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Review article

A comprehensive review on standardization of Khus powder by analytical method

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Abstract

Vetiver (*Chrysopogon zizanioides*) is a plant that has been used in a variety of disciplines, including medicine, aromatherapy, trade, environmental protection, and agriculture, due to its historic importance and versatility. Vetiver has a rich history in traditional medical practises; nevertheless, just a few research studies have confirmed its usefulness in illness therapy. The goal of this study was to gather current data on phytochemistry and pharmacology. It was discovered that several traditional vetivers use were validated when examined using various disease-based pharmacological models with extracts of roots, leaves, and root oil as test materials. Plants are valued in the pharmaceutical business for their structural diversity and vast variety of pharmacological activity. Phytochemicals refer to physiologically active substances found in plants. These phytochemicals serve as direct medicinal agents. This research focuses on the collecting of Vetiver plants, the qualitative and quantitative analysis of the phytochemicals, and the technique of detection.

Introduction

Vetiveria zizanioides and *Chrysopogon zizanioides* are together known as "vetiver plant," and they are members of the species-diversified Poaceae family, which has 11,337 species and 707 genera, [1] a perennial herbaceous graminaceous plant native to South and Southeast Asia, as well as tropical and subtropical India. Vetiver has been successfully used to stabilise artificial batters for roads and railroads in Africa, Asia, Australia, Central America, Latin America, and southern Europe. Due to its intriguing qualities, the vetiver plant has become extensively distributed over the past few decades not just in the equatorial area but also in other unique places of the world. [2] When it comes to the characteristics of vetiver plants, the root system is made up of a vast, thick, and quickly expanding web of fibrous filaments that may reach a depth of three metres. Vegetables with deep roots may grow on slopes, improve water penetration rates, and produce the necessary grip action to lessen the likelihood of soil layer slide during periods of heavy rainfall [3]. Terpenes/terpenoids, aromatic and aliphatic molecules that are low-molecular-weight fragrance chemicals that give plants their varied qualities, are the main components of essential oils. The goal of the current study was to identify the chemical makeup of *Vetiveria zizanioides* root oil [4].
The plant is cultivated for its fragrant roots, which are a source of vetiver oil, an essential oil. Because of its strong scent, this oil is mostly employed in upscale perfumeries, where it is highly valued for its fixative and perfumery properties. Major producers are Haiti, China, and Indonesia. Indonesia produces between 60 and 75 tonnes of vetiver oil per year, making it the world's largest source of vetiver oil. An estimated 250 tonnes of vetiver oil are traded annually

worldwide; the major producers are Haiti, Indonesia, China, Japan, India, and Brazil, while the major buyers are the USA, Europe, India, and Japan. In commerce, vetiver oil is commonly referred to as vetiver oil [5]. Numerous illnesses have been treated using vetiver, or *Vetiveria zizanioides,* an aromatic herb. Plants are employed as digestive, carminative, stomachic, constipating, haematinic, expectorant, antispasmodic, antiasthmatic, anthelmintic, antimicrobial, and diuretic, according to Ayurvedic literature. In addition to treating ulcers, the roots are utilised to chill the brain. The herb is also used to treat dysmenorrhea, amenorrhea, and anaemia. Due to its calming qualities, vetiver oil has long been used in aromatherapy to treat sleeplessness, nervous tension, anxiety, and stress [6].

