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ORIGINAL ARTICLE

Unveiling the Antimicrobial Activity of Methanol Extract from *Cyperus articulatus* Rhizomes

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ABSTRACT
We analyzed the antibacterial activity of different species of bacteria and fungi by a methanol extract made from the rhizome of the Cyperus articulatus linn. prior to extract cannabis, which was obtained from the Cyperaceae originated. Bacteria, fungi, and some funguses were examined. Classification was performed on two species of gram-positive bacteria, namely Staphylococcus aureus and Streptococcus aerugenosa. Additionally, classification was performed on four species of gram-negative bacteria, including Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi, and Proeus vulgaris. The methanol extracts were pre-processed in the pdf using a Soxhlet Instrument once the rhizomes were obtained from rural Birbhum, West Bengal, India. After the methanol extract seeps into it, it is saved, while the dried condensed extract is stored for later use. The extract's antibacterial potential was assessed using the paper polymorphism test as well as the microdilution method. For comparison, standard antibacterial and antifungal medicines such as streptomycin, ampicillin, and amphotericin B were used. The rhizome extract's antibacterial activity against both pathogens and fungus were comparable to that of the traditional medicines. Furthermore, due to the fact that the distillation extract antibacterial activity was always more considerable against pathogenic fungi, it becomes significant to remark.
Keywords: *Cyperus articulatus, Disc diffusion assay, antimicrobial activity, Minimum inhibitory concentration (MIC).*

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INTRODUCTION

For a great length of time, individuals have relied on herbal medicines as a technique of assisting them in maintaining their health and treating a selection of disorders. As a result, herbal remedies have grown increasingly popular [1]. Among the various reasons that have led to the widespread use of herbal medicines, this scenario is one of them. These medicines that are generated from plants have had a tremendous influence on human health as a consequence of drawing inspiration from long-standing traditions and folklore while making them. The creation of these drugs is a direct result of this happening [2]. The examination of medicinal plants that have been used in traditional practices has made it possible to identify a number of essential medicines. This achievement was made possible by the fact that these plants have been used. In order to achieve this objective, an investigation into plants that have therapeutic properties has been carried out [3]. In the immediate aftermath of the great advancements that have been accomplished in the realm of scientific research, this is the instant consequence that has evolved as a result of those achievements. There are a substantial number of medicinal plants that possess antibacterial characteristics, and a growing number of people from all over the world are starting to become more aware of this fact. They are becoming more alert every day. An increasing number of individuals are becoming conscious of this occurrence [4].

In spite of the fact that there are synthetic antimicrobial therapies that are available, the utilization of herbal remedies continues to be the primary method of therapy for a significant number of people even in this day and age. This is due to the fact that homemade herbal treatments are completely natural and do not contain any artificial components [5]. It is estimated that over eighty percent of the world's

population makes use of herbal medicine, as indicated by the data that was made available to the public by the World Health Organization (WHO). This data is derived from the numbers that were provided in the previous sentence. The inappropriate use of synthetic drugs, on the other hand, has led to the development of drug resistance in both plants and people. This drug resistance has been seen in both species. Both types of species are affected by this phenomenon. A problem of this sort has been identified as one of the issues that have been seen in both species. [6] Furthermore, there have been instances of patients developing resistance to the medications that are being administered. In addition, there is evidence that certain plant species have developed resistance to the drugs that they are commonly treated with. Moreover, this is in addition to the findings that were discussed before. It is possible for synthetic antibiotics to have a variety of unfavourable side effects, some of which include hypersensitivity, immunosuppression, and allergic reactions, amongst other reactions. There are a great number of potential negative consequences, and these are only some of them. Because of this, there is an immediate need for research into non-traditional antimicrobials, such as those that are produced from plants. This demand is a direct result of the situation. This is the situation that has arisen as a result of the relevance of the subject matter. There are other antimicrobials that are derived from animals, which is another form of antimicrobial [7].

