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Natural Products from Heath Forest's Sedges with Toxicological Properties: Identification and Characterization of Bioactive Compounds

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Abstract

Superabsorbent Heath woods, also known as kerangas woodlands, vary from the nearby dipterocarp woods in terms of composition and structure. They thrive on sandy, nutrient-poor soil and are widespread throughout the tropics. In order to fully understand each species' potential, it is necessary to do a thorough investigation of the component makeup of the plant species that are present in these forests. *Lepironia articulata*, *Dapsilanthus disjunctus*, and *Eleocharis ochrostachys* are only a few examples of the several sedge plant species found in heath woods. Then, using a photo-linked outstanding durability chromatography of liquids technique Diode Array Detector (HPLC-DAD), they were able to identify phenolic compounds and specific phenolic acids. The sedges were formerly prized for their beneficial traditional qualities since they contain a number of bioactive components. In this study, the researchers employed an alkaline approach to extract the water-soluble chemicals from the sedge species. Additionally, it was discovered that *Lepironia articulata* and vanillic acid were the two main phenolic ingredients in those plants, However, *Eleocharis ochrostachys*' primary polyphenol constituent was trans-p-coumaric acid. The results showed that caffeine, ferulic acid, 4-hydroxybenzoic acid, vanillic acid, and the sedge variety had high quantities of trans-p-coumaric acids. The phenolic ingredient in them that dominated in the sedge plant extracts was trans-p-coumaric. By discussing FT-IR and HPLC analysis applications in this study, researchers hoped to ignite their interest the discipline of organic vegetation identification.

Keywords: Allelopathy, *Dapsilanthus disjunctus*, *Eleocharis ochrostachys*, heath forests, *Lepironia articulata*, natural poison

Full length article *Corresponding Author, e-mail: sachin_sls@iimtindia.net

1. Introduction

Natural products produced by plants, animals, and fungi, as well as their compounds that are bioactive, have been thoroughly researched and examined over the past few decades for use as treatments in a variety of medical conditions for example, cardiovascular, hypertension, diabetes, reproductive, malignancies, and neurological conditions [1]. Brain diseases such as Huntington's and dementia, alzheimer and Parkinson's disease, and a condition called amyotrophic lateral sclerosis all result in the slow breakdown and degradation of neuron functionality and organization, which leads to the death of neuronal cells. Because dementia is caused by multifactorial pathological processes, targeting various pathways for effect and a neuroprotection technique, which entails shielding nerve cells from dying and restoring their functionality, could be intriguing options for both the avoidance and cure of neurodegenerative conditions [2]. Natural products have come about as promising neuroprotective substances for Neurodegenerative disease therapy. Therapeutic potential of organic goods and their biologically active components to produce a preventative impact on neurological disorders. The increasing malfunction and loss of neural function and structure that results in the death of neurons are referred to as degeneration [3]. Neurodegeneration occurs in a variety of central nervous system (CNS) illnesses. the scientific appearance of acute and ongoing neurological conditions is determined by the demise of different kinds of neurons associated to functioning neural networks. A category of neurological illnesses known collectively as "neurodegenerative diseases" primarily affect neurons in the CNS and are characterised by a steady decline of cells in the CNS that impairs likely brain processes [4]. Acute neurotoxicity is a condition when cells are quickly injured and typically die as a result of a severe shock or trauma such as head trauma, strokes, trauma to the head, cranial or subarachnoid hemorrhaging, and ischemia of the brain. Rapid neurotoxicity is a disorder whereby cells are quickly injured and typically die as a result of a severe shock or

