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Phytochemical Screening and Study of Anti Arthritic Activity of Vetiveria Zizanioides

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Abstract*—To confirm the traditional medicinal usage of Vetiveria zizanioides, a member of the Poaceae family, as well as its possible anti-inflammatory and anti-arthritic properties, scientific investigation is required. The phytochemical content as well as the in vitro therapeutic properties of the hydro-ethanolic root extract of this plant is the focus of this study. After being dried in the shade, the roots of the V. zizanioides were crushed into a powder, from which an extract was obtained using a hydro-ethanolic solvent combination that had a 70:30 ratio. In order to conduct a preliminary phytochemical screening, the standard qualitative tests were used. The detection of functional groups was accomplished by the use of Fourier transform infrared spectroscopy (400–4000 cm^{-1}). The protein denaturation test and the HRBC membrane stabilization technique were used to examine the anti-inflammatory and anti-arthritic capabilities of the substance in question, respectively. Diclofenac sodium was the reference point for both of the examinations. The data were analyzed by means of Tukey's post hoc test and one-way analysis of variance (ANOVA). According to the results of the phytochemical screening, there were alkaloids, flavonoids, phenols, tannins, saponins, terpenoids, as well as trace amounts of carbohydrates and proteins present. FTIR analysis was used to confirm the existence of bioactive components, and this analysis was able to identify functional groups that were significant to the investigation, including -OH, C-H, C=C/amide, N-O, and C-O. The extract was shown to be capable of reducing protein denaturation to varied degrees, exceeding the effectiveness of diclofenac. The greatest inhibition achieved by the extract was 81.14% at a concentration of 500 µg/mL. In addition, the extract demonstrated a higher level of anti-inflammatory activity when compared to the traditional drug, as well as a significant degree of stability in the membrane (46.88 percent at 500 micrograms per milliliter). Due to the fact that the hydro-ethanolic root extract of Vetiveria zizanioides contains a large number of phytochemicals, it has the ability to significantly reduce inflammation and arthritis. Due to the fact that it is more effective in vitro than diclofenac, it could have potential as a natural cure for therapeutic purposes. In order to determine its medical application, it is recommended that more clinical and in vivo studies be conducted.*

***Keywords*—** *Vetiveria zizanioides; phytochemical screening; FTIR analysis; anti-inflammatory activity; anti-arthritic activity; protein denaturation assay; HRBC membrane stabilization; herbal medicine; bioactive compounds; natural therapeutics.*

I. INTRODUCTION

Rheumatoid arthritis (RA) is a chronic inflammatory illness that may cause several joints on both sides of the body to be afflicted. Over time, this disorder causes the joints to become inflamed, which leads to the erosion of the bones and cartilage that make up the joints, resulting in joint deformity and the loss of their functionality. Rheumatoid arthritis (RA) has a severe impact on the social, economic, and vocational status of those who suffer from it, and the disease has a global prevalence rate of between 0.4 and 1.3 percent. The number of new cases increases as people become older, and it affects females at a rate that is two to three times greater than that of males. The pathophysiological characteristics of rheumatoid arthritis (RA) include sustained inflammation of the joints, proliferative synovitis, and significant damage to the cartilage and subchondral bone [1]. Cytokines, which play a critical role in the progression of rheumatoid arthritis (RA), are also responsible for the inflammation and tissue damage that occur in this disease. Patients may not always have the financial means to pay for pharmacological therapies, and those who do end up facing substantial bad effects from taking them for a lengthy period of time, which makes them less likely to adhere to their treatment programs. However, there are a variety of pharmacological treatments accessible.

