ASSESSMENT OF GENETIC DIVERSITY USING RANDOM AMPLIFIED POLYMORPHIC DNA (RAPD) MARKERS, PHYTOCHEMICAL SCREENING AND ANTIBACTERIAL STUDY OF *ACROSTICHUM AUREUM LINN.*

Dissertation submitted in partial fulfillment of the requirements for the award of the degree of "Master of Science" in

Botany

By

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2023-24

CERTIFICATE

This is to certify that the dissertation entitled **"Assessment of Genetic Diversity Using Random Amplified Polymorphic DNA (RAPD) markers, Phytochemical Screening and Antibacterial Study of *Acrostichum aureum Linn.*"** is an authentic record of work carried out by **Shwetha U** under my supervision and guidance in the partial fulfillment of the requirement of the M.Sc. Degree of Mahatma Gandhi University, Kottayam. I, further certify that no part of the work embodied in this dissertation work has been submitted for the award of any other degree or diploma.

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BONAFIDE CERTIFICATE

This is to certify that the dissertation entitled **"Assessment of Genetic Diversity using Random Amplified Polymorphic DNA (RAPD) Markers, Phytochemical Screening and Antibacterial Study of *Acrostichum aureum* (L.)"** submitted by **Ms. Shwetha U,** M.Sc. Botany, St. Teresa's College (Autonomous), Ernakulam in partial fulfillment for the M. Sc. Degree, is the bonafide record of the research work under taken by her in this institution under my guidance and supervision.

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DECLARATION

I hereby declare that the work which is being presented in the dissertation, entitled **"Assessment of Genetic Diversity using RAPD Markers, Antibacterial Study and Phytochemical Screening of *Acrostichum aureum Linn.*",** in fulfilment of the requirements for the award of the degree of Master of Science in Botany and submitted to St. Teresa's College (Autonomous), Ernakulam is an authentic record of my own work carried out during M.Sc. period under the supervision of Dr. Elsam Joseph.

The matter embodied in this dissertation has not been submitted by me for the award of any other degree of this or any other University/ Institute.

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ABSTRACT

Acrostichum aureum Linn. commonly known as golden leather fern which is grows in mangrove swamps belonging to the family Teridaceae. The purpose of this study was to understand the Genetic Diversity of *Acrostichum aureum Linn.* using Random amplified polymorphic DNA (RAPD) markers. A number of 05 genotypes were selected from different locations of Kerala. Each populations of the plants were named as AC01 to AC05. A Numerical Taxonomy system for the personal computer (NTSYS) was used to determine phylogenetic tree using UPGMA software. It was found that the RAPD markers system was helpful for identifying variance and genetic diversity. In addition to genetic diversity, the present study was used to find phytochemical compounds using Ethanolic Solvent and also used to explain the Antibacterial property of the plant extract to determine effectiveness of inhibition against the selected Pathogen *Staphylococcus aureus* and *Escherichia coli*. Phytochemical Screening of the Ethanolic extract of the plant revealed the presence of many significant phytochemicals. The Antibacterial study of Ethanolic extract of *Acrostichum aureum Linn.* showed the highest activity against *Escherichia coli* followed by *Staphylococcus aureus.* Thus *Acrostichum aureum Linn.* can be used as a Medicinal Agent since it possesses significant antibacterial activity with certain vital Phytochemicals.

INTRODUCTION

Vascular plants are generally agreed upon to be distinguished from thallophytes, and their main divisions are well-defined. Pteridophytes that are alive can be easily separated into two groups: fern associates, which include lycopods, horsetails, psilotates, and true ferns. The only exception to this has been the Gnetales, which were originally excluded from the cycadophytines but are now typically regarded as belonging to them. However, it appears that the fossil record presents us with forms that obscure this distinct image, leading to divergent interpretations of the phylogeny and relationships between the major families of gymnosperms and pteridophytes (Kramer *et al*., 1990).

A pteridophyte is a type of vascular plant that reproduces by spores and has both xylem and phloem. Pteridophytes are called "cryptogams" because they do not produce seeds or blooms; this indicates that their mode of reproduction is concealed (Dudani *et al*., 2011). Pteridophytes are in an intermediate position between bryophytes and higher land plants, while land plants have complicated internal organization. The life cycle requirements and events of pteridophytes are similar to those of bryophytes. Water is necessary for the fertilization of the sexual generation of bryophytes and pteridophytes, known as gametophytes (Rashid. A.,1999).

Acrostichum aureum Linn.

Globally, mangrove ferns such as *Acrostichum aureum Linn*. (Pteridaceae) can be found in tropical and subtropical regions (Uddin *et al*.,2012). *Acrostichum aureum Linn.* a big terrestrial plant of the Pteridaceae family, is frequently associated with mangroves in Kerala. It is typically observed in flooded areas during rainy seasons and at high tides (Thomas 2012). Large mangrove swamps and other damp areas are home to the golden leather fern or Acrostichum aureum. Swamp fern and mangrove fern are some other common names for it. The enormous fronds of the golden leather fern can reach a length of 1.8 meters, or six feet. The pinnae are alternating, dark green, leathery, and widely spread, while the leaves are glossy, broad, and pinnate. The inner fronds are almost straight, while the outside ones arch sideways. Sporangia, or reproductive organs, are present on the upper five to eight pairs of pinnae of some of the larger fronds. These have a felted look to the pinnae and are brick red

(De Winter & Amoroso 2003). It can withstand higher saline levels and is found in salt marshes and along riverbanks. But fresh water is where the spores germinate more readily.

TAXONOMY

Table 1: classification of *Acrostichum aureum L.*

Kingdom	Plantae
Phylum	Tracheophyta
Class	Polypodiopsida
Order	Polypodiales
Family	Pteridaceae
Genus	*Acrostichum*
Species	*Acrostichum aureum Linn.*

Plate 1: *Acrostichum aureum Linn.*

BENEFITS

The edible mangrove plant fern *Acrostichum aureum Linn.* is primarily found in tropical and subtropical climates worldwide. The purpose of this review was to present comprehensive information about the phytochemistry, biological activity, and traditional usage of *Acrostichum aureum Linn.* Results showed that *Acrostichum aureum Linn.* is traditionally used worldwide to treat a variety of conditions, such as boils, wounds, non-healing ulcers, snakebite, bleeding, worm infections, asthma, sore throats, constipation, and elephantiasis. *Acrostichum aureum Linn.* has been shown to contain sterols, glycosides, saponins, alkaloids, tannins, flavonoids, phthalates, and terpenoids, among other secondary metabolites. The plant has been shown to contain beneficial phytochemicals such as kaempferol, di-(2-methylheptyl) phthalate, β-sitosterol, (2S,3S)-sulfated pterosin C, (+)-pinoresinol-4-O-sulfate, lupeol, α-amyrin, and phytol. Research conducted both in vitro and in vivo has demonstrated the potent antioxidant, anti-inflammatory, anti-ulcer, tyrosinase inhibiting, anthelmintic, anti-diarrheal, analgesic, anti-tumor, anti-fertility, anticancer, antibacterial, antiviral, and wound healing characteristics of various extracts and phytochemicals found in *Acrostichum aureum Linn.* (Akinwumi *et al* 2022).