traumatic occurrence such as head injury, strokes, trauma to the brain, cranial or subarachnoid bleeding, and ischemia of the brain. Neurodegeneration has been linked to a variety of molecular pathways, include oxidative stress, neurological inflammation, excitotoxicity, dysfunction of the mitochondria, aberrant protein folding and collection, and death [5]. These physiological processes have been linked to the onset and development of neurological diseases. Extensive research has been conducted to date in order to clarify the underlying causes and prospective therapy targets for neurodegenerative diseases. As a result, neurological protection techniques and related mechanisms are most effective in preventing or delaying the development of dementia by interacting with the pathologic alteration processes. Natural products have been recognized and used as having medicinal powers since ancient times [6]. Natural products and their chemical constituents has been the subject of much research in the last several decades to ascertain their function in physiology, nutritive qualities, and potential wellness and medical advantages [7]. Over the recent years, many analysts have shown the preventative impact of organic materials and bioactive molecules against a wide range of ailments, including cardiovascular, diabetic, reproductive, cancer, and neurological problems. Natural ingredients are showing promise as antioxidants for the treatment of neurological conditions [8]. This study concentrated on the curative abilities of natural goods and their bioactive elements in order to exhibit neuroprotective properties on neurodegenerative pathologies. This study [9] looked at the impact data from Sentinel-2 and fuzzy logic frameworks were used to assess the condition of forests using several flora metrics. The beat-wise geographic disruptions of human and natural causes in the research region were determined using a GIS-based analytical hierarchy process (AHP). The [10] study, diverse forests seem to provide more of the majority of the ecosystem's goods and services, such as increased biodiversity and enhanced mitigation of risks, soil characteristics, and multiple-use benefits. Cervid browsing, particularly ruins sapling stands, is the most important threat. With current regenerating procedures and materials, it is possible to generate single-story mixed woods. This forest fire erupted under unusual meteorological scenarios. The climatic condition was evaluated at both the synoptic and local scales using climatic fields derived from ECMWF's ERA-Interim world forecast [11]. The study [12] examined the present state of Sumatran pine and the potential as a rehabilitated and restored species, as well as provides a map of its artificial and natural distribution in Java. Furthermore, the article will demonstrate the genetic diversity of the species, Identify electrical advancements in forestry, both at plantations and crop sizes, describe tree improvement programs, including their role in farming customs, and pine products both wood and non-timber. The study [13] intended to characterize these leftovers and assess their efficacy as soil amendments or medium for development constituents. Residues are classified largely depending on their source and the extraction process utilized. The study [14] effected of forestry on antler farming and the effects of caribou herding on forests for the purpose of the research. We investigated the viewpoints of antler herding and forests professionals on the effects of woodland initiatives on antler farming and the relationship between woodland and animal farming. The study [15] is to define the oil that is essential (EO) and the water distillation water that remains (HRW), the result of manufacture, of Artemisia campestris subsp. maritima via Portugal, as well as to gauge the antioxidants, antifungal, antibacterial, and wound recovery operations of both takes at non-toxic levels. The study [16] was to isolate and characterize protein from the outer layer of a cylindrical goby-N. melanostomus in the Black Sea. The findings showed that the skin of N. melanostomus had a significant amount of protein (80%), was rich in glycine (34%), alanine (11%), proline (9%), and both hydroxyproline (7%), all of those that are typical of connective proteins. The study [17], they take a close look at the various vegetable and fruit waste streams, their defining features, the bioactive materials found in waste streams, extraction methods, and potential uses for the extracted compounds. The study [18] focused on the uniqueness of these contemporary approaches as well as the impact of their material characteristics. Along with to the primary concern of liquid choosing, physical variables used by the various procedures impact their extraction outcomes in various ways.

2. Materials and methods

2.1 Plant Materials and Sample Preparation

For this research, gathered a range of plant grasses from Terengganu's heath forests, included *Eleocharis ochrostachys*, *Dapsilanthus disjunctus*, or *Lepironia articulata*. Following that, the International Islamic University's botanical gardens unit, faculty of landscaping and architectural design, and kulliyyah of building and environment validated the existence of the grass taxonomy. In order to the phenolic compounds that existed, please in the turf samples using the high-performance LC (HPLC) method, the researchers bought the phenolic acid reference chemicals.