Taxonomy

The 48 species of the Andropogoneae tribe, which includes the genus *Chrysopogon,* are dispersed across the world. In early 1999, Veldkamp made an estimate of around 45 species. Three other species were subsequently added: *C. copei, C. purushothamanii,* and *C. castaneus.* This *Chrysopogon zizanioides* was known as *Vetiveria zizanioides* (L.) Nash for more than a century, with the letter L standing for Linnaeus. The systematic classification of plants and animals was pioneered by the botanist Linnaeus. Then, Vetiver was accurately identified as *Vetiveria zizanioides* by Nash and Stapf (1903–1906). Subsequently, plant taxonomist J. F. Veldkamp combined the Vetiveria genus with the Chrysopogon genus, reclassifying the former since there was no discernible distinction between the two. Consequently, in contrast to Vetiveria, it is presently recognised as *Chrysopogon zizanioides* because this genus was established earlier. The word "veter" (from Tamil, India) refers to the removed grass. *Eukaryota, Kingdom-Plantae, Phylum-Spermatophyta, Subphylum: Angiospermae,* Class- *Monocotyledonae*, Order: *Cyperales,* Family: Poaceae, Genus: *Chrysopogon,* Species*: Chrysopogon zizanioides* is the taxonomy of *Chrysopogon zizanioides*. Given that vetiver has 2n = 20 chromosomes, it is a diploid plant. It is roughly divided into two types: South Indian nonflowering and North Indian wild-growing flowering plants [7].

Chemical constituents

This plant contains phytochemical components such as khusimone, khusimol, vetivene, khositone, terpenes, epizizianal, vetivenyl vetivenate, vetiverol, vetivone, iso-khusimol, vetiver oils, benzoic acid, tripene-4-ol, and ßHumulene. Zizaene and prezizaene. The most prominent active components detected are khusimol, vetivone, eudesmol, khusimone, zizaene, and prezizaene, which are thought to be the oil's fingerprint. Vetiver essential oil, also known as *Chrysopogon zizanioides* (L.). One of the most crucial raw ingredients in the fragrance sector is nash. Vetiver is utilised in perfumes both as an ingredient on its own and as a fixative. Since it has such a calming effect, vetiver oil has long been used in aromatherapy to relieve tension, stress, anxiety, and insomnia. A complex mixture of more than 150 sesquiterpenoid components makes up vetiver oil. The use of vetiver oil in aromatherapy dates back many years. The nation of origin has a big influence on the oil's chemical composition and scent quality. The oil consistently contains the sesquiterpenes khusimol, beta-vetivone, and alpha-vetivone, with concentrations as high as 35 percent. Out of the sixty components identified thus far, these three are the most recent. As such, they are considered to be fingerprints of the oil because of their relationship to it, even if they lack the typical vetiver scent qualities [8]. Chemical constituents and their structure are presented in table 1.

Pharmacological action

Anti Inflammatory

The action that reduces pain is anti-inflammatory. These medications can lessen inflammation at specific dosages. It is the body's reaction to pain and damage. These medications are used as long-term pain relief. *Vetiveria zizanioides* (L) essential oil shown anti-inflammatory properties. It primarily functions as an anti-inflammatory in the neurological and circulatory systems. Additionally, it is a useful aid for inflammatory conditions brought on by dry winds, dehydration, and sunstroke [17].

Antioxidant activity

Antioxidant compounds limit the oxidation potential of other molecules. Numerous free radicals are produced during the oxidation process, which might harm a cell. The chain reactions are stopped by these antioxidants. By scavenging free radicals in vivo, the plant extract of *Vetiveria zizanioides* demonstrates antioxidant potential. Free radicals damage DNA and preoxidize lipids, which can lead to a variety of illnesses. *Vetiveria zizanioides* roots are used in ethanolic extract for several antioxidant activities, including reducing oxidation ability, trapping superoxide anion radicals, and overall antioxidant capacity [18].