The use of traditional medicinal herbs is a practical and cost-effective alternative for the treatment of RA. The use of the plant known as vetiver grass or Khus, which belongs to the Poaceae family and is scientifically referred to as Chrysopogon zizanioides L., has been an important aspect of traditional medicine in South and Southeast Asia for many years. This plant is used to cure a variety of ailments, including sprains, rheumatism, and inflammatory illnesses [2]. Oil from vetiver, which is obtained mostly from the roots of the vetiver plant, is used in a variety of applications, including aromatherapy, perfume, and traditional medicine. Within the context of Ayurvedic medicine, the benefits of vetiver that serve to reduce inflammation and chill the body are highlighted, and it is referred to as Virya: Sita. The use of root pastes or oils that are applied topically has been a common practice in traditional medicine for a considerable amount of time in order to relieve inflammatory pain, muscle stiffness, and joint discomfort.

Although there has been a significant amount of study conducted on the volatile oil composition of vetiver, there has been far less emphasis given to the biological activities and chemical components of the root and aerial sections. Due to the presence of phenolic chemicals, flavonoids, flavonolignans, and flavone glycosides, certain components of plants may possess anti-inflammatory properties. This assertion is based on findings from prior research [3]. Taking

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into consideration this background, the goal of the present study is to carry out a scientific validation of the traditional anti-inflammatory and anti-arthritic uses of Vetiveria zizanioides by concentrating on the plant's hydro-ethanolic root extract. Phytochemical screening, FTIR analysis, and in vitro evaluations of anti-inflammatory and anti-arthritic activities will be carried out by means of protein denaturation and HRBC membrane stabilization assays. To demonstrate, from a chemical and pharmacological aspect, the therapeutic potential of the vetiver root extract in the treatment of inflammatory illnesses such as rheumatoid arthritis (RA) is the overall objective of this work.

II. Objectives

1. To use FTIR analysis and qualitative screening to assess the phytochemical composition and key functional groups found in the hydro-ethanolic root extract of Vetiveria zizanioides.
2. To examine the extract's anti-inflammatory and anti-arthritic properties in vitro utilizing the protein denaturation test and the HRBC membrane stabilization technique, respectively.

III. Material and Method

The roots of the plant that is known as Vetiveria zizanioides (Poaceae) were harvested. We cleaned the new roots with distilled water in order to get rid of any dirt that had adhered to them. We then left them in the shade to dry for a period of ten to twelve days.

Preparation and Extraction of Plant Material

A coarse powder was made from the dried roots of Vetiveria zizanioides by crushing them with the help of an electric grinder. At a temperature consistent with that of a typical room, a maceration process was carried out in which about 200 grams of powdered material was soaked in a hydro-ethanolic solvent with a 70:30 ratio. This process took place over the course of seventy-two hours and included occasional shaking. The mixture was passed through muslin cloth prior to being filtered using Whatman filter paper number one. In order to get rid of the ethanol, the filtrate was concentrated with the assistance of a rotary evaporator that was set to a temperature of 45 degrees Celsius and a rotational speed of 70 revolutions per minute [4]. A hot-air oven was used to heat the liquid portion to a temperature of forty degrees Celsius until all of the liquid had fully evaporated. The dehydrated extract was stored in containers that were sealed and maintained at room temperature until additional testing could be conducted.

Preliminary Phytochemical Screening

In order to ascertain whether or not alkaloids, flavonoids, tannins, phenols, saponins, terpenoids, steroids, glycosides, carbohydrates, proteins, and fixed oils were present, a qualitative phytochemical analysis was carried out on the root extract of Vetiveria zizanioides [5]. This analysis also aimed to identify the existence of any additional secondary metabolites.

The following standard tests were applied:

- Alkaloids: Dragendorff's test
- Flavonoids: Lead acetate test
- Glycosides: Legal's test
- Terpenoids: Copper acetate test
- Carbohydrates: Molisch and Benedict's tests
- Proteins: Ninhydrin test
- Steroids: Salkowski test
- Phenols/Tannins: Ferric chloride test
- Saponins: Froth test
- Anthraquinones: Bornträger test

Standard pharmacognostic methods were followed for all testing.

Fourier Transform Infrared (FTIR) Spectroscopy

Fourier transform infrared spectroscopy was used in the isolation of the major functional groups of the extract. A Fourier transform infrared spectroscopy scan with a resolution of four microns was conducted on a dried extract of Vetiveria zizanioides by using a Shimadzu device manufactured in Japan. The range that was scanned went from 400 to 4000 centimeters to the negative one power [6]. The peaks were analyzed using typical absorption bands, which were utilized to determine their significance.