GENETIC DIVERSITY

A wide range of variability that arises between genotypes with regard to the overall genetic composition of a single species or between species is collectively referred to as genetic diversity. Evolutionary changes are based on genetic diversity. A plant species' genetic diversity and its distribution are influenced by various factors, such as its breeding system, evolution, and environment. For genetic variety to be used and conserved effectively, a deeper comprehension of genetic diversity and its distribution is necessary (Ramanatha Rao, 2002).

The molecular underpinnings of the fundamental biological processes in plants must be understood in order to effectively conserve, manage, and use plant genetic resources (PGR). The evaluation of genetic diversity is often carried out at the molecular level by a

variety of laboratory-based methods, including direct measurement of levels of variation through allozyme or DNA analysis (Mondini, L. *et al*., 2009). There are several different types of DNA molecular markers used in molecular investigations of genetic variation. A genomic locus is called a molecular marker if its existence clearly separates the chromosomal feature it represents from the surrounding areas at the 3' and 5' extremities. A genomic locus can be discovered using a probe or specialized initiator (primer) (Barcaccia, G., *et al*. 2000).

Since increasing population pressure, agricultural land urbanization, and rapid modernization are the main causes of food insecurity in the developing world, the significance of plant genetic diversity (PGD) is now understood. Workers are most interested in genetic variety. There are significant concerns about the declining genetic variability and diversity among crop plant species, and it will be difficult to enhance crop varieties going forward.

MOLECULAR MARKERS

Each and every organism has an own DNA sequence. Variations are mutations that happen in an organism's DNA sequences, and they cause the emergence of new alleles in a population. Polymorphism is the existence of two or more alternative variants of a certain DNA sequence that can exist in various people or communities. Balanced polymorphism is the state in which non-identical alleles for a trait are preserved at frequencies higher than 1% in a population. The degree of polymorphism in a species' gene pool determines both its chances of surviving and its capacity for evolution. The majority of plants have significant genetic variation in their natural populations, and these variations set each plant apart (Sebastian, V. A *et al*., 2009).

A gene or DNA sequence that has a defined position on a chromosome and is always linked to a certain gene or trait is called a genetic marker. A genetic marker can be a long DNA sequence, such as mini- and microsatellites, or a short one, such as a single nucleotide polymorphism (SNP). DNA polymorphism is shown by molecular markers (Al-Samarai, F. R., 2015).

Allozymes and other physical and biochemical features have long been used to quantify genetic diversity within a species' gene pool. But these days, allozymes are

Insufficient to evaluate genetic diversity among populations (Heun *et al*. 1994). These population-level investigations are now feasible because to a variety of molecular-based methods, such as RFLP (Restricted Fragment Length Polymorphism), ISSR (Inter Simple Sequence Repeat), and RAPD (Random Amplified Polymorphic DNA).

Because of their simplicity and speed, RAPDs are among the molecular markers that have been utilized most frequently in genetic studies . Every technique has benefits and drawbacks of its own. The degree to which these techniques can address genetic Distinctions, the kinds of data they provide, and the extent of their applicability. Molecular markers find application in a wide range of fields, including population history, genetic mapping, paternity testing, cultivar identification, marker-assisted crop breeding, and the detection of mutant genes linked to hereditary disorders.

RANDOM AMPLIFIED POLYMORPHIC DNA (RAPD) MARKERS

RAPD random amplified polymorphic DNA markers are preferred among popular molecular markers because they can identify polymorphisms in nucleic acid sequences. For random amplification of polymorphic DNA, it is an independent, straightforward, fast, and affordable method that takes a tiny amount of DNA. PD pronounces this method as rapid. It is a kind of PCR reaction in which the amplified DNA segments are chosen at random. Since the primer will bind to the targeted gene at an unknown location in the sequence, knowledge of the targeted gene's DNA sequence is not necessary for RAPD analysis, which is primarily used in molecular marker studies related to genetic diversity. This makes the method popular for comparing the DNA of biological systems that have not received much attention from the scientific community or in systems where relatively few DNA sequences are compared. The key to the achievement is the use of many genetic markers, which just needed a small quantity of DNA and did not require cloning. Any type of molecular characterization of the species' genome, including sequencing, is done using the RAPD method. Markers are longer oligonucleotide primers, typically 10 BP, that bind to complementary sequences throughout the genome to create band profiles. When the area between the opposing primer sides is within the amplifiable distances, PCR amplification takes place.

The following benefits have led to the widespread usage of RAPD markers:

- DNA probes and sequence data are not required for the synthesis of specific primers.
- It is fast, easy to use, and efficient because it does not require any blotting or hybridization processes.
- Only small amounts of DNA (about 10 mg) are required for each reaction, and it can be automated.
- A large quantity of fragments.
- Easily obtainable are arbitrary primers
- When compared to other marker technologies, the unit costs per test are minimal (Kumar, N. S., 2011).

PHYTOCHEMISTRY

The study of phytochemicals, or substances originating from plants, is known as phytochemistry. The vast majority of secondary metabolites that are present in plants, their roles in plant and human biology, and their biosynthesis are all subjects of interest to phytochemists. Plants produce phytochemicals for a variety of purposes, such as defense against pest infestations and plant illnesses. Although there are many different types of chemicals in plants, the majority belong to four main biosynthetic classes: terpenoids, polyketides, phenylpropanoids, and alkaloids. One may classify phytochemistry as a branch of either chemistry or botany. Ethnobotany can be used to lead activities in the wild or in botanical gardens. Studies on phytochemicals aimed at human use (i.e., drug development) may be classified as pharmacognosy, while studies on the ecological roles and evolution of phytochemicals would probably be classified as chemical ecology. Plant physiology is another discipline that benefits from a knowledge of phytochemistry.