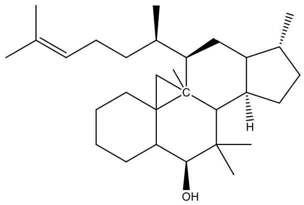
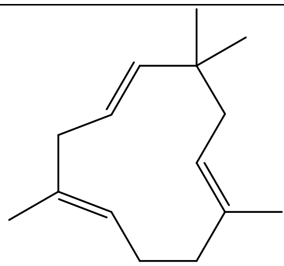
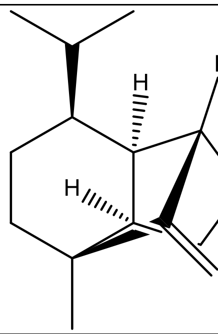
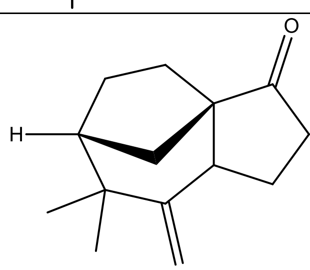
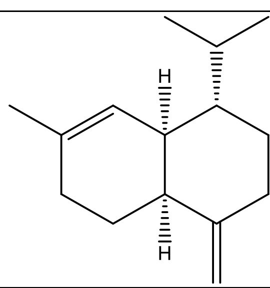
Antifungal activity

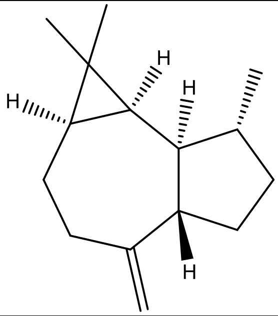
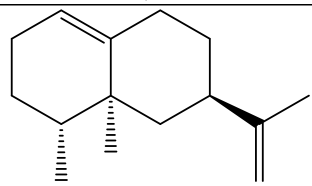
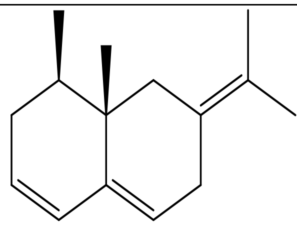
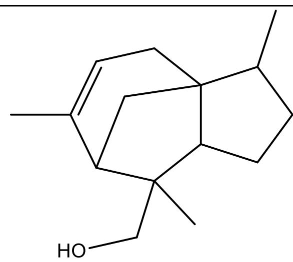
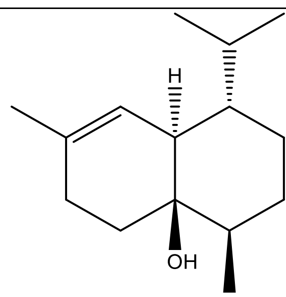
Fungi-induced infections are treated with antifungal medications. When a fungal infection affects only the skin, it is often not too dangerous, but when it spreads to the interior organs, it can be fatal. In an aqueous and ethanolic extract, *Vetiveria zizanioides* shown antifungal activity against standard cultures of *Asperigulls nigra, Asperigulls clavatus*, and *Candida albicanus*. The usual technique was the ager plate method with *griseofulvin* and *nystatin* [19].

Hepatoprotective activity

Vetiveria zizanioides methanolic and ethanolic extracts have demonstrated hepatoprotection. It has been shown that a dose of 300–500 mg/kg of this grass in methanolic extract is beneficial. 20% damage was shown by ethanolic extract at a dose of 3.7 mg/kg [20].

Table 1. Chemical constituents and their structure.

Component name	Structure	Synthetic name	Molecular formula & Molecular weight	Reference
Cycloartenol		(1R,3aS,3bS,5aR,7S,9aR,10aS,12aR)-3a,6,6,12a-Tetramethyl-1-[(2R)-6-methylhept-5-en-2-yl]tetradecahydro-2H,10H-cyclopenta[a]cyclopropa[e]phenanthren-7-ol	$C_{30}H_{50}O$ 426.72g/mol	9
Alpha-Humulene		(1*E*,4*E*,8*E*)-2,6,6,9-Tetramethylcycloundeca-1,4-8-triene	$C_{15}H_{24}$ 222.37 g/mol	10
Sativene		(1R,2S,3S,6S,8S)-6-methyl-7-methylidene-3-propan-2-yltricyclo[4.4.0.02,8]decane	$C_{15}H_{24}$ 204.3 g/mol	11
Khusimene		(1R,8R)-7,7-dimethyl-6-methylenetricyclo[6.2.1.01,5]undecan-2-one	$C_{15}H_{24}O$ 220.350 g/mol	12
Gamma-Muurolene		(1S,4aS,8aR)-1-isopropyl-7-methyl-4-methylene-1,2,3,4,4a,5,6,8a-octahydronaphthalene	$C_{15}H_{24}$ 204.35 g/mol	12