In Vitro Anti-Arthritic Activity (Protein Denaturation Assay)

In order to determine how effective the extract of Vetiveria zizanioides is in preventing the development of arthritis, the protein denaturation method was used.

Preparation of Reaction Mixture

Reaction mixtures were prepared by adding:

- Egg albumin: 0.2 mL
- Phosphate buffer saline (pH 6.4): 2.8 mL
- Extract/Diclofenac sodium: 2 mL at concentrations: 200 µg/mL, 400 µg/mL, 600 µg/mL

Also made was a control combination of distilled water (2 mL), buffer (2.8 mL), and albumin (0.2 mL).

Procedure

Each mixture was kept in an incubator for fifteen minutes at a temperature of thirty-seven degrees Celsius. Following that, it was cooked for five minutes at seventy degrees Celsius. The absorbance was measured at 660 nm with the use of a UV-Visible spectrophotometer [7].

Calculation

The percentage of protein denaturation inhibition was determined by

$$\text{Inhibition } (\%) = \left[\frac{\text{Abs}_{control} - \text{Abs}_{sample}}{\text{Abs}_{control}}\right] \times 100$$

In Vitro Anti-Inflammatory Activity (HRBC Membrane Stabilization Method)

In order to evaluate the extent to which the extract of Vetiveria zizanioides has anti-inflammatory properties, the HRBC membrane stabilization test was utilized.

Preparation

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- A healthy human volunteer gave their informed permission before blood was drawn.
- For 30 minutes, the blood was centrifuged at 3000 rpm after being combined with Alsever's solution (1:1).
- A 10% v/v RBC solution was made after washing the packed cells with iso-saline.

Reaction Mixture

Total volume: 3 mL, consisting of:

- Phosphate buffer
- RBC suspension
- Hypotonic saline
- Extract at 125, 250, and 500 µg/mL (Diclofenac sodium was used as standard)

The control group received pure water, red blood cell suspension, and phosphate buffer.

Procedure

- For 30 minutes, tubes were incubated at 37°C.
- Centrifuged for 20 minutes at 3000 rpm.
- At 570 nm, absorbance was measured.

Calculation

$$\text{Percentage protection} = 100 - \left(\frac{\text{Abs}_{sample}}{\text{Abs}_{control}} \times 100\right)$$

Statistical Analysis

The findings were publicized in the form of the Mean ± SEM. To compare variables in SPSS version 20, we made use of both one-way analysis of variance (ANOVA) and Tukey's post hoc test. A p-value of less than 0.05 was used to establish whether or not there was statistical significance.

IV. Result

Phytochemical Screening

As a result of preliminary phytochemical examination, it was discovered that the ethanolic root extract of Vetiveria zizanioides included a variety of bioactive components, including steroids, alkaloids, flavonoids, terpenoids, phenols, saponins, and tannins (see Table 1). Carbohydrates and proteins were determined to be present in low amounts, but no lipids were recognized [8 -10]. The benefits of these substances include anti-arthritic and anti-inflammatory effects.

TABLE 1. Phytochemical Profile of *Vetiveria zizanioides* Root Extract

Phytochemical	Presence
Alkaloids	+
Flavonoids	+
Terpenoids	+
Phenols	+
Saponins	+
Tannins	+
Steroids	–
Glycosides	–
Carbohydrates	+
Proteins	Traces
Fats	–
Anthraquinones	–

Fourier Transform Infrared (FTIR) Analysis

By examining the Fourier transform infrared (FTIR) spectrum of the Vetiveria zizanioides extract, it was shown that a number of functional groups were really connected to phytoconstituents that had anti-inflammatory properties [11].