ANTIBACTERIAL

Chemicals with antibacterial action are those that, while not harmful to nearby tissue, either kill or inhibit the growth of bacteria locally. The term "antibacterial" describes drugs or other chemicals that either directly destroy or stop the growth and reproduction of bacteria. There are many different types of antibacterial agents, such as disinfectants, antiseptics, antibiotics, and other substances. These materials are used to maintain hygiene and stop the spread of bacterial illnesses in a variety of contexts, such as healthcare, food manufacturing, and domestic cleaning. To prevent bacterial resistance and potential environmental impact, it is crucial to use antibacterial products sparingly and carefully.

OBJECTIVES

1. Isolation and PCR amplification of genomic DNA using Random Amplify polymorphic DNA (RAPD) gathered from various locations of Kerala.

2. Analysis of genetic diversity in ***Acrostichum aureum Linn***.

3. Preliminary Phytochemical Screening of ***Acrostichum aureum Linn.*** plant Extract.

4. Determination of Antibacterial Study of ***Acrostichum aureum Linn***.

REVIEW OF LITERATURE

GENETIC DIVERSITY

Hughes *et al.*, (2008) published a study titled "Ecological Consequences of Genetic Diversity." Their findings demonstrated the significant influence that genetic diversity has on ecological processes, including nutrient and energy fluxes, species competition, population recovery, community organisation, and primary productivity from disturbance. Genetic variation has significant ecological effects in 13 ecosystems, communities, and populations. Sometimes the ecological consequences are comparable in magnitude to the effects of species variation.

According to Booy, G. *et al*., (2000)'s work on Genetic variety and the Survival of a Population, populations whose quantitatively inherited fitness-related traits are crucial must have genetic variety in order to be fit. They considered a wide range of variables that can affect how crucial genetic diversity is to population survival. The degree of environmental heterogeneity, the species' evolutionary and population history, and the mating system all have a significant impact on the amount of genetic variation in a population. The significance of genetic variation at the population level, which is assumed to determine species survival and, consequently, species variety, underlies the significance of species diversity for ecosystem functioning. People are still debating this at the population level. An examination of the significance at the population level is provided in this review.

A study of the genetic relatedness and diversity among 45 genotypes of different green gram sources for eight features was carried out by Natarajan, C., *et al.* in 1988. High genotypic coefficients of variation were observed in the number of pods, plant height, and seed yield. For plant height, days to blooming, pod length, and 100 seed weights, significant heritability differences were noted. Plant height was found to exhibit the greatest genetic advancement, after seed yield. According to correlation studies, there is a positive and

significant correlation between the number of seeds in each pod, racemes and pods per plant, and plant height. The greatest factor influencing genetic differences in the materials that were chosen was seed weight.

Ali, Y., *et al*., (2008) conducted research on genetic variability, association, and diversity in wheat (Triticum aestivum L.) germplasm. For eight metric traits—plant height, number of productive tillers per plant, number of spike lets per panicle, spike length, number of grains per earring, fertility percentage, 1000 seed weight, and yield per plant—they assessed seventy local and exotic wheat genotypes for variation parameters, correlations, and path coefficients. For every trait under investigation, significant genotypic differences were found, suggesting that there is a considerable degree of variance among genotypes for every trait. The results of the study demonstrated a significant and positive correlation between genotype, the number of productive tillers per plant, and the number of grains per panicle with the greatest direct effect, and seed yield per plant. Since these two traits account for the majority of the yield per plant, greater attention should be paid to them in order to boost wheat grain production. Despite having a substantial correlation with grain yield, the length of the spike and the number of spike lets per spike had a high indirect effect on the number of grains per spike and the number of productive tillers per plant, so these factors were excluded from the selection criteria. There are notable genetic differences across genotypes for the majority of attributes, according to the distribution patterns of all genotypes in the various groups.

The impact of genetic variety within a dominant species and plant species diversity on the biomass of plant communities in the Great Lakes sand dune environment was examined by Crawford, K. M., and Rudgers, J. A. (2012). They conducted two studies to examine the diversity's independent impacts. In the first, they altered the genetic diversity of *Ammophila breviligulata*, a species that dominates the dune. Nevertheless, they alter the total number of plant species, excluding *A. breviligulata*. They then created communities that changed the number of species and genetic diversity levels in *A. breviligulata* in order to investigate the consequences of interactions. Even so, there was no separate impact on The combined impacts of species diversity and genetic diversity in *A. breviligulata* on biomass output in this system had a considerable impact on the total aboveground biomass production of plant communities. The findings imply that biomass production may be affected by the interplay between genetic variation within a dominant species and plant species diversity.

MOLECULAR MARKERS

In 2009 research titled "Potential molecular markers in plant biotechnology," Kumar, P., et al. discussed the benefits, drawbacks, and uses of molecular and biochemical markers in comparison to other marker types. Utilizing molecular genetics and technological studies of plants are increasingly relying on markers, which show DNA polymorphisms. There are several kinds of markers, including molecular markers based on DNA, biochemical markers, and morphological markers. The two types of these DNA-based markers are PCR-based markers (RAPD, AFLP, SSR, SNP, etc.) and non-PCR-based markers (RFLP). The most popular are microsatellite DNA markers because they are straightforward to utilize by PCR, followed by denaturing gel electrophoresis to determine allele size, and they offer a great degree of information from several of each locus's alleles. Despite being limited to tandem markers, Single Nucleotide Polymorphism, or SNP, is a relatively novel marker type that has gained popularity. These novel and distinct sorts of markers are significant for comprehending genomic variability and variety between similar and distinct plants because of their fifteen daily evolution.

1999 saw studies on molecular markers and their use in wheat breeding by Gupta, P.K., *et al*. They discussed the various molecular markers that are now being used to map genes and identify various wheat characteristics. The molecular indicators DNA amplification fingerprinting (DAF), sequence-tagged sites (STS), amplified fragment length polymorphisms (AFLPs), random amplified polymorphic DNAs (RAPDs), restriction fragment length polymorphisms (RFLPs), and microsatellites (STMS) are among the topics covered. Additional markers are also covered, such as single nucleotide polymorphism (SNP), expressed sequence tag (EST), and microsatellite-initiated polymerase chain reaction (MP-PCR). They also went over fresh data regarding cereal synteny. genomics, marker validation, marker-assisted selection, and their application to wheat and grain breeding in particular.

