography and bioautography assay in *Vetiveria zizanoides* methanol extracts demonstrated well defined growth inhibition zones against *S. aureus* 1 in correspondence with flavonoids observed at Rf value ranging from 0.63-0.75.

Conclusion: The present study opens future prospects of these plant constituents to be used as potential antimicrobial drug against infectious agents and can be used in treatment of various infectious diseases.

Keywords: Agar well diffusion, Clinical isolates, *Phragmites karka*, Phytochemical analysis, TLC bioautography, *Vetiveria zizanoides*.

INTRODUCTION

Pathogenic microorganisms causes a number of diseases by their qualities of invasiveness and toxigenesis. Synthetic antibiotics used against them can cause oxidative stress that can lead to damage to DNA, proteins and lipids in human cells, but this effect can be alleviated by antioxidants. Thus as an alternate to this, medicinal plants can be used as they contain phytochemicals and antioxidants that can pave a way for development of antimicrobial drug as well as used in cancer therapy as plants have been used as a source of sophisticated traditional medicines from thousands of years.

Medicinal plants represent a rich source of antimicrobial agents and are widely used either directly as folk remedies or indirectly in the preparation of modern pharmaceutical by all sections of the population. World Health Organization (WHO) noted that more than 80% of the world's population depends on traditional medicine for primary healthcare. Since ages various ailments and infectious diseases have been known to be treated using herbal remedies for the betterment of mankind throughout the world. Thus, scientists are increasingly turning their attention to natural products, either as pure compounds or as standardized plant extracts, looking for new leads to develop better drugs against microbial infections [1]. Grasses from various families are also studied for their potential role in pharmaceutical drugs. The grasses have traditionally been used by the Indian tribes for treating various ailments, diseases and disorders. It has also been used in traditional medicine of Asia and Africa.

Phragmites karka (Retz.) belongs to the family Poaceae and is widely distributed in India, Australia and Europe. It is a perennial reed with long rhizomes and robust, erect culms to 3 m. The leaves are 15-30 cm long and nearly 2.5 cm broad; inflorescence is a large plume-like panicle with capillary branches and small, slender spikelets. It occurs mostly on waterlogged, saline areas or along the swamps, shallow water lakes, or on dry barren lands. In Ayurvedic system of medicine, *Phragmites karka* is used for different ailments such as diuretic, diabetic and anti-emetic [2].

Vetiver or khus (*Vetiveria zizanioides*) is a tall, perennial, miraculous grass native to India first developed for soil and water conservation by the World Bank during mid 1980s. It produces spongy, much branched, root system (khus roots) with fine rootlets, containing

fragrant oil which is a perfume by itself [3]. It is the major source of the well-known oil of vetiver, which is used as a valuable fixative in blending of cosmetics, and in perfumery making agarbattis, soaps, soft drinks, pan masala. Vetiver essential oil also offer other benefits including strengthening of bones, treatment of rheumatism, gout, arthritis, muscle aches, dryness, cramps and dry skin [4]. Different parts of the vetiver plant have traditionally been used by the Indian tribes for treating various ailments, diseases and disorders including boils, burns, urinary tract infection, malarial fever, epilepsy, fever, headache, toothache, weakness, rheumatism, and as an anti-helminthic [5]. The present study aimed at evaluating the phytochemical screening, *in vitro* antimicrobial activity and TLC autobiography analysis of *Phragmites karka* and *Vetiveria zizanoides* extracts.

MATERIALS AND METHODS

Plant materials and extracts preparation

Fresh leaves of *Phragmites karka* and *Vetiveria zizanoides* were collected from Okhla bird sanctuary, Noida, Uttar Pradesh, India. The plant specimens were identified taxonomically and authenticated. The leaf samples were washed thoroughly 2-3 times with running tap water and once with sterile water, air-dried and crushed into fine powder. Fifty grams of each of the air-dried and coarsely powdered plant material was extracted successively with 200 ml each of methanol, and diethyl ether using a soxhlet evaporator for 48 h [6]. After complete solvent evaporation, extracts were dissolved in 10% dimethyl sulphoxide (DMSO) to a final concentration of 50 mg/ml and stored at 4 °C in sterile screw-capped bottles for further use.

Microbial cultures and growth conditions

Clinical isolates of *Escherichia coli*, *Salmonella typhi*, *Salmonella paratyphi*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Acinetobacter* sp were used. The cultures of bacteria were maintained on nutrient agar slants at 4 °C and subcultured on to nutrient broth for 24 h prior to testing. The fungal isolate includes *Aspergillus niger*, *Aspergillus* sp and *Candida albicans*. The cultures were maintained on potato dextrose agar at 4 °C. These microbial isolates served as test pathogens for antibacterial activity assay.

