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Evaluation of Antimicrobial efficacy of Chrysopogon zizanoides on Staphylococcus aureus, Escherichia coli and candida albicans -an in vitro study.

C.m, Anitha[1], Santo Grace Umesh[2], Swetha Shunmuga sundararaj[3], Hareni Murali[4], Lakshmi Ramachandran[5], Janani Karthikeyan[6]

[1,2,5,6]Assistant Professor, Department of Periodontics, SRM Dental College Bharathi Salai, Ramapuram, Chennai.

[3,4]Undergraduate Student, Department of Periodontics, SRM Dental College Bharathi Salai, Ramapuram, Chennai.

ABSTRACT

Aim: *To Compare and analyze the antimicrobial efficacy of Crude,Ethanolic and aqueous extract of Chrysopogon zizanioides, on Staphylococcus aureus, escherichia coli and candida albicans .*

Materials and methods : *The Selected herb for our research is Vetiver (Chrysopogon zizanioides). Undry Plant materials were processed and homogenized to fine powder, filtered through Whattman filter paper no.1 and subjected for antimicrobial sensitivity(crude extract). Then slowly heated 10 grams of dried powder was dissolved in distilled water for six hours, filtered through eight layers of muslin cloth, centrifuged at 5000 rpm for 15 min after which the supernatant was collected and autoclaved at 121°C and 15 lbs pressure before storing at 4°C(aqueous extract). The inal extract will be concentrated by distilling off the solvent using a rotary evaporator. Finally, 2 ml of stored plant extracts are dissolved in 1L of Dimethyl sulfoxide 3(organic solvents based xtracts-Ethanol) and Sterile Distilled water (aqueous extracts) to be tested for Antimicrobial efficacy.*

Result: *It was found that the ethanolic extract had the maximum antimicrobial activity when Compared to Crude ad aqueous extracts against the tested microorganisms. The maximum zone of inhibition against Staphylococcus aureus was noticed on using 10% ethanolic extract, Escherichia coli while using 20% ethanolic extract, and 15% ethanolic extract against Candida albicans.*

Conclusion: *This investigation of Chrysopogon zizanioides has shown an influence on oral microorganisms and has confirmed its significance especially in the area of influence on tested Organisms ,Staphylococcus aureus, escherichia coli and candida albicans*

Keywords: *Chrysopogon zizanioides, Antimicrobial sensitivity test, Vettiver, Anitimicrobial efficacy.*

INTRODUCTION

Chrysoogon zzanioides (commonly known as Vetiver) belongs to the Poaceae family, which comprises 707 genera and 11,337 species Most commercially used vetiver genotypes are sterile and propagated through rhizome buds. This grass exhibits vigorous growth under a wide range of soil types and climatic conditions. Remarkably, nearly all vetiver cultivated globally is a genetically identical clone known as 'Sunshine'—named after the town of Sunshine, Louisiana. This cultivar is predominantly used in commercial essential oil production Vetiver is a high-biomass, C4 plant with efficient photosynthesis It features a long (3–4 m), dense, and complex aerenchymatous root system capable of penetrating deep soil layers, which contributes to excellent soil stabilization. It can withstand extreme environmental conditions, including temperatures ranging from -20°C to 60°C (Truong, 2000; Lavania et al., 2004)[1,2] and is highly tolerant of acidic, alkaline, and saline soils (Truong, 1999; 2000). Chemically, vetiver is rich in sesquiterpenes, primarily found in its roots, while its leaves contain significant phenolic compounds. Different parts of the plant are used in traditional medicine for a variety of conditions. For example, root extracts are used to treat headaches and toothaches; leaf paste for lumbago, sprains, and rheumatism; stem decoction for urinary tract infections; leaf juice as an anthelmintic; root ash for acidity; and the vapors are used in cases of malarial fever. The plant is also applied to treat boils, burns, epilepsy, fever, scorpion stings, snake bites, and mouth sores (Jain, 1991) [3]

Pharmacologically, vetiver shows diverse bioactivities. Ethanolic root extracts have demonstrated anticonvulsant properties by enhancing GABAergic transmission, offering relief from generalized tonic-

clonic and partial seizures (Gupta et al., 2013). [4]The essential oil exhibits potent antioxidant activity, confirmed by DPPH and metal chelating assays (Hyun-Jin et al., 2005). [5]The hexane extract of oil-free roots has shown anti-tuberculosis activity, effective even in dried root form (Saikia et al., 2012).[6] Vetiver oil has also demonstrated superior antifungal and cytotoxic effects compared to several other essential oils (Powers et al., 2018)[7], along with strong free radical scavenging and ferric-reducing antioxidant capacity (Luqman et al., 2012).[8] Furthermore, the ethanolic extract of vetiver has shown notable antimicrobial activity, particularly against Gram-negative bacteria—a result attributed to its flavonoid content (Devprakash et al., 2011).[9]

MATERIALS & METHODS

Selected Herb

The selected medicinal herb for this study was *Vetiver* (*Chrysopogon zizanioides*). Fresh plant materials were thoroughly washed under running tap water and then air-dried in direct sunlight for 20 days. Once completely dried, the materials were homogenized into a fine powder and stored in airtight containers (Nair and Chanda)[10]. Alternatively, dry herbs were powdered for extraction.