Using RAPD and ISSR markers, Wang, J. B (2006) found that the invasive clonal plant Eichhornia crassipes in China lacked genetic variety. Inter simple sequence repeat (ISSR) and random amplified polymorphic DNA (RAPD) markers were

employed to examine the genetic makeup of six populations of invasive Eichhornia crassipes plants that were taken from a Southern Chinese introduction site. A total of 172 RAPD and 145 ISSR bands were produced, respectively, using 25 RAPD and 18 ISSR primers. Nevertheless, neither marker revealed any polymorphism bands within or between groups. This implies that all populations of *E. crassipes* in southern China have essentially the same genotype and that there is very little genetic variability within the species. This study proposed various other factors associated with the expansion of *E. crassipes* in China, in addition to genetic diversity.

In 1994, Cloutier and Landry conducted research on the use of molecular markers in plant tissue culture. Many crops have benefited from the successful application of plant tissue culture techniques. Plant molecular genetics research is expanding rapidly. Molecular many of the present tissue culture restrictions are being studied with the use of markers. They have been applied to the investigation of the mechanisms behind somatic lineage variation in the genomes of mitochondria, chloroplasts, and nuclear organelles. For the exact identification of plant origins from protoplast fusion, microspore or anther culture, and other tissue culture research, where this information is crucial, molecular markers are effective tools. Several important plant species now have populations of doubled haploid lines thanks to advancements in tissue culture techniques. It has been demonstrated that doubled haploid populations are helpful for creating molecular maps and identifying significant agronomic features. In addition to discussing the possibilities for advancing molecular techniques and innovative molecular markers like SCAR and STS as well as high-resolution map sequencing strategies, this study highlights the use of molecular markers in plant tissue culture.

In 2010, Tanya, C. and Kumar, R. conducted a review on molecular markers and their uses in aquaculture and fishing. The statistical analysis of molecular markers has transformed the analytical tools needed to investigate genetic diversity. Different In fisheries and aquaculture nowadays, markers such as protein, DNA, or molecules are employed. Numerous significant scientific findings regarding current aquaculture practices are made possible by these markers, including: 1) Species identification; 2) Research on genetic variation and population structure in natural populations; 3) Comparison of hatchery and wild populations; 4) Evaluation of 16 demographic bottlenecks in wild populations; and 5) Propagation assisted rehabilitation programs. The study covered the fundamentals of molecular

genetics, a summary of frequently used markers and their uses, as well as the limitations of these markers in fisheries and aquaculture research.

In 2013, Pourmohammad, A. conducted research on the use of molecular markers in therapeutic plants. Many essential medicinal plant species are authenticated using DNA-based methods. Geographical circumstances affect the active components of the effectiveness profile of medicinal herbs. Molecular markers based on DNA are useful in enhancing medicinal plant species. Since genetic information is specific to each species and unaffected by age, physiological state, or environmental variables, DNA markers are considered more dependable than other methods. It has been reported that RAPD markers can be used to identify specimens of Andrographis paniculata, *Allium schoenoprasum L*., and *Codonopsis pilosula* that have been collected from various geographical locations. Similar to this, ISSR markers allow for the differentiation of several Arabidopsis thaliana specimens. The genus Capsicum's intraspecific genetic diversity and the DNA fingerprinting of pepper cultivars were evaluated using RFLP technology. The genome coverage, mechanism of polymorphism, and discriminating power of markers vary. Therefore, depending on the technology available, they can complement one another. Medicinal plant materials can be independently described by the use of molecular methods.

RANDOM AMPLIFIED POLYMORPHIC DNA(RAPD) MARKERS

In 2003, Xu, C. Y., *et al*. used RAPD analysis to investigate the genetic diversity of Chinese alligator weed. They examined the genetic diversity of an invasive weed species, Alternanthera philoxeroides, collected from eight distinct locales in southern China, using random amplified polymorphic DNA (RAPD). 108 RAPD primers amplified 391 bands from samples taken from various locales at rather high spatial intervals; however, no genetic variation was detected between samples. The genetic diversity of alligator weeds is extremely minimal, according to molecular evidence. Widespread in China, alligator weed is a very invasive plant. They believed that low genetic diversity does not always equate to endangered plant species and that the low genetic diversity of alligator weed does not hinder its widespread dispersion in China. Moreover, this study's molecular findings implies that the alligator weeds found in southern China might have come from a single clone or from a very small number of

clones. Since its arrival in China, the spread is most likely the result of significant vegetative propagation.

Advances in molecular biology techniques have resulted in the production of numerous highly useful DNA markers for the identification of genetic polymorphisms, as reported by Bardakci, F. (2001). The polymerase chain reaction (PCR) is the foundation of randomly amplified polymorphic DNA (RAPD) technology, which is one of the most widely used molecular methods for the creation of DNA markers. It is not necessary to know the DNA sequence beforehand because the RAPD marker is the amplification product of an anonymous DNA sequence employing single, short, and random oligonucleotide primers. The RAPD technique is valuable despite the ongoing dispute regarding the reproducibility of RAPD profiles. Its low cost, rapid development of a large number of DNA markers, and minimal equipment requirements make it so.

The indica and japonica types of rice (*Oryza sativa L.*) are the two subspecies that are most commonly known. Using RAPD markers, Mackill, D.J. classified Japonica rice cultivars in 1995. There were two wild varieties (*O. rufipogon* Griffith and *O. nivara* Sharma et Shastry) among the 134 cultivars, most of which were japonicas. Thirty bands exhibiting unique polymorphisms were obtained using ten oligonucleotide primers. By using cluster analysis, the varieties of indica and japonica were divided into distinct groupings. There was less clustering in the Japanese group. Despite the fact that tropical japonica cultivars typically group together, there were no obvious distinctions between tropical and temperate species. The distances between each of the two japonica subgroups and the indica group were significantly greater than the distance between them, according to canonical discriminant analysis, and they were roughly equal. Improved resolution of closely related strains was achieved with additional primers. It was determined that while the RAPD marker is helpful for taxonomy, a large number of primers are needed to distinguish between closely related japonica rice cultivars. Crosses between chosen temperate and tropical parents can yield the highest genetic diversity for genetic mapping, or the utilisation of F1 hybrid vigour within japonica cultivars.

Waycott, M. (1995) used RAPD and allozyme analysis to evaluate genetic variation and clonality in the seagrass Posidonia australis. In Warnbro Sound, Western Australia, researchers examined the genetic makeup of seagrasses that are native to the South

Australian coast. Allozyme and his RAPD (randomly amplified polymorphic DNA) study revealed high levels of genetic variation within tiny sections 18 of the meadow. P. australis exhibits a genetic variation level that is either higher or comparable to that of other hydrophilic species. This study's evidence of genetic heterogeneity within the population suggests that P. australis meadows may not always need to be monoclonal. Posidonia australis has a genetic variety, and the meadows under study are multiclonal. Polyclonal populations have a high chance of successful outcrossing and sexual reproduction, which makes this significant. Determining the level of outbreeding in these populations and evaluating the efficacy of sexual reproduction in both monoclonal and polyclonal populations are therefore highly interesting.