Antimicrobial activity assay

Antimicrobial activity of *Phragmites karka* and *Vetiveria zizanioides* solvent extracts was determined by agar well diffusion method with slight modifications in organic solvents like methanol, diethyl ether and aqueous extracts [7]. Control wells containing the same volume of hexane, acetone, methanol, distilled water and DMSO were made. After 24 h incubation at 37 °C for bacteria and 28 °C for fungal isolates, all plates were observed for zones of growth inhibition, and the diameter of these zones was measured in millimeters. All tests were performed in triplicate and the antimicrobial activity was expressed as the mean of inhibition.

Phytochemical analysis

The extracts were subjected to phytochemical screening to test the presence of metabolites such as reducing sugars, alkaloids, anthraquinones, glycosides, flavonoids, tannins, steroids, saponins, triterpenoids and phlobatanins by using wet reactions following the procedures described by Trease and Evans [8] and Sofowora [9].

TLC bioautography assay

Methanolic extract of *Vetiveria zizanioides* possessing significant antimicrobial activity against *S. aureus* 1 was investigated by thin layer chromatography (TLC) bioautographic agar-overlay method. About 10 µl of the extract was chromatographed on pre-coated aluminium silica gel G 25 plates with toluene: ethyl acetate (93:7) as mobile phase. The developed TLC plates were dried and thinly overlaid with molten Mueller-Hinton agar inoculated with an overnight culture of the test bacteria [10].

Statistical analysis

The antimicrobial activity of the solvent and aqueous extracts was indicated by clear zones of growth inhibition. The resultant clear zones around the agar wells were measured in mm. All the experiments were conducted in triplicate and the data are presented as mean values±standard deviation.

RESULTS AND DISCUSSION

Antimicrobial activity assay

The antimicrobial activity of *Phragmites karka* and *Vetiveria zizanioides* extracts was studied using nine bacterial and four fungal clinical isolates by agar well diffusion method. The methanol extract from *V. zizanioides* presented the highest activity against both *S.*

aureus (15±0.12 mm) and *Salmonella paratyphi* (13.9±0.08 mm). Clinical isolates *S. aureus*, *E. coli*, and *P. aeruginosa* were found to be susceptible to both the solvent extracts of *Phragmites karka* and *Vetiveria zizanioides* (table 1). Previous reports also revealed the antimicrobial efficacy of both the investigated grasses extracts against *S. aureus*, *E. coli* and *P. aeruginosa* [11,12]. The growth of *Klebsiella* sp. and *Salmonella typhi* was only inhibited by the diethyl ether extract of *Phragmites karka* and *Vetiveria zizanioides*. Similarly, the growth of *Candida albicans* and *Rhizopus nigricans* was only inhibited by methanol extract of both the grasses. Moreover, none of the extracts were active against *Aspergillus sp.*, *Aspergillus niger* and *Klebsiella* sp. 2 (table 1). Findings in this study supported the observations of some researchers about *Vetiveria zizanioides* which exhibited antifungal activity against *Candida albicans*[11,13]. Aqueous extracts exhibited almost nil inhibitory effect against the assayed bacterial and fungal clinical isolates except in isolates *E. coli* where slight inhibitory activity was reported. Similar results were reported for aqueous extracts by various researchers [14, 15].

Among the Gram positive bacteria, the solvent extracts from both the grasses showed maximum activity against *S. aureus* 1, producing the maximum zone of inhibition 15±0.12 mm in *Vetiveria zizanioides* ethanol extract and 13±0.09 mm in diethyl ether extract of *Phragmites karka*. Similar results were reported by Sangeetha and Stella [13] where the leaf extract of vetiver showed the highest mean zone of inhibition against *Staphylococcus aureus*. Among Gram negative organisms tested, *Salmonella paratyphi* was more sensitive with 13.9±0.08 mm zone of inhibition in *Vetiveria zizanioides* ethanol extract as compared to *P. aeruginosa* (11±0.05 mm) and *Acinetobacter sp* (8.4±0.07 mm). Our results are in fair correlation with the studies in which both grasses showed antimicrobial activities against Gram-ve and Gram+ve bacteria. The differences in the antimicrobial activities with the reported one may be due to different geographical environment, age of the plant, different method followed for isolation of oil, cultivar type, seasonality etc.

Phytochemical analysis

Results of preliminary phytochemical analysis revealed the presence of flavonoids, saponins, cardiac glycosides, tannins, alkaloids and steroids either in both *Vetiveria zizanioides* methanol and diethyl ether extracts or in any of them (table 2). Flavonoids and steroids were detected in both solvent and aqueous extracts of *Phragmites karka*. None of the plant extracts tested had shown the presence of phlobatanins. Tannins, cardiac glycosides and alkaloids.