2.2 Sequential Alkaline Extraction of Sedges Species

In earlier progressive caustic extraction using solvent method was adapted to extract phenolic compounds present in the sedge variety. Using a sodium hydroxide (NaOH) solution for 12 hours at 60°C in the oven is a technique for collecting free and related phenolic compounds from crop samples. Hydrochloric acid (HCl) was then used to sterilize the alkaline extracts when the pH was 2. The obtained centrifugation of samples, and the resulting liquid was extracted twice, once with ethanol and once with ethyl acetate as the solvents. Following the extraction process, the samples that were dried of the dehydrated compounds were redissolved in alcohol for an HPLC examination.

2.3 Identification of Phenolic Functional Groups

Utilizing an FT-IR analyzer the functional chemicals present on the methanol extracts were identified. Materials were evaluated at absorption ranges that ranged from 400 to 4000 cm^{-1}.

2.4 Determination of Total Phenolic Content (TPC)

To assess the TPC of the aforementioned extract, the study team made a little adjustment to the Folin-Ciocalteu technique. Declining harvester was used to calculate TPC. After the outcomes were determined using the TECAN plate reader, the TPC results were represented as "Gallic Acid Equivalence (GAE)" per ounce of dry matter of the test specimens.

2.5 Identification of Individual Phenolic Compounds

The researchers evaluated the specimens utilizing HPLC equipment to determine each of the phenolic acid components contained in the extracts. The Diode Array Detector (DAD) with a Automatic Sampling and Sequential Compressor Injector make up the HPLC system. For every minute of the HPLC run, the sample extracts were run on a Zorbax SB-C18 Reverse Stage cartridge at 25°C. The investigators utilized a gradient-based elution procedure with two mobile phases to analyze the extracts: Phase an included diluting 1% of formic acid was used. Both 100% acetonitrile and water/acetonitrile (90:10 v/v) showed its presence in Stage B. Assessments have been carried out using the following types of removals drops: linear from 25.10 to 35 minutes with 100% B, strait from 35-35.1 min with 100% B to 0% B, and equal from 0 to 20 seconds with 0% B to 40% B. While the extracts were being separated at a flow rate of 0.4 ml/min, the contents of phenolic acids in each sample were be continuously evaluated at 280 nm. Researchers were able to identify these substances by comparing the duration of retention of the phenolic acids to those of other chemicals and ultraviolet (UV) radiation.

2.6 Statistical analyses

The findings of this investigation were statistically examined by the investigators. Because all studies were done in three copies, the amounts of the phenolic acid constituents identified in this research were reported as the Mean deviation (SD). The scientists used the XLSTAT-Pro (2014) application (Addinsoft, France). Every experiment indicated pertinent median phenolic acid values with a degree of 99 percent accuracy (p0.001).

3. Results and discussions

3.1 Analysis of Phenolic Functional Groups

Regarding the likely functional groups that are contained on the compounds that are active, the FT-IR spectroscopy investigation offers a wealth of pertinent information. Three different the sedge species, were used in this investigation. The compounds that are active present in the dried leaves of these three sedge species were analyzed for their categories of function by the researchers. Following the FT-IR study, different plant species underwent successive acid extraction and part distilling. The findings of the functional category analysis are shown in Table 1. The researchers also provided a comparison between peak absorption frequencies with those from the FT-IR data library in the table.