Allo-aromadendrene		(1aR,4aS,7R,7aS,7bS)-1,1,7-trimethyl-4-methylidene-2,3,4a,5,6,7,7a,7b-octahydro-1aH-cyclopropa[e]azulene	$C_{15}H_{24}$ 204.35 g/mol	13
Valencene		(3R,4aS,5R)-4a,5-Dimethyl-3-(prop-1-en-2-yl)-1,2,3,4,4a,5,6,7-octahydronaphthalene	$C_{15}H_{24}$ 204.35 g/mol	14
BetaVetivenene		(8R,8aS)-8,8a-dimethyl-2-(propan-2-ylidene)-1,2,3,7,8,8a-hexahydronaphthalene	$C_{15}H_{22}$ 204.35 g/mol	15
Cedren-13-ol, 8-		(2,6,8-trimethyl-6-tricyclo[5.3.1.01,5]undec-8-enyl)methanol	$C_{15}H_{24}O$ 220.35g/mol	16
Cubenol		(1S,4R,4aR,8aR)-4,7-dimethyl-1-propan-2-yl-2,3,4,5,6,8a-hexahydro-1H-naphthalen-4a-ol	$C_{15}H_{26}O$ 222.37 g/mol	13

Antitubercular activity

The root extract of *Vetiveria zizanioides* has *antimycobacterial* action against Mycobacterium TB at a 500 microgram/milliliter dosage. *Antitubercular* activity detected at extremely low concentration (50 µg/mL) in the Hexane fraction Long-term effects of the dosage persist even after the oil has been kremoved [21].

Mosquito repellent activity

Indian natives employed the root oil as a repellent to mosquitoes. Oil nanoemulsion with a range of 150–160 nm is homogenised, forms a thin layer, and evaporates. For a very long period, this movie worked as a mosquito repellent. Various investigations have demonstrated that varying concentrations of *Vetiveria zizanioides* root oil are also beneficial in inhibiting the proliferation of Anapheles Stephens eggs that are deposited by gravid [22].

Antidepressant activity

There is antidepressant efficacy in vetiver ethanolic extract. When used with fluoxetine, it has much more positive benefits. Plantae zizanioides acted as a mood enhancer [23].

Antihyperglycaemic activity
Vetiveria zizanioides (L) Nash roots were used to create an ethanolic extract having antihyperglycemic properties. The ethanolic root extract of *Vetiveria zizanioides* has antihyperglycemic properties. This extract's impact on rats under controlled conditions is beneficial [24].

Antibacterial activity
The antibacterial properties of *Vetiveria zizanioides'* ethanolic root extract Using the zone of inhibition, linn is assessed. The antibacterial properties of flavonoids, terpenoids, and tannins are present in the ethanolic root extract of *Vetiveria zizanioides.* Four bacterial strains—two gramme positive *S aureus*, *B. subtilis*, and two gramme negative bacteria, *P. aeurogenosa*, E. coli—perform the task. The results of the study demonstrate the antibacterial action of flavonoids against gram-negative bacteria. They bind to microbial cell membranes to produce complexes that impair their function. Root extract contains tannins, which exhibit antibacterial action through the formation of complexes with polysaccharides, microbial enzyme adhesion, and cell transport protein. Thus, it can be said that the active ingredient in the ethanolic root extract of *Vetiveria zizanioides* with antibacterial activity in vitro is tannin [17].