Table 1: Antimicrobial activity of various extracts of *Vetiveria zizanioides* and *Phragmites karka* determined by agar well diffusion method

Test Microorganisms	Zone of Inhibition (in mm) <i>Vetiveria zizanioides</i>		Zone of Inhibition (in mm) <i>Phragmites karka</i>		M	DE	A	M	DE	A
Bacterial isolates										
<i>Acinetobacter sp</i>	8.4±0.07	-	-	9.4±0.12	-	-	-	-	-	-
<i>E. coli</i>	-	-	-	-	9±0.05	-	-	-	7.1±0.08	-
<i>Klebsiella sp. 1</i>	-	-	-	-	9.7±0.07	-	-	-	-	-
<i>Klebsiella sp. 2</i>	-	-	-	-	-	-	-	-	-	-
<i>Salmonella typhi</i>	-	10±0.07	-	-	-	-	-	-	-	-
<i>Salmonella paratyphi</i>	13.9±0.08	10±0.12	-	10±0.09	-	-	-	-	-	-
<i>S. aureus 1</i>	15±0.12	-	-	10±0.11	13±0.09	-	-	-	-	-
<i>S. aureus 2</i>	13.5±0.11	-	-	11.5±0.18	-	-	-	-	-	-
<i>P. aeruginosa</i>	11±0.05	12.7±0.10	-	-	12.4±0.13	-	-	-	-	-
Fungal isolates										
<i>Aspergillus sp.</i>	-	-	-	-	-	-	-	-	-	-
<i>Aspergillus niger</i>	-	-	-	-	-	-	-	-	-	-
<i>Candida albicans</i>	10±0.08	-	-	-	-	-	-	-	-	-
<i>Rhizopus nigricans</i>	-	-	-	8.4±0.12	-	-	-	-	-	-

Zone of inhibition is expressed as mean±standard deviation, M: methanol extract, DE: diethyl ether extract, A: aqueous extract,-: no inhibition

Were observed only in *Vetiveria zizanioides* extracts. Most of the phytoconstituents were observed in solvent extracts from both grasses whereas, very few phytochemicals were present in aqueous extract. Similarly, various researchers revealed the presence of alkaloids, flavonoids, saponins, terpenoids, tannins and phenolics in *Vetiveria zizanioides* extracts [14, 16].

TLC Bioautography assay

Bioautographic assay is used to screen for antimicrobial activity where the components are separated on the surface of chromatographic plates and overlaying the TLC plate with molten bacterial agar. TLC analysis revealed the presence of flavonoids and

saponins in the methanol extract of *Vetiveria zizanioides* tested (data not shown). TLC bioautography assay demonstrated strong inhibition zone of *Vetiveria zizanioides* methanol extracts against the growth of *S. aureus* 1. Inhibition zones against the growth of *S. aureus* 1 was observed on the TLC plates as clear spots on pink background when sprayed with aqueous solution of 2, 3, 5 Triphenyl tetrazolium chloride. Spot with R_f value ranging from 0.63-0.75 corresponds to the spots representing flavonoids on spraying the plate with 1% ethanolic solution of aluminium chloride. This result suggests that the antibacterial activity present in *Vetiveria zizanioides* extract may be due to the presence of flavonoids. These findings corroborated with the observations of Mishra *et al.*[17] who reported the antibacterial efficacy of flavonoids and tannins against

S. aureus. Flavonoids are found to be effective antimicrobial substance which may be due to their ability to complex with extra cellular and soluble proteins and to complex with bacterial cell wall; more lipophilic flavonoids may disrupt the microbial membrane [17]. Tannin in the plant extract was found to possess antibacterial activity as reported by Basari and Fan[18]. It is possible that the observed inhibition was likely due to one or more active compounds which overlap possibly due to the solvent system used for screening. In addition to the components with antimicrobial activity several compounds on the reference chromatogram were visible in UV light at 235 nm (data not shown) and others that were visible by using vanillin/sulphuric acid reagent, many of these compounds did not coincide with the antimicrobial components.

Table 2: Phytochemical analysis of various extracts of *Vetiveria zizanioides* and *Phragmites karka*

Phytoconstituents	<i>Vetiveria zizanioides</i> <i>Phragmites karka</i> M DE A M DE A					
Flavanoids	+	-	-	+	+	+
Steroids	-	+	-	+	+	+
Tannins	+	-	-	-	-	-
Saponins	+	+	+	-	-	-
Reducing sugars	+	+	-	-	-	-
Phlobatanins	-	-	-	-	-	-
Cardiac glycosides	+	-	-	-	-	-
Alkaloids	+	-	-	-	-	-

M: methanol extract, DE: diethyl ether extract, A: aqueous extract, +: present, -: not present

CONCLUSION

Herbs are integral part of nature; plants contain natural substance that can promote health. The present study indicated that the solvent extracts of *Vetiveria zizanioides* and *Phragmites karka* can be used as a potential antimicrobial drug against infectious agents and can be used in treatment of various infectious diseases. They are safe and sustainable methods that may be applied to control the growth of microorganisms directly to the infection site are generally inexpensive and non toxic for humans and effective in small concentrations. These can be further screened for their antioxidant potential which could prove useful for the treatment of cancer and anti-inflammatory, anti-rheumatic disease, thus expanding new horizons for alternate and complementary medicines.

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CONFLICT OF INTERESTS

We declare that we have no conflict of interest

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