According to the findings, there were five primary chemical groups present in the vegetative species: aromatic bitcoins, alcohols, aromatic hydrocarbons, aliphatic ketones, and aliphatic hydrocarbons. Following the FT-IR examination of the leaves that have dried, the progressive alkaline extraction also showed the presence of several operational groups in the various solvent extracts (Table 1). First, the major peak was seen in the hydrocarbon fraction at 1711.28 $cm^{-1}$and 2956.28 cm^{-1} With regard to aliphatic ketones, The C=O stretching was attributed for the unusual bulge at 2950-2975 cm^{-1}, while aliphatic hydrocarbons were associated with the CH_3 asymmetrical bond signal at 1700–1725 cm1. Secondly, the major peak was also visible in the ethanol phase at 1603.55 cm1. The C=C vibration (present in aliphatic hydrocarbons) might be the source of the distinctive peak seen between 1700 and 2000 cm1. At 1051.67 cm1 and 1219.42 cm^1, respectively, the alcohol fractional extract revealed the existence of two major maxima.

Alcohols were ascribed to the -C-O stretching and -OH deformation at 1200 cm^{-1}, and the primary peak noticed between 1050 and 1150 cm^{-1},was allocated to the C-O stretching. Butanol extract failed to demonstrate the existence of any groups that function. As a result, after FT-IR analysis of the D. Three types of functions were found in disjunctus extracts. The researchers discovered this when they used FT-IR to examine successive alkaline extraction conducted from dried *L. articulata* leaves, the major peak was seen in the extract of hexane at 2956.54 cm^1. The CH3 asymmetric bond was attributed to the peak measured between 2950 and 2975 cm^1. Two significant peaks in the ethanolic extract could be seen at 1125.15 cm^1 and 1221.01 cm^1. While the peak recorded at 1050–1150 cm1 was connected with the C–O stretch, the height identified at 1200 cm^1 was associated with alcohol. The portions of butanol and ethyl acetate lacked any functional groups. Three function categories were consequently suggested by the components.

After that, the primary signal at 2956.78 cm^1 was discovered by FT-IR analysis of the hydrocarbons component of the subsequent alkaline extraction from dried *E. ochrostachys* stems. The CH_3 asymmetry peak was identified between 2950 and 2975cm^1. The primary peak of the butanol fractional extract was seen at 2963.99 cm1. The CH3 asymmetrical bond was given credit for the height at 2950-2975 cm^1At 1092.74 cm^1, the ethanol fraction had the primary peak. The C-O stretching and -OH deformation were attributed to the distinctive peak at 1100 cm1. Ethyl a fraction, however, lacked any groups that were useful.

Table 1: The FT-IR peak intensities for *Dapsilanthus disjunctus*, *Lepironia articulata*, and *Eleocharis ochrostachys* were obtained using sequential alkaline solvent extraction into a mixture of ethyl acetate (DEA), butanol, and methanol

Sedge species	Solvent	Group frequency (cm^{-1})	Functional group	Bond	Peak (wave number cm^{-1})	Intensity
Eleocharis ochrostachys	Hexane	2951-2976	Aliphatic hydrocarbon	CH3 asymmetric	2855.77	92.729
	Butanol	2952-2977	Aliphatic hydrocarbon	CH3 asymmetric	3064.98	89.594
	Ethanol	1101	Alcohol	-C-O stretch &-OH deformation	1193.75	58.527
Dapsilanthus disjunctus	Hexane	1706-1726	Aliphatic ketones	C=O stretch	1712.30	98.032
		2951-2976	Aliphatic hydrocarbon	CH3 asymmetric	2857.29	99.349
	Ethyl acetate	1701-2001	Aromatic hydrocarbon	C=C vibration	1604	97.745
	Ethanol	1052-1152	Aliphatic hydrocarbon	C-O stretch	1052.66	92.901
Lepironia articulata	Hexane	2952-2975	Aliphatic hydrocarbon	CH3 asymmetric	2857.53	96.433
	Ethanol	1051-1151	Aliphatic ether	C-O stretch	1126.17	95.719
		1201	Alcohol	-C-O stretch &-OH deformation	1222.04	98.948

Table 2: Total phenolic content for *D. disjunctus, L. articulata and E. ochrostachys*