It is possible to locate it all throughout the Deccan Peninsula in India, and the word "Piri piri" is commonly used to refer to it in Hindi for the purpose of referring to it. It is something that can be found anywhere. The dry, hilly, and rocky regions of Eastern, Central, and Southern India are among the most suitable habitats for the development of *Cyperus articulatus* Linn. These locations are among the most optimal environments in the globe. Additionally, it is important to point out that these places are among the most suitable for the species [8]. The settings that these locations offer to the animals are of the highest conceivable quality that anything could possibly be able to supply for them. Within the realm of Ayurvedic medicine, it is utilized for the purpose of treating a wide variety of conditions, such as dyspepsia, tumours, fever, loss of consciousness, and bronchitis, amongst others. As a result of the fact that it contains properties that are bitter, pungent, laxative, and carminative, this is the result. This approach is utilized in the treatment of a variety of disorders that are addressed in the context of therapy [9].

Furthermore, there is a lack of knowledge concerning the antibacterial capabilities of the rhizomes of *Cyperus articulatus* [10], despite the fact that the plant has been utilized for a considerable amount of time and has a wide variety of potential applications in the field of medicine. Putting a methanol extract of *Cyperus articulatus* rhizome through a number of tests against a broad variety of pathogenic microorganisms with the intention of identifying whether or not it exhibits antibacterial qualities was the primary objective that we had in mind.

MATERIAL AND METHODS

Plant Material

The Botanical Survey of India in Shibpur, India, verified the genuineness of those rhizomes after they were collected from a rural region in the Birbhum, which is located in the Indian state of West Bengal. Rhizomes that had been collected and allowed to air-dry were then subjected to mechanical grinding in order to produce a fine powder. Following the powdered material, a Soxhlet equipment was utilised in order to extract the substance utilising methanol. Immediately following this, a spinning vacuum evaporator was employed in order to completely extract the solvent while simultaneously reducing the pressure to a significant degree. Following the transfer of the concentrated extract *Cyperus articulatus*, which had a yield of 35.42% on a weight-to-weight ratio, it was placed in a vacuum desiccator and kept for further use in the research.

Preliminary phytochemical studies

A number of phytochemical assays were carried out in order to determine the bioactive components that were present in the extract. The approach was supplied by Kar et al. for these bundles of experiments; however, the process that was published was modified in some way [11].

Bacterial and fungal stain

The methanol extract was tested with the assistance of two gram-positive bacteria, namely *Staphylococcus aureus* 22945 and *Streptococcus aeruginosa* U59, as well as four gram-negative bacteria, namely *Escherichia coli* K88, *Pseudomonas aeruginosa*, *Salmonella typhi* 12, and *Proteus vulgaris* CC-52, and two fungi, namely *Aspergillus niger* 36 and *Candida albicans*. The purpose of this evaluation was to determine whether or not the methanol extract had the ability to inhibit the growth of these microorganisms [12].

Each and every microbial strain was freeze-dried, and after that, it was stored on stab slant agar at a temperature of four degrees Celsius without being exposed to any oxygen. A period of 18 hours was spent maintaining the cultures on NA media at a temperature of 37±1 degrees Celsius. This was done in order to ensure that the bacteria that were mentioned before were preserved.

Media Preparation and antibacterial Activity

Through the utilization of agar disc diffusion in conjunction with the methanol extract, it was shown that the rhizome had antibacterial properties. The procedure was started by pouring thirty milliliters of nutrient-rich soup into a conical flask. This was done in order to get the process kicked off. This action was taken in order to cause the bacterial strain to become active. In the following step, the flask was placed on a rotary shaker for a duration of twenty-four hours [13].

As a prerequisite for the production of Muller Hinton Agar (MHA), it was essential to make use of a sterile conical flask in order to dissolve 3.8 grams of MHA powder in one hundred milliliters of distilled water. The autoclaved medium was ready to be utilized once it had reached a temperature that was consistent with what is regarded to be normal. After some time had passed, the melted MHA was mixed with two hundred microliters of the inoculum that had been produced, and the resulting mixture was then transferred to Petri plates [13].

The agar disc diffusion method involved soaking paper discs with a diameter of 0.6 centimeters in 50 microliters of the methanol extract, let them to dry, and then placing them on top of MHA plates that had been contaminated with bacteria. This process was repeated until the discs were completely dry. Moreover, there were control discs that contained antibiotics like streptomycin and ampicillin. These discs were distributed around the laboratory. Following that, I let the plates to undergo an incubation at 37 degrees Celsius for an entire night. Throughout the course of the experiment, each bacterial strain was given a control plate that was devoid of any extract and comprised solely of pure solvents. The inhibitory zones that encircled the discs were subjected to measurements in order to ascertain the degree of antibacterial activity that was present. Following the completion of the trials three times, we determined the average of the data and recorded it [14].