PHYTOCHEMICAL ANALYSIS

In this study D., A.. (2023). is to analyze the phytochemicals of *Hybiscus sabdariffa* and *Hyphaene thebaica* in a qualitative and quantitative manner. Using methanol as a solvent, the soxhlet hot percolation method was used to extract phytochemical components from the plants. Alkaloids, cardiac glycosides, flavonoids, glycosides, phenols, resins, saponins, steroids, tannins, and reducing sugar were all subjected to qualitative phytochemical examination. Using conventional protocols, quantitative phytochemical analysis was performed on alkaloids, total phenolics, total flavonoids, tannins, saponins, and glycosides. Both plant species included significant amounts of alkaloids, phenolics, flavonoids, tannins, saponins, and total glycosides, according to the phytochemical examination. The outcome Significant amounts of phytochemicals found in the extract of Hibiscus sabdariffa leaves have been shown to reduce sugar, resin, and phenol, whereas significant amounts of phytochemicals found in the extract of *Hyphaene thebaica* leaves have been shown to reduce steroids. Thus, these plants may have anti-oxidant and antihypertensive properties.

Sakshi Pathak and Bharti Jain's 2023 Journal of Plant Science Research study examines the phytochemical composition of powdered dry *Moringa oleifera* leaves. The purpose of the study is to identify and measure the phytochemical components that are contained in the powder. The leaves of Moringa oleifera are abundant in a variety of bioactive substances, and the plant is well-known for its nutritional and therapeutic qualities. The authors

carried out a thorough investigation using accepted analytical techniques to identify the presence of phytochemicals such flavonoids, phenolic compounds, alkaloids, saponins, terpenoids, and others. Antioxidant, anti-inflammatory, antibacterial, and other health-promoting qualities are well-known for these chemicals. These results underscore the potential of *Moringa oleifera* leaf powder as a functional food or dietary supplement and advance our understanding of its nutritional and therapeutic benefits. The significance of Moringa oleifera as a naturally occurring source of bioactive chemicals and its possible therapeutic uses in a range of medical diseases are highlighted by this study.

Irene Chinda Kengne, Aimé G. Fankam, Elodie Konack Yamako, and Jean De Dieu Tamokou's paper, which was published in 2023 in Advances in Pharmacological and Pharmaceutical Sciences, examines the phytochemical composition and antioxidant and antifungal characteristics of *Lavigeria macrocarpa*, a tree species, and two herb species, *Tristemma mauritianum* and *Crassocephalum bougheyanum*. The purpose of the study is to assess the pharmacological potential of the bioactive chemicals found in these plant species. The authors identified and measured a variety of phytochemical elements, including alkaloids, phenolic compounds, flavonoids, and others, by performing phytochemical analysis using accepted techniques. The study's findings showed that the three plant species have a considerable phytochemical variety, suggesting the existence of bioactive substances with possible therapeutic uses. The extracts also showed significant antioxidant capacity and noteworthy antifungal efficacy against the tested fungal strains, suggesting that they may be used to treat fungal infections and disorders associated with oxidative stress.

MATERIALS AND METHODS

PLANT MATERIAL

A total of 10 genotype of *Acrostichum aureum Linn.* were collected from different regions of Kerala. These plants were then successfully maintained for further analysis. Plant populations are named AC1 (*Acrostichum aureum Linn.*1) to AC10 (*Acrostichum aureum Linn.*10). These selected plants were properly maintained under favourable growth conditions of growth. Thus, a healthy plant population of all 10 samples was available for study at any time.

Large species of fern that thrives in mangrove swamps and other damp places is called Acrostichum aureum, or golden leather fern. Mangrove fern and swamp fern are two other frequent names. *Acrostichum aureum Linn*. reaches a maximum height of 3.4 m. Mature frond stalks are robust, upright, and scale-covered (Kimura *et al.,* 2017).

Plate 2: *Acrostichum aureum Linn.* habit

Plate 3: *Acrostichum aureum Linn.* single plant

Table 2: Details of *Acrostichum aureum Linn*. accession collected from different regions of Kerala for genetic diversity analysis

Sl. No	Location	Latitude	Longitude
AC1	Kannadipparamba varam road Kannur, Kerala.	11.919	75.411
AC2	Edavanakkad, Vypin Ernakulam, Kerala.	10.091	76.210
AC3	Grand Ayur Island Alappuzha, Kerala	9.820	76.362
AC4	Changaram wellanda Alappuzha, Kerala.	9.797	76.288
AC5	Malabar Botanical Garden, Kozhikode	11.243	75.827