Total phenolic content	Sedge species
23.87 ± 0.15	*Eleocharis ochrostachys*
785.98 ± 7.04	*Dapsilanthus disjunctus*
985.64 ± 5.97	*Lepironia articulata*

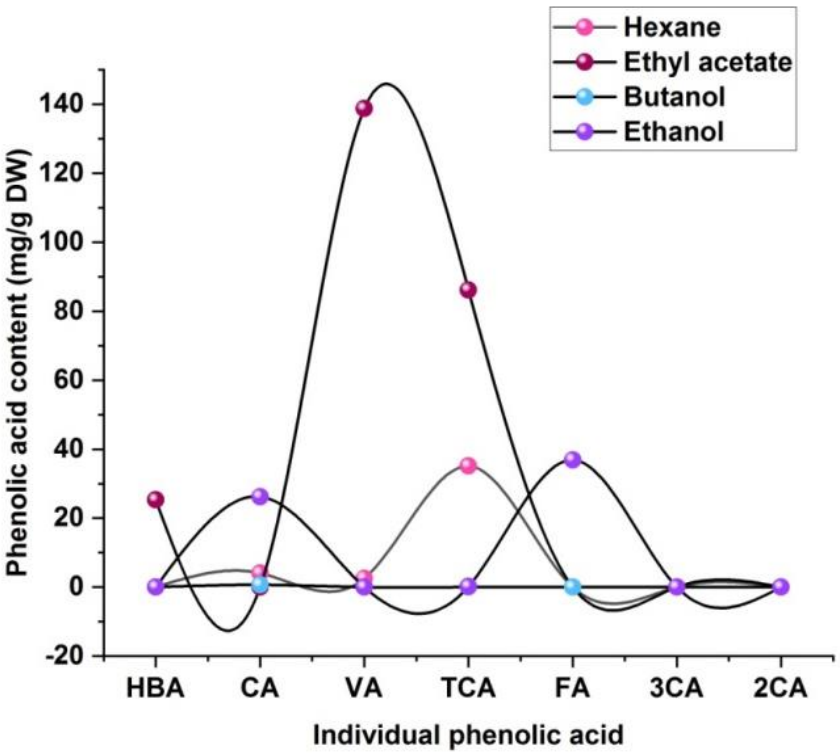

Fig. 1: Sequential alkaline extraction of compounds from a solution of ethyl acetate, butanol, and ethanol revealed the levels of phenolic acid level in *E. ochrostachys*