Qualitative and quantitative analysis
Qualitative and quantitative analysis of phytochemicals can be done using Gas Chromatography-Mass Spectroscopy (GCMS). GCMS can be applied to solid, liquid and gaseous samples. First the samples are converted into gaseous state then analysis is carried out on the basis of mass to charge ratio. High Performance Liquid Chromatography is applicable for compounds soluble in solvents. High performance thin layer chromatography is applicable for the separation, detection, qualitative and quantitative analysis of phytochemicals [25].

Gas chromatography
Volatile chemicals are separated using this method. The chemical species' distribution in the gas phase yields the rate of kinetics for that species. In gas chromatography, a sample is injected into the chromatographic column's head after being evaporated. The flow of the gaseous, inert mobile phase moves the sample through the column. A liquid stationary phase is present in the column itself and is adsorbed onto an inert solid's surface [25].

High Performance Liquid Chromatography (HPLC)
The abbreviation HPLC stands for High-Pressure Liquid Chromatography. By doing this, chemicals are separated according to how they interact with the solvent of the mobile phase and the solid particles in a densely packed column. The analyte must be eluted through the column and through the detector at pressures as high as 400 bars. Compounds that cannot evaporate or that break down at high temperatures can benefit from HPLC. In a single procedure, HPLC offers both quantitative and qualitative analysis [26].

High Performance Thin Layer Chromatography (HPTLC)
As the method developed, high performance thin layer chromatography (HPTLC) became a crucial tool for drug analysis. HPTLC is a quick separation method that may be used to evaluate a broad range of samples because of its flexibility. This method has several benefits since it is easy to use and takes little time to assess both complicated and basic sample cleanups. Without regard to time constraints, HPTLC assesses the complete chromatogram using a range of criteria. Additionally, several samples and standards are developed independently and simultaneously on each plate, increasing the dependability of the results. Drugs including ethinyl estradiol, cyproterone alfuzosin, tramadol, and pentazocine have all been quantified using HPTLC [27].

Optimum Performance Laminar Chromatography (OPLC)
The benefits of TLC and HPLC are combined in OPLC. Depending on the model, the system may handle up to 4 or 8 samples at once while simultaneously separating samples weighing between 10 and 15 mg. A pump is used in OPLC to push a liquid mobile phase through a stationary phase, like bonded-phase media or silica. Flat planar columns may be utilised in the same applications as cylindrical glass or stainless-steel columns thanks to the OPLC column housing construction. A solvent delivery pump forces the mobile phase through the flat column at a constant linear velocity while it is under pressure up to 50 bars. Everything needed to successfully separate the compound sample of interest is included in the work station, including two 96-well plate sample holders, an automated sampling system that takes a sample from each well and deposits it on the OPLC planar sorbent bed, an OPLC purification unit, a solvent delivery system with a mobile phase degasser and pump, a four-channel diode array detector to keep an eye on the eluent, and six 96-well plates to hold the separated compounds [28].

Methods of detection
Spectroscopy is used in the detection of phytochemicals. The following are frequently used in the study of phytochemicals.

UV spectroscopy
The measurement of a light beam's attenuation after it passes through a sample or after it is reflected off a sample surface is known as ultraviolet and visible spectroscopy. Since ultraviolet light has the energy to raise outer electrons' energy levels, it is typically used to study molecules or inorganic complexes in solution. This is the outcome of the electrical energy levels changing. By using Beer- Lambert's law to measure the absorbance at a certain wavelength, one may ascertain the analyte solution's concentration. The area of the electromagnetic spectrum between 100 and 400 µm is

included in optical spectroscopy. One of the most often used techniques in pharmaceutical analysis is UV-visible spectrophotometry. Characterizing the absorption, transmission, and reflectivity of several significant materials, including pigments and other plant-based chemicals, is helpful. In order to characterise the optical or electrical characteristics of materials for this qualitative application, at least a portion of the UV-visible spectrum must be recorded [29].