Preparation of Crude Extracts

Fresh parts of *Vetiver* were finely ground using a grinder to obtain a homogenous mixture. The resulting pulp was filtered using Whatman filter paper No. 1. The filtrate was then subjected to antimicrobial sensitivity testing.

Preparation of Aqueous Extracts

The aqueous extract was prepared by heating 10 grams of the dried powder in distilled water over low heat for six hours. After allowing the mixture to rest for two hours post-heating, it was filtered through eight layers of muslin cloth and centrifuged at 5000 rpm for 15 minutes. The supernatant was collected, concentrated to one-fourth of its original volume, and then autoclaved at 121°C under 15 lbs of pressure. The extract was stored at 4°C until further use.

Preparation of Organic-Based Extracts

Successive extraction of 10 grams of powdered plant material was carried out using a Soxhlet apparatus and solvents of increasing polarity, including chloroform, acetone, and ethanol (100 ml each). The plant powder was extracted at 80°C for 8 hours with each solvent. All solvent extracts were then combined and concentrated using a rotary evaporator to remove residual solvents. The final extract was stored at 4°C in airtight bottles.

For antimicrobial assays, 2 ml of each stored plant extract was dissolved in 1 liter of dimethyl sulfoxide (for organic solvent-based extracts) or sterile distilled water (for aqueous extracts), producing the final test solutions.

Test Microorganisms

The microbial strains used in this study were:

- *Staphylococcus aureus*
- *Escherichia coli*
- *Candida albicans*

Antimicrobial Activity Assay

A lawn culture of each microbial strain was prepared on Mueller-Hinton Agar (MHA) plates using sterile cotton swabs from inoculums standardized to 0.5 McFarland turbidity. The antimicrobial activity was assessed using the well diffusion method. Plates were incubated at 37°C for 24 hours, after which zones of inhibition were measured to determine the antimicrobial efficacy of each extract.

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RESULTS

Staphylococcus **Escherchia Coli**

Figure 1:

Candida albicans:

Figure

Table 1: Zone of inhibition

Dilution	Staphylococcus aureus	Escherichia coli	Candida albicans
10% Ehanolic Extract	18 mm	17 mm	18 mm

15% Ehanolic Extract	16 mm	15 mm	19 mm
20% Ehanolic Extract	18 mm	18 mm	18 mm
Aqueous Extract	16 mm	18 mm	19 mm
Crude Extract	17 mm	16 mm	17 mm

DISCUSSION:

This study investigated the antimicrobial activity of crude, aqueous, and varying concentrations of ethanolic extracts (10%, 15%, and 20%) of Chrysopogan zizanioides (vetiver) against Staphylococcus aureus, Escherichia coli, and Candida albicans. Similar studies were conducted by Jeyashree et al who studied the antimicrobial activity of root and shoot of an aromatic plant Vetiveria zizanioides (recently reclassified as Chrysopogon zizanioides L. Roberty), [11]on two pathogenic bacteria E.coli (MTCC 443) and Staphylococcus aureus (MTCC 737) and two potent pathogenic fungi, Candida albicans and Cryptococcus neoformans.. The results showed that the extracts of vetiver are pharmacologically important that could be applied for human ailments. Some of Literature evidences have shown that Vetiver oil Capsules are used for antidepressant activities [12]

The ethanolic extracts at all tested concentrations (10%, 15%, and 20%) demonstrated measurable and good antimicrobial activity. The 10% ethanolic extract showed strong inhibition across all three organisms, with 18 mm zones for both Staphylococcus aureus and Candida albicans, and 17 mm for E. coli. The 15% ethanolic extract showed slightly lower activity against S. aureus and E. coli (16 mm and 15 mm respectively), though it had the highest zone (19 mm) against Candida albicans. The 20% ethanolic extract maintained a consistent inhibition zone of 18 mm across all tested organisms, suggesting a broad and balanced antimicrobial spectrum at this concentration.