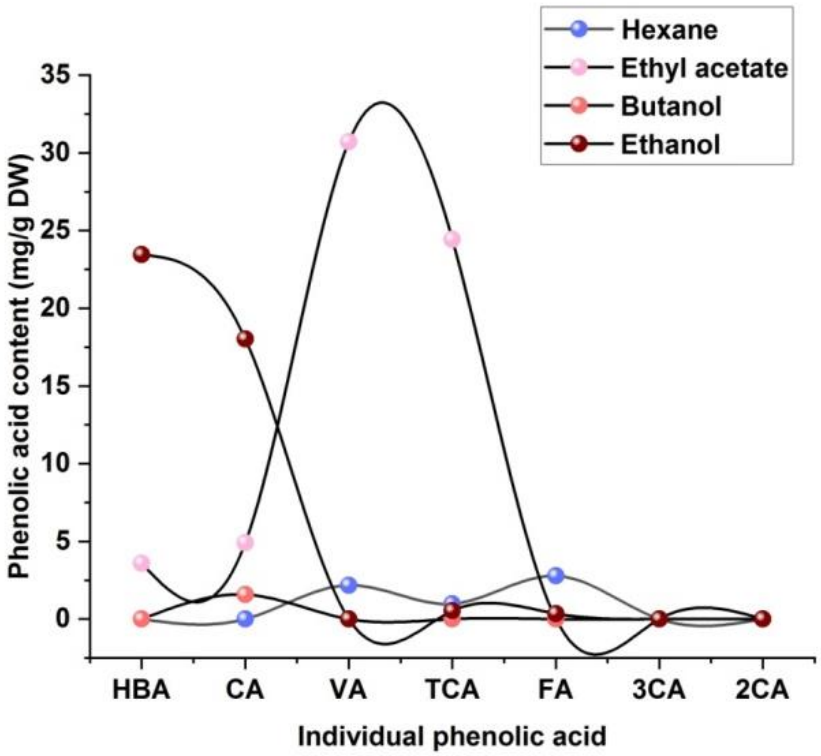

Fig. 2: *D. disjunctus* had no phenolic acid present after repeated alkaline solvent the extraction from a solution of ethyl acetate (ETA), butanol, and alcohol

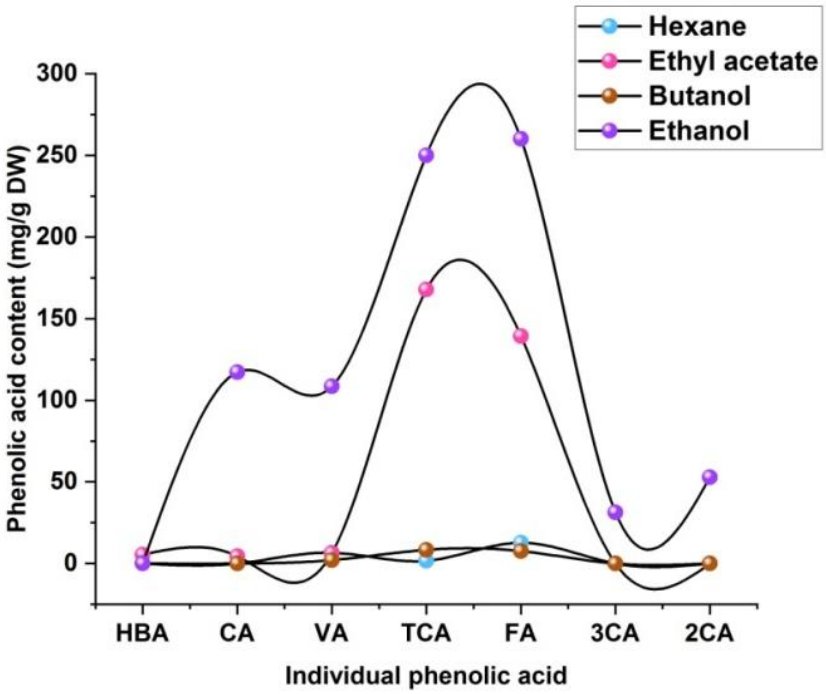

Fig. 3: The phenolic acid content of *D. disjunctus* was obtained using sequential alkaline extraction with solvent from an a solution of what is and acetate of ethyl solution

Thus, following FT-IR studies of the leaf extracts, 2 functional categories were able to be identified. The sedge taxa demonstrated the existence of several functional groupings, in sum. Except for butanol, the dried leaf solvent portion of *D. disjunctus* included a variety of chemically active groups, including aliphatic hydrocarbons, aliphatic ketone, volatile hydrocarbons, and alcoholic. Nevertheless, neither the butanol nor the ethyl acetate (ETA) fractions of contained any functional groups, nor did the ethyl acetate fraction.

3.2 Phenolic Compound and Individual Phenolic Acid Analysis in the Sedge Genus

Total phenolic content (TPC) was calculated using the Folin Ciocalteu method for all three species of sedges collected from heath trees and then translated to gallic acid equivalents. The results showed that the TPC values for *L. articulata* and the D. articulata variation were 984.63 and 5.96 g GAE/g DW, respectively. Disjunctus variety (Table 2) and *E. ochrostachys* variety. The outcomes of each phenolic compound's highest-pressure liquid chromatography (HPLC) analysis are shown in Fig. 1, 2 and 3. The leaf extractor of A had the highest proportion of vanillic acid. Articulata, with concentrations of 138.72 g/g DW (Fig. 2) and 114.72 g/g DW (Fig. 3), respectively. Trans-p-Coumaric acid having the greatest amount in the neutral draws of *E. ochrostachys* causes (Fig. 1). It was intriguing to observe that all five major phenolic acids—"ferulic acid, vanillic acid, 4-hydroxybenzoic acid, caffeine acid, and trans-p-coumaric acidity"were found in high concentrations among grass species.