IR spectroscopy

The functional group included in the sample is identified using infrared spectroscopy. The measurement of a sample's mid-infrared light absorption wavelength and intensity is known as infrared absorption spectroscopy. Molecule vibrations can be excited to greater energy levels by mid-infrared light due to its high energy. Since many IR absorption bands have wavelengths that correspond to certain kinds of chemical bonds, IR spectroscopy is most useful for analysing organic and organometallic compounds qualitatively. IR spectroscopy is a useful technique for identifying freshly generated molecules and for verifying the authenticity of certain compounds [30].

Nuclear Magnetic Resonance Spectroscopy (NMR)

The main focus of NMR is on the magnetic characteristics of certain atomic nuclei, such as the hydrogen atom nucleus, the proton, the carbon atom, and an isotope of carbon. Many researchers have been able to examine molecules by using NMR spectroscopy, which records the differences between the different magnetic nuclei and provides a clear image of the locations of these nuclei inside the molecule. Additionally, it will show which atoms are found in nearby groupings. In the end, it can determine how many atoms are in each of these settings. Preparative or semi-preparative thin-layer chromatography, liquid chromatography, and column chromatography have all been used in the past in an effort to isolate individual phenols, the structures of which are then ascertained by NMR off-line [31].

X-Ray crystallography

The experimental method known as x-ray crystallography makes use of the fact that crystals scatter X-rays. X-rays can be dispersed by the electron cloud of an atom of similar size because they have the right wavelength (between 10 and 8 Angstroms). The electron density may be reconstructed using the diffraction pattern that results from X-ray scattering off the periodic assembly of molecules or atoms in the crystal. To finish the reconstruction, more phase information might be retrieved from the diffraction data or from a supplementary diffraction experiment. After gradually incorporating a model into the experimental electron density and refining it in light of the data, a rather accurate molecular structure is produced [32].

Fluorimetry and Phosphorimetry

The pharmaceutical industry is always searching for micro sample-based, sensitive analytical methods. One method that achieves great sensitivity without sacrificing accuracy or specificity is fluorescence spectrometry. There has been a noticeable rise in the quantity of publications published recently about the use of fluorimetry and phosphorimetry in the quantitative study of different medications in dosage forms and biological fluids [33].

Mass spectroscopy

One effective technique for material identification is mass spectrometry. Among the most vital instruments in the biological sciences, mass spectrometry may be used for everything from protein characterisation to small molecule analysis. Effective for identifying new chemicals, quantifying known compounds, and clarifying the structure and chemical characteristics of molecules, mass spectrometry is a potent analytical tool. The MS Spectrum may be used to determine the sample's molecular weight. Certain kinds of mass spectrometers can also produce structural data. By simultaneously defining the molecular weight and a diagnostic fragment of the molecule, this procedure is helpful for both the structural elucidation of organic compounds for peptide or oligonucleotide sequencing and for highly specific monitoring of the presence of previously characterised compounds in complex mixtures [34].

Conclusion

Many nations, particularly in Asia, have long employed vetiver as an aromatic and therapeutic herb. It has recently gained widespread reputation as the perfect plant for protecting the environment, conserving water and soil, and both. The fact that vetiver is being studied more and more for conservation purposes and for medicinal purposes indicates that vetiver has a great deal of potential to serve people all over the world in terms of economic, environmental, and therapeutic advantages. Further advantages are hampered by the complexity and decreasing availability of root oil extraction for small institutes. Given its wide range of applications, vetiver conservation efforts might ultimately prove to be a win-win situation for all parties involved something that will only be achievable with cooperation from both developed and developing nations. Further research suggestions and innovative extraction methods are needed to improve the quality, accessibility, and usefulness of vetiver.

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Conflicts of interest

The authors report no conflicts of interest in this work.

Ethical approvals

This study does not involve experiments on animals or human subjects.

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Data availability

All data generated or analyzed during this study are included in this published article.

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