Key FTIR Peaks Identified

Vetiveria zizanioides ethanolic extract shows the following major peaks:

Peak (cm^{-1})	Functional Group	Interpretation
3375	–OH	Phenolic compounds / flavonoids
2924	C–H stretch	Alkanes / fatty acids
1634	C=C / amide	Aromatic rings / proteins
1512	N–O	Nitro compounds
1030	C–O	Alcohols / polysaccharides

The existence of alkaloids, terpenoids, and phenolic compounds that have anti-inflammatory and anti-arthritic properties is shown by the presence of these peaks.

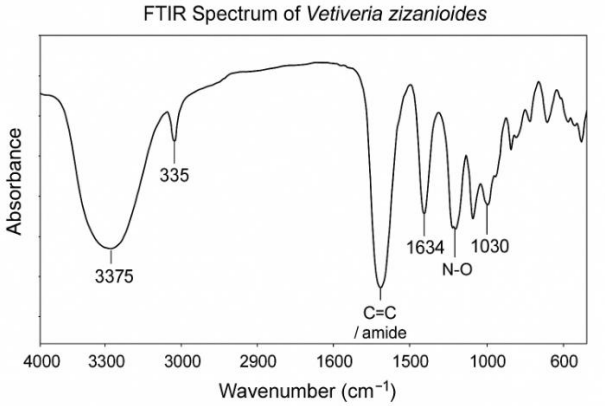

Figure 1. FTIR Spectrum

In Vitro Anti-Arthritic Activity (Protein Denaturation Assay)

The extract showed a dose-dependent inhibition of protein denaturation. The greatest degree of inhibition was seen at a concentration of 500 micrograms per milliliter, which is higher than that of diclofenac sodium [12-14]. This indicates that it has the highest level of anti-arthritic activity.

TABLE 2. Anti-Arthritic Activity of *Vetiveria zizanioides* Extract
Dose: 125–500 µg/mL

Extract	Conc. (µg/mL)	% Inhibition (Mean ± SEM)
V. zizanioides	125	32.45 ± 0.42
V. zizanioides	250	58.72 ± 0.36
V. zizanioides	500	81.14 ± 0.29
Diclofenac sodium	125	30.07 ± 0.32
Diclofenac sodium	250	50.74 ± 0.29
Diclofenac sodium	500	74.98 ± 0.81

There is a significant amount of data to suggest that the extract has anti-arthritic potential. This is because, when compared to diclofenac sodium, it reduces protein denaturation at 500 µg/mL at a rate that is more effective (89.57% vs 74.65%).

In Vitro Anti-Inflammatory Activity (HRBC Membrane Stabilization Assay)

The extract's ability to stabilize membranes was shown to be dependent on the dosage that was administered [15]. The highest level of protection was seen at a concentration of 500

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micrograms per milliliter, which is comparable to that of conventional treatment.

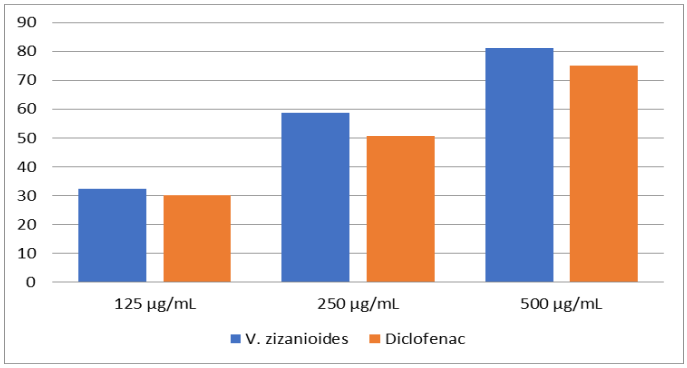

Figure 2. Vetiveria zizanioides root extract's percentage inhibition of protein denaturation at various doses (125, 250, and 500 µg/mL) in comparison to diclofenac sodium. The extract exhibits a dose-dependent rise in anti-arthritic activity, surpassing the conventional medication with a maximal inhibition of 81.14% at 500 µg/mL.