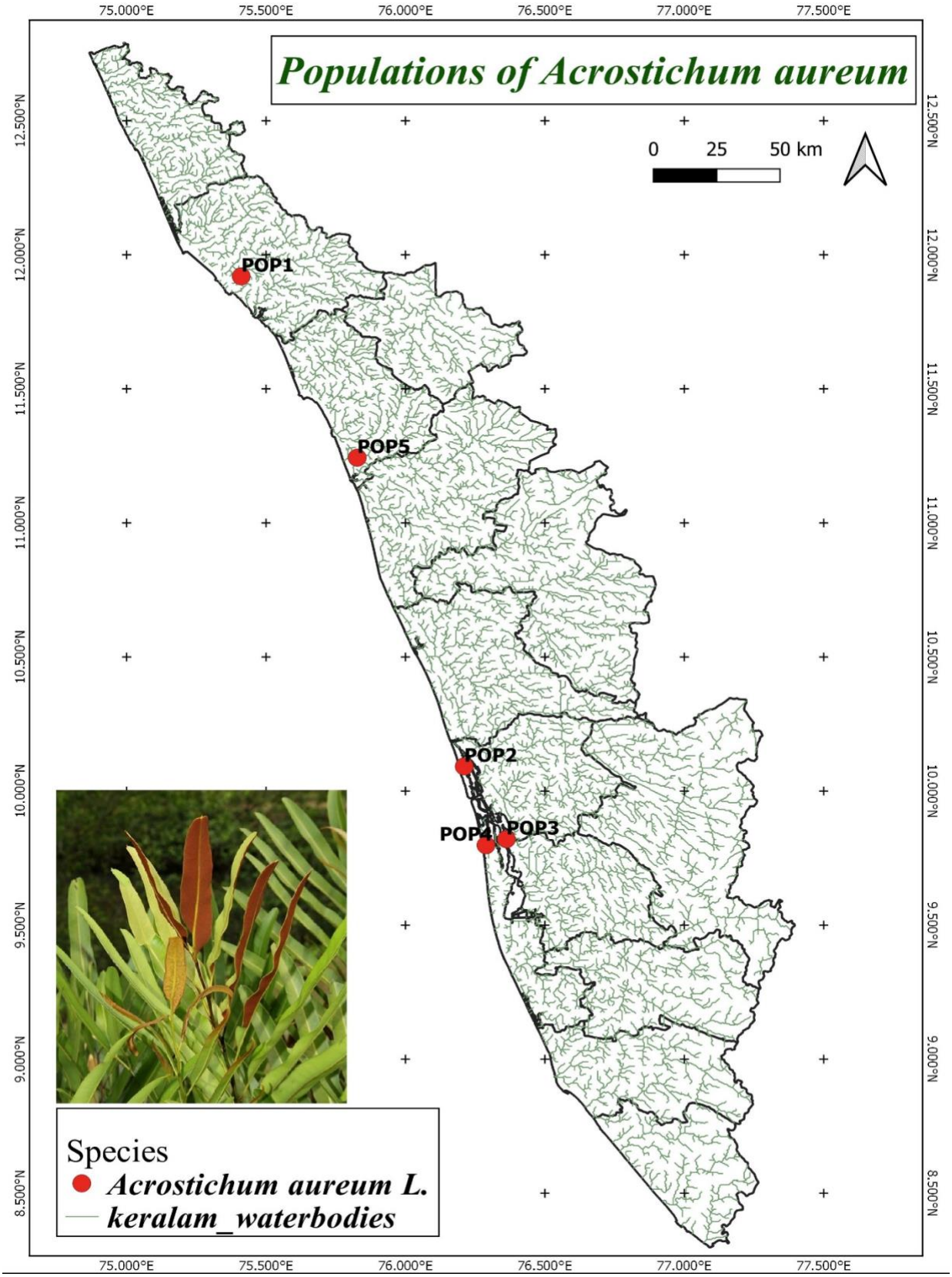


Figure 1: ***Acrostichum aureum L.*** **Kerala waterbodies**

PREPARATION OF GENOMIC DNA FROM PLANT TISSUE

Tender, healthy leaves' sample from all accessions were taken. It was then rinsed well and pat dried with sterile filter paper. One gram of leaf material was weighed and then chopped it into pieces in a mortar that has already cooled. Polyvinylpyrrolidone (PVP) was added to CTAB Extraction Buffer prior to the extraction, and preheated. Pulverized the tissue using a mortar and pestle. 500–1000 µl of extraction buffer was added to form a slurry, and then transferred to a 2 ml centrifuge tube. The tubes were then kept in the water bath for 45 minutes at 65°C, with periodic stirring.

The liquid was brought down to the room temperature and added with 70 µl of the chloroform-isoamyl alcohol (24:1) combination. It was stirred thoroughly and mixed well,then centrifuged for 15 minutes at 25°C at 12,000 rpm. The top aqueous phase was then transferred to a fresh tube and filled with 1/10 of the CTAB/NaCl solution. The mixture was stirred gently. Same amount of chloroform was added to it and centrifuged it for ten minutes at 4°C at 12,000 rpm. The top aqueous phase was again transferred to a fresh eppendorf tube. The same volume of chloroform was added and centrifuged for ten minutes at 4°C at 12,000 rpm. Tubes were inverted for better mixing and the supernatant was transferred to a new eppendorf tube. Cold 100% isopropanol was added through the walls of the tubes. The mixture was kept for one hour at 20°C. Centrifuged for 15 minutes at 4°C at 7850 rpm. The pellets were dried in the air after collecting them twice with cold 70% ethanol at 1000 rpm for five minutes. Pellets were then suspended in 200 µl of TE buffer. It was then incubated in a water bath at 37°C for one to two hours after adding 4 µl of RNase.

500 µl of phenol: Chloroform: Isoamyl mixture (24:25:1) was incorporate into it and centrifuged for 10 minutes at 4 °C at 10,000 rpm. Supernatant was collected to a new eppendorf tube, then it was carefully mixed with 500 µl of chloroform. Centrifuged for ten minutes at 1000 rpm. The supernatant was collected and mixed with double the volume of cooled ethanol 0.1 litre of 7.5 M sodium acetate.

The sample was then stored overnight at -20 °C. It was then centrifuged for 15 minutes at 4 °C at 10,000 rpm and the pellets were collected. After cleaning the pellets were collected, cleaned in cold 75% ethanol and kept for drying. 50 µl of TE buffer was added to it and kept at -20 °C for long term storage.

Table 3: Stock solutions required for genomic DNA extraction

Solutions	Composition	Quantity
Tris buffer pH 8	Tris 1M H_2O	12.11 gm 100 ml
EDTA	Na_2EDTA H_2O	18.61 gm 100 ml
CTAB Extraction buffer pH 8.0 (stored at room temperature)	CTAB 2% W/V Tris buffer 100 mM Na_2EDTA 20 mM PVP 1% NaCl 1.4 M H_2O	2 gm 10 ml 4 ml 1 gm 8.2 gm 100 ml
TE buffer	Tris buffer 10mM Na_2EDTA 1M H_2O	1 ml 0.2 ml 100 ml
Sodium acetate	Sodium acetate 3 M H_2O	24.61 gm 100 ml

QUANTIFICATION AND VISUALIZATION OF DNA

Using a nanodrop spectrophotometer (Multiskan sky), optical density (O.D.) at A260 and A280 was measured in order to quantify DNA. The software called Nanodrop (Thermo Scientific, USA) was used to record the samples' concentration and purity. The samples of DNA were diluted to a 50 ng/µl concentration and kept for usage at -20°C. To prepare DNA samples, 6X loading dye was mixed with 1X final concentration. To assess the quality of the isolated DNA, 1µl loading dye and 5µl of isolated genomic DNA were loaded onto a 0.8% agarose gel stained with ethidium bromide. The gel was then electrophoresed in 1x TAE buffer for 30 minutes at 100 V. The gels were captured on camera using a Bio-Rad Gel Documentation system.