The findings showed that ethanol-based preparations of *E. ochrostachys* stems included higher concentrations of phenolic chemicals than those produced by ethyl acetate, hexane, or butanol extraction. The *D. disjunctus* extracts were also found to have the highest concentrations of trans-p-coumaric acid, ferulic acid, caffeic acid, and 4-hydroxybenzoic acid among the various chemical components (Fig. 2). The findings showed that extracts dissolved in hexane and propane had the lowest quantities of polyphenol acid, whereas ethyl acetate had the highest concentration. Disjunctus extracts of 3-coumaric acid and 2-coumaric acid are absent from the D sample. Vanillic acid, p-coumaric acid as a Trans, 4-hydroxybenzoic acid, and ferulic acid (2.78 g/g DW) were also present in greater amounts in the *L. articulata* extracts than other substances.

The findings showed that the predominant phenolic components in the sedge species extract were trans-p-coumaric and vanillic acids. However, neither 3-Coumaric acids nor 2-Coumaric acids were present in the specimens from D, nor were *L. articulata* or disjunctus. The most effective solvents for using to extract phenolic components from the sedge variety were discovered to be vodka and ethyl acetate. It was decided that polyphenols that are were the best choice for utilization. Due to the fact that it helps identify and quantify the numerous phytochemical substances in extracts from plants, HPLC is a separation method that is often utilized. The herbaceous extracts' inhibitory action against g-negative and g-positive pathogenic bacteria is further highlighted by the amount of phenolic acids in them. The usage of phenolic acids may

reduce the use of various synthetic fungicides as reducing their impact on the natural world.

The membrane's electric prospective, particularly regulates the interchange of external and interior cellular components, is another factor that the phenolic chemicals influence. As a result, phenolic acids have a greater biological relevance and should be researched to find out how they might stop the development of disorders. Ferulic acid, one of the phenolic acids, has shown strong antibacterial action against a variety of infectious microorganisms. The minimal inhibitory concentration (MIC) of a chemical determines its ability to kill bacteria. Researchers have previously reported that p-Hydroxybenzoic acid has strong antibacterial action versus species of bacteria and fungi in addition to strong antioxidant effects against free radicals. Additionally, the endocrine and antimutagenic activities of the p-hydroxybenzoic acid were greater. Cinnamic, p-hydroxybenzoic, and p-coumaric acids were examined for their antimicrobial characteristics, and these activities were compared to those of their glucuronate-protected counterparts and methylation derivatives. Scientists have found phenolic chemicals, which may be utilized as allelopathic medications, in a number of Eucalyptus species, including. In a separate experiment, researchers discovered that the extract of ethanol was the primary contributor to the *D. disjunctus* species had an effect on locomotion and showed effectiveness on par with that of renowned anti-anxiety drugs.

4. Conclusions

The researchers in the present research emphasized how FT-IR and HPLC studies may be utilized to find being present several phytochemical components in various extracts of plants. Here, the scientists conducted an early investigation where they discovered that the three sedge taxa they chose were members of 5 functional categories. To find out whether there are any more bioactive substances present in the sedge variety that could be useful in various contexts, additional study must be done. Future efforts to isolate the bioactive substances will need the use of several chromatographic and purification methods. According to the study's findings, sedge taxa have the potential to be employed as an inherent herbicide or toxin as well as an extra or substitute source for phenolic chemical compounds.

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