Sabouraud Dextrose Agar (SDA) was used instead of yeast in order to conduct antifungal tests on yeasts. The technique that was used was the same as the one that was used in the previous instance. In order to serve as a positive control, amphotericin B was utilized, and the minimum fungicidal concentrations (MFCs) were measured and recorded. It was necessary to take this action in order to guarantee that the outcomes were reliable. A temperature of thirty-five degrees Celsius was maintained in an incubator for a period of twenty-four hours, during which time the plates were kept in their original state unaltered. [14] After a period of forty-eight hours had elapsed, research was carried out on the discoveries that were discovered.

Minimum Inhibitory Concentration (MIC)

The chemical is stated to have a minimum inhibitory concentration (MIC) when there is no turbidity seen in the test tube, which is evidence that it has bacteriostatic effect. A methodology based on the broth dilution method was utilised, with minor adjustments based on Haniffa's method from 2012. Before achieving a concentration that could be used in nourishing broth, the plant extract was initially prepared at the highest concentration possible in sterile distilled water. After that, it was serially diluted (twofold) in order to get the desired concentration. After that, a bacterial suspension at a volume of 0.2 millilitres was added to each dilution. When the test tubes had been incubated at 37 degrees Celsius for 18 hours, the turbidity of the liquid was examined. The value of the minimum inhibitory concentration (MIC) was measured. This value represents the concentration at which there was no turbidity noticeable.

RESULTS AND DISCUSSION

The methanol extract from the rhizome of *Cyperus articulatus* was found to contain many bioactive components, including alkaloids, saponins, tannins, and cardiac glycosides, according to the preliminary phytochemical screening. **Table-1** summarizes these results. Notably, these phytochemicals have a reputation for strong antioxidant activity and have been found to have a range of biological impacts, such as anti-inflammatory and anticancer capabilities.

Tabular data from the disc diffusion test of *Cyperus articulatus* rhizome methanol extract is shown in **Tables 2** and **3**. **Table 2** clearly shows that the methanol extract was active against several pathogens, including *Salmonella typhi, Pseudomonas aeruginosa, Staphylococcus aureus* ATC 2245, and *Escherichia coli* K88.

On the basis of the data obtained from the disc diffusion experiment, which were given in Table-3, a comparison was made between the crude extracts and the standard antibiotics Streptomycin (10µg/disc) and Ampicillin (10µg/disc). It is clear from the table that the extract possesses a significant antifungal

efficacy. The outcomes were comparable to those of the conventional antifungal medication, Amphotericin B (10µg/disc), and there was a significant antifungal effectiveness against fungus strains such Aspergillus Niger 36 and Candida albicans.

The extracts shown remarkable efficacy against *Escherichia coli* K88 and *Pseudomonas aeruginosa*, which were two of the bacteria and fungus that were brought to the laboratory for examination. The extracts of *C. articulatus* include chemical components that are responsible for their antibacterial and antifungal properties. After conducting phytochemical experiments, it was determined that the presence of alkaloids, saponins, and tannins in the methanol extract of *C. articulatus* is likely responsible for at least some of the antibacterial activities that the extract possesses.

There are a number of bacterial and fungal strains tested, and the MIC and MBC/MFC values for the methanol extract are shown in **Table-4**.

In conclusion, a comparison was made between the efficacy of two antibiotics that are routinely used: the action of streptomycin against bacteria and the activity of amphotericin B on fungus. The lowest inhibitory concentrations are useful in determining the necessary extract concentration for growth inhibition, which is the concentration at which the extract does not result in visual turbidity.

When the lowest MBC/MFC value after reinoculation was used to calculate the concentration at which no growth was seen after culture, this concentration shows that a cidal effect was present. When compared to the typical use, the examination of the effectiveness of the methanol extract against the microorganisms that were tested and its potential for use as an alternative source of an antibacterial agent can be more instructive.

Table 1: An examination of the phytochemical properties of *Cyperus articulatus* extract

Phytochemicals	Alkaloid	Terpenoids	Tannin	Saponin	Steroid	Flavonoid	Phenolic	Tannins	Cardiac glycosides
Cyperus articulatus	++	+	++	+++	-	+	-	+	++

Table 2: *Cyperus articulatus* methanol extracts have been shown to have antibacterial potential through the use of disc diffusion assay.