Table 4: Stock solution required for Agarose gel electrophoresis

Solution	Composition	Quantity
TAE Buffer (10x) pH 8	Tris base Acetic acid Na2EDTA 0.5Mm H2O	21.6 gm 11 gm 8 ml 100 ml
Gel loading buffer	Bromo phenol blue 0.25% Xylene cyanole 0.25 % Sucrose 40%(w/v) H2O	250 gm 250 gm 40 gm 100 ml
Ethidium bromide	Ethidium bromide H2O	1gm 100 ml

PCR AMPLIFICATIONS

Five accessions of 8 *Acrostichum aureum Linn*. genomic DNA were amplified by PCR utilising RAPD which was created by Bio serve Biotechnologies in accordance with the Operon Technologies, Inc. USA sequence. A 25 µl volume was used for PCR amplification procedures, which included 1 µl of template DNA, 12.5 µl of Takara Master Mix, 1 µl of 10 pmol primers, and enough Milli Q water to get the volume to 25 µl. All of the reagents were combined with a quick spin. A Bio-Rad thermal cycler was used to carry out the amplification process.10-mer short oligonucleotide primers chosen at random from the operon series were used for RAPD amplification. The steps in the RAPD amplification sequence were as follows: 39 cycles of 1 minute at 36°C and 2 minutes at 72 °C were followed by 1 cycle of 2 minutes at 95°C, 35°C, and 75°C. A 7-minute extension at 72°C occurred after the last cycle. Samples were stored at 12°C.

Table 5: List of RAPD primers used in the study

Primer	Sequence	No of BP	GC content %
OPA 5	5'-AGGGGTCTTG-3'	10	60
OPC8	5'-TGGACCGGTG-3'	10	70
OPG3	5'-GAGCCCTCCA-3'	10	70
OPB 17	5'-AGGGAACGAG-3'	10	60
OPC11	5'-AAAGCTGCGG-3'	10	60

Table 6: Conditions for PCR reaction		
PCR steps	**Temperature**	**Time**
Initial denaturation	94℃	2 min
Denaturation	95℃	2 min
Annealing	35℃	2min
Extension	72℃	2min
No. of cycles of denaturation, annealing, extension	39 cycles at 93℃,36℃,72℃	1min,1min, and 2min
Final extension	72℃	7 min

DATA ANALYSIS

The bands were qualitatively scored using gel photos derived from ISSR and RAPD analysis. Data were recorded in a binary matrix sheet with "0" denoting absence and "1" denoting presence, based on clearly repeatable bands from each accession that were scored as unit characters. The data set contained both polymorphism and monomorphic bands to demonstrate an objective assessment of genetic variation. The software package Numerical Taxonomy and Multivariate Analysis System (NTSYSpc) (version 2.02) was utilized to evaluate the binary data that was acquired from the marker systems.

The “J” coefficient was chosen in NTSYS, and a dendrogram was created using UPGMA (Unweighted Pair Group Method with Arithmetic Mean) to analyze diversity patterns and interpret data.

PIC (Polymorphism Information Content)

Due to the polymorphism that the dominant marker reveals, we elaborate on a binary matrix of bands' presence or absence. The following general equation can be used to estimate the Polymorphism Information Content (PIC) if we take the frequency of bands present as P and the frequency of absence as q.

PIC 1-(P2-Q2) (Lemos *et.*al,2019).

By dividing the number of isolate alleles where the band was discovered by the total number of isolates, the frequency of an allele was determined. To evaluate genetic diversity, PIC is a useful metric. When measuring a genetic marker's information for linkage studies, PIC value is frequently employed. It is possible to assess the degree of gene variation whereby using the PIC index.

A locus with a PIC >0.5 is considered to have great variety.

PIC <0.25 denotes a low level of diversity at the locus.

PIC of 0.25 to 0.5 suggests that the locus has an intermediate level of diversity. PIC analyses can be used to assess markers, allowing the best marker to be chosen for phylogenetic and genomic mapping (Anderson 1993) (Powell *et al*., 1996).

EMR (EFFECTIVE MULTIPLEX RATIO)

According to Milbourne et al. (1997), the EMR of a primer is "the product of the fraction of p olymorphic loci and the number of polymorphic loci of the individual assay."
It is the result of multiplying the fraction of polymorphic fragments (β) by the total number of fragments per primer (n).

$E=n\beta$

were β = total number of polymorphic DNA and n = total number of bands.

MARKER INDEX

The marker index MI was computed for each of the ten markers in order to assess the overall utility of a particular marker system. Rather than the degree of polymorphism found, the high MI is a reflection of the marker's ability to assess a greater number of bands simultaneously. PIC and EMR produce MI (Varshney et al., 2005).

MI=PIC *EMR

The information content of polymorphism (PIC) and the effective multiplex ratio (EMR) were.

PHYTOCHEMICAL ANALYSIS

Plant Material Collection

The plant material was collected from Malabar Botanical Garden and Institute for Plant Science, Calicut, Kerala, India. The fresh leaves were collected and washed to ensure the absence of any impurities. The sample was shade dried for two weeks and then powdered using a blender. The extract were prepared by using soxhlet apparatus and stored in air tight container.

Extract Preparation

A Soxhlet apparatus was used to extract the 15g of grounded, dried leaf powder using an organic solvent (ethanol). The boiling point was set up at 40°C. The extract was air dried and stored in a glass container for further analysis.

Phytochemical Analysis

Secondary metabolite screening of extracts was carried out to identify the presence of various secondary metabolites by employing standard protocols.

Test For Alkaloids

Mayer's test: Few drops of Mayer's reagent were added to 0.5 mL of extract. The formation of a yellowish or white precipitate indicates the presence of alkaloids.

Dragendorff ’s test: Few drops of Dragendorff’s reagent is added to 0.5mL of extract. The formation of an orange red precipitate indicates the presence of alkaloids. An orange red precipitate was formed, indicating the presence of alkaloids.

Test for flavonoids

Sodium hydroxide test: 1ml of 10% of Sodium hydroxide is added to 0.5ml of extract. Initially, a deep yellow colour appeared but it gradually became colourless by adding few drops of dilute HCL, indicating that flavonoids were present.

Test For Glycosides

Salkowski test: 1 ml of Chloroform and 1ml of Concentrated Sulphuric acid is added to 0.5ml of extract. The formation of a reddish-brown precipitate indicates the presence of Glycosides.

Test for Tannins

Ferric chloride test: Few drops of 10% ferric chloride solution is added to 0.5ml extract. Occurrence of blackish blue or blackish green colour indicates the presence of tannins.

Test for Terpenoid

Salkowski test: 1 ml of Chloroform and 1ml of Concentrated Sulphuric acid is added to 0.5ml of extract. The formation of a reddish-brown precipitate indicates the presence of Terpenoid.