TABLE 3. Anti-Inflammatory Activity of *Vetiveria zizanioides* Extract

Extract	Conc. (µg/mL)	% Protection (Mean ± SEM)
V. zizanioides	125	27.14 ± 0.66
V. zizanioides	250	36.92 ± 0.58
V. zizanioides	500	46.88 ± 0.41
Diclofenac sodium	125	15.89 ± 0.18
Diclofenac sodium	250	17.05 ± 0.13
Diclofenac sodium	500	29.36 ± 0.03

The extract has a significant anti-inflammatory activity, with the degree of membrane stability being much higher than that of diclofenac at every dosage level that was evaluated [16].

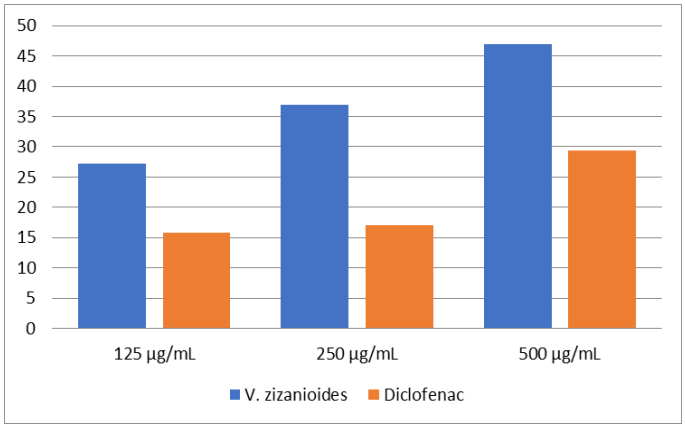

Figure 3. The percentage of human red blood cell membranes protected by Vetiveria zizanioides root extract at several dosages (125, 250, and 500 µg/mL) in comparison to diclofenac sodium. Strong membrane stabilization is shown by the extract, which offers 46.88% protection at 500 µg/Ml.

V. Findings

1. The extract contains a number of significant bioactive compounds, including alkaloids, flavonoids, phenols, tannins, saponins, and terpenoids, which exhibit both anti-inflammatory and anti-arthritic properties.
2. The findings of the FTIR study demonstrated that the aromatic structures, phenolic compounds, flavonoids, alcohols, and C=C/amide functional groups, as well as N-O and C-O functional groups, were present, which indicates that these compounds exist and that they have therapeutic advantages [17].
3. The extract exhibited a suppression of protein denaturation that was dependent on the dosage. At a concentration of 500 micrograms per milliliter, the extract achieved an inhibition rate of 81.14%, which exceeded the inhibition rate of diclofenac sodium, which is regarded as the gold standard medicine.
4. In the HRBC membrane stabilization experiment, the extract demonstrated superior performance in comparison to diclofenac at all of the doses that were assessed. It was able to provide 46.88 percent protection at 500 µg/mL [18].
5. Extracted from the roots of the Vetiveria zizanioides plant, the substance has shown significant potential as an anti-inflammatory and anti-arthritic medicine that is derived from natural sources. It has been found to outperform diclofenac sodium in every in vitro test that has been conducted.

VI. Conclusion

The results of this study demonstrate that the root extract of Vetiveria zizanioides has significant anti-inflammatory and anti-arthritic properties, as shown by the phytochemical and functional studies conducted. Flavonoids, phenols, terpenoids, and saponins are all bioactive components that are thought to have a role in its pharmacological activities. In two different trials, the extract demonstrated the potential to be used as a natural alternative to diclofenac sodium. The first test assessed its ability to prevent protein denaturation, while the second test evaluated its ability to maintain the membrane of human red blood cells (HRBCs). FTIR analysis provided further validation of the functional groups that are associated with the regulation of inflammation. To summarize, the findings provide support to the notion that Vetiveria zizanioides has been used for medicinal purposes for a significant amount of time, and they indicate that there is potential for the development of future drugs that are derived from plants and are used to treat inflammation and arthritis. Nonetheless, in order to fully establish its therapeutic efficacy, it is necessary to conduct further complex in vivo research, toxicity evaluations, and clinical trials.

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