Organisms	**ZONE OF INHIBITION**					
	50 mg/ml	100 mg/ml	200 mg/ml	400 mg/ml	Streptomycin (50µl)	Ampicillin(50µl)
S. aureus	2.0	8.0	12.0	16.0	20.0	22.0
S.aeruginosa	--	4.0	6.0	10.0	18.0	16.0
E. coli	4.0	6.0	10.0	14.0	18.0	16.0
P. aeruginosa	4.0	8.0	12.0	18.0	22.0	22.0
S. typhi	4.0	6.0	10.0	16.0	21.0	18.0
P. vulgaris	2.0	5.0	8.0	13.0	18.0	16.0

*Values are mean of triplicates experiment

Table 3: Evaluation of the Antifungal Capabilities of Methanol Extracts Derived from *Cyperus articulatus* Made Using the Disc Diffusion Assay

Organisms	**ZONE OF INHIBITION IN DIAMETRE**				
	50 mg/ml	100 mg/ml	200 mg/ml	400 mg/ml	Amphotericin B
A.niger	--	2.0	6.0	12.0	18.0
C.albicans	--	4.0	10.0	14.0	21.0

Table 4: Values for the Minimum Bactericidal Concentration (MBC) and the Minimum Inhibitory Concentration (MIC) of the Methanol Extract of *Cyperus articulatus* (MECA)

Organisms	Extract	MECA		Streptomycin	
		MIC	MBC	MIC	MBC
Bacterial strain	*S. aureus*	21.32	5.1×10^{-7}	4.0	2×10^{-8}
	S.aerugenosa	16.1	6.2×10^{-7}	2.3	3×10^{-9}
	E. Coli	14.23	7.2×10^{-7}	4.5	6×10^{-8}
	P. aeruginosa	12.69	5.3×10^{-7}	5.3	3×10^{-9}
	S.typhi	11.36	6.5×10^{-7}	1.3	2×10^{-8}
	P. vulgaris	15.6	7.2×10^{-8}	2.6	3×10^{-9}
Fungal strain	*A.niger*	7.8	5×10^{-7}	1.2	3×10^{-9}
	C.albicans	4	6×10^{-8}	1.6	3×10^{-9}

*Values are mean of triplicates experiment

CONCLUSION

In summary, with Table 1 provided information, it can be inferred that the methanol extract from the plant *Cyperus articulatus* exhibits effective antibacterial performance, especially against some fungi, and certain tested bacteria. Based on Table 1 information, it can be also noted that the plant also has a specific chemical located in the rhizome that may be responsible for such antibacterial properties. In conclusion, since the plant demonstrates effective activity against bacteria and fungi in the test, it is reasonable to draw a conclusion that *Cyperus articulatus* has probable antagonistic impacts. It is also important to acknowledge the possibility of using the plant to fight bacterial and fungal infections. The fact that the methanol extract was tested against most of the tested pathogens and effective can indicate that *Cyperus articulatus* rhizome have bioactive agents. These agents can affect the pathogens' cells process, thus prevent propagation or destructing it. To prove the effectiveness and extract active agents, the following research should be systematic and oriented toward isolating an antibacterial agent. The next phase can be the isolation of the chemical substance from the *Cyperus articulates* rhizome be executed efficiently and the precise methodologies used. Then, the isolated substances can be purified using various techniques as High-Performance Liquid Chromatography and GC-MS, among others, can be productive. It will allow as to identify with high accuracy molecular structure and activity of such components. Integration with testing agent activity can be conducted for these developed from the isolated components. Its action mechanism may help us identify how the material interacts with more refines to achieve the desired response to the agents' use in treating infections. The purified and isolated substances will be employed on a large plate against a burge scale bacterial and fungi culture at the same time to finally understand its chemical structure. The structure of the isolated components will determine its possible synthesis and for the investigation agent in the future. These researches are the final point to detect agent vitality in combating bacteria. Considering the seriousness of the issue of antibiotic resistance, the research will be very useful. *Cyperus articulatus* as the focus of the research is desirable, and the results can be potentially utilized as a pharmaceutical agent.

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DECLARATION OF INTEREST

When it comes to their work, the writers have not mentioned any potential conflicts of interest that may be present. They have total authority over the entirety of the document, including its composition and the content of the paper. In every respect, they are in total control of it.

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