The aqueous extract showed relatively good inhibition, particularly against E. coli (18 mm) and Candida albicans (19 mm), although its effect on S. aureus was slightly lower (16 mm). This contradicts the common expectation that aqueous extracts may be less effective due to limited solubility of active phytoconstituents in water. The results suggest that certain water-soluble compounds in vetiver also contribute significantly to its antimicrobial activity.

The crude extract showed moderate and relatively balanced activity, with inhibition zones of 17 mm, 16 mm, and 17 mm for S. aureus, E. coli, and C. albicans respectively. This indicates the presence of bioactive compounds in their natural, unprocessed form still retain antimicrobial potential, albeit slightly less optimized compared to purified ethanolic or aqueous extracts.

This shows that Staphylococcus aureus was most susceptible to the 10% and 20% ethanolic extracts (18 mm).E. coli showed highest inhibition from the 20% ethanolic and aqueous extracts (18 mm).Candida albicans responded best to the 15% ethanolic and aqueous extracts (19 mm) Similar studies were conducted by Muthuvivekandavel et al who focussed on analyzing the phytoconstituents from aqueous and ethanolic extracts of Vetiveria zizanioides along with its anti-microbial properties. Antibacterial activity against selected bacterial pathogens were determined by agar well diffusionmethod (inhibitory zone). It was found that the bacterial growth inhibited significantly at higher level than the aqueous extract. Based upon the in-vitro assays it could be concluded that the ethanolic extract of Vetiveria zizanioides showed significant antibacterial activities against various zones of inhibition.[13]

Our study demonstrates the antimicrobial efficacy of crude, aqueous, and ethanolic extracts of *Chrysopogon zizanioides* (vetiver) against *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*. The ethanolic extract, particularly at 10% and 20%, exhibited strong and broad-spectrum antimicrobial activity. The 10% ethanolic extract showed the highest inhibition against *S. aureus* and *C. albicans* (18 mm), while the 20% extract showed consistent inhibition (18 mm) across all organisms. These results indicate that the ethanolic extract is effective in disrupting microbial activity, likely due to better solubility and extraction of bioactive compounds such as sesquiterpenes and phenolic constituents.

These findings are supported by Premjanu et al., who reported significant antimicrobial activity of ethanolic vetiver root extract, with inhibition zones reaching 23 mm against *S. aureus* and 22 mm against *E. coli* [14].

Similarly, vetiver essential oils have demonstrated minimum inhibitory concentrations (MICs) as low as 39 µg/mL against *S. aureus* [15], reinforcing its broad antimicrobial potential.

Interestingly, our study found that the aqueous extract had notable antimicrobial activity, especially against *C. albicans* (19 mm) and *E. coli* (18 mm), contrary to the common notion that aqueous extracts are less effective due to poor solubility of certain phytochemicals. This is supported by Jha et al., who found that vetiver essential oil could increase membrane permeability and disrupt cell integrity in methicillin-resistant *S. aureus* (MRSA), highlighting the presence of active water-soluble components [16].

The moderate but consistent antimicrobial activity of the crude extract also suggests that unprocessed plant material retains bioactive potential, although extraction using specific solvents enhances efficacy. This observation aligns with data by Dahham et al., who emphasized that extraction methods influence the spectrum and strength of antimicrobial effects by concentrating specific bioactive molecules [17].

Comparing these findings with studies on *Zingiber officinale* (ginger), similar trends were observed. The study titled *"Antimicrobial activity of ethanolic extract of Zingiber officinale – an in vitro study"* demonstrated that ginger ethanolic extract effectively inhibited the growth of *S. aureus*, *E. coli*, and *C. albicans*, with inhibition zones ranging from 14 mm to 20 mm depending on extract concentration and organism [18]. This aligns with our findings on vetiver, especially regarding high inhibition against *S. aureus* and *C. albicans* using ethanolic extracts.

In summary, our findings validate *Chrysopogon zizanioides* as a potential source of antimicrobial agents, particularly when extracted with ethanol. The results contribute to a growing body of evidence suggesting its applicability in pharmaceutical or dental settings for managing microbial infections.

Limitation:

Since ethanol by itself has antimicrobial properties and therefore the antimicrobial effect of vetiver may not be established.

The current study proved the antimicrobial effect of vetiver on aerobic bacteria. Further research has to be carried out to demonstrate the antimicrobial effect on gram negative anaerobic bacteria which are the major cause for periodontal disease.

CONCLUSION:

The antimicrobial potential of vetiver is evident across all extract types, with ethanolic extracts generally providing stronger, more consistent results. These findings support its traditional use in herbal medicine and underscore its potential as a natural antimicrobial agent. Further work should focus on identifying and characterizing the specific active compounds, optimizing extraction methods, and exploring synergistic effects in formulation development.

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