Test for Steroids

Salkowski test: 1 ml of Chloroform and 1ml of Concentrated Sulphuric acid is added to 0.5ml of extract. The formation of a reddish-brown precipitate indicates the presence of Steroids.

Test for Saponins

Foam test: 0.5ml of sample is added to 2ml of Distilled water and shaken for 15 minutes. The formation of foam indicates the presence of saponin.

Test for Phenol

Ferric chloride test: 0.5ml of sample is added to 1 ml ferric chloride solution. The formation of blue-black colour indicates the presence of phenol.

ANTIBACTERIAL ANALYSIS

Preparation of Bacterial Culture

In the current study, the plant extract were evaluated for antimicrobial activity against *Staphylococcus* strain and *Escherichia coli,* a Gram positive and a Gram negative bacteria respectively. 3gm of nutrient broth was dissolved in 100ml of distilled water in a conical flask. The broth is sterilized by autoclaving for 15 minutes. Both of the obtained bacteria strains were inoculated in the nutrient broth in laminar air flow and incubated in appropriate conditions for 24hrs.

Preparation of Petri Plates

The selected species of plant *Acrostichum aureum Linn.* were analysed for the antimicrobial activity for gram negative *Escherichia coli* and gram-positive *Staphylococcus aureus* by disc diffusion methods. Agar medium was prepared by dissolving 4gm MHC Agar and 2.6gm of nutrient broth in 100ml distilled water. The mixture is sterilized in an autoclave for 15 minutes. Just after sterilization the mixture was poured into petri plates in laminar air flow. The petri plates were allowed to solidify under aseptic conditions.

Antimicrobial Test by Disc Diffusion Method

The antibacterial activity of the extract was tested against the gram negative and gram-positive bacterial strain using agar well diffusion method (Perez *et al.,1990*). The standard inoculums suspension (10^6 CFU/ml) was streaked over the surface of the sterile Muller Hinton Agar plates using sterile cotton swab to ensure confluent growth of the organisms. The wells of 6mm size were cut in the Agar Plates and the wells were loaded with various concentrations of extract (100µl). The DMSO was taken as the control. All the plates were incubated at 37°C for 24 - 48hrs. The zone of inhibition of bacterial growth was measured in millimeters (mm) and recorded. After incubation, the plates were observed for the formation of zone of inhibition and the zone sizes were measured in mm.

RESULT

Genomic DNA Isolation

Genomic DNA of was isolated from the samples of *Acrostichum aureum Linn.* from 5 natural populations. The purity and concentration of the DNA samples are given in the table 7. Figure 2 representing the qualitative estimation of these isolates.

Table 7: Quantitative Estimation of Genomic DNA

Sl. No.	Plant ID	Concentration of Genomic DNA (ng/µl)	Purity (A260/A280)
1	AC-01	377	1.70
2	AC-02	244	1.75
3	AC-03	300	1.71
4	AC-04	450	1.78

5	AC-05	400	1.77

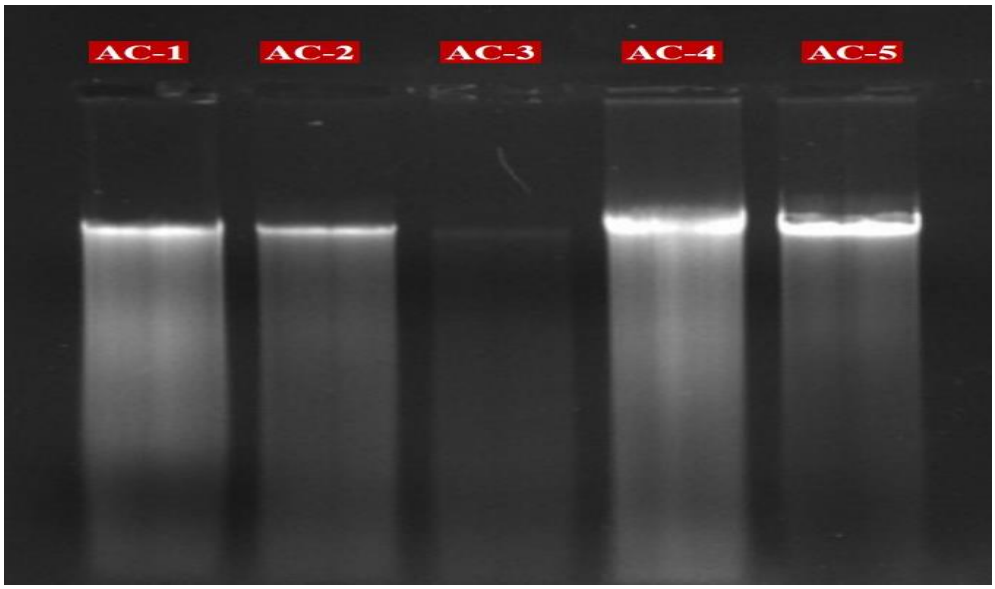


Figure 2: Gel image of genomic DNA of *Acrostichum aureum L.*

RAPD BAND ANALYSIS

The present study analyzed genetic diversity among *Acrostichum aureum Linn.* populations in various geographical regions of Kerala. Samples from five populations were subjected to amplification using RAPD primers. The banding pattern obtained by RAPD primers clearly distinguished populations into different clusters, indicating genetic diversity among them. Diversity estimates provide useful information for understanding the genetic structure of the plant using RAPD. In this study, five RAPD primers were screened for polymorphism survey in pooled DNA accessions of *Acrostichum aureum L*.

The PCR analysis for 05 samples taken in the study with 5 RAPD primers (random Amplified Polymorphic DNA) primers generated 51 scorable bands. An average of 10.2 bands per primer was generated. Among the five RAPD primers OPC 11 and OPG 03 produced a maximum number of bands (12) whereas OPB 17 produced less no: of bands (7). The primers OPA 05 produce 09 bands, and OPC 08 produced 11 bands respectively.

Table 8: Data for RAPD primers used for analysing 05 accessions of *Acrostichum aureum*						
RAPD Primer	**Number of bands**	**No. of Polymorphic bands**	**Percentage of Polymorphism**	**PIC**	**EMR**	**MI**
OPA05	09	05	55.5%	0.142	45	6.39
OPB17	07	02	28.57%	0.14	14	1.96
OPC08	11	11	100 %	0.974	121	117.914
OPC11	12	12	100 %	0.68	144	97.92
OPG03	12	10	83.3%	0.323	120	38.796
Total	51	40	367.37%	2.259	444	262.98
Average	10.2	8	73.474%	0.451	88.8	52.596

Out of 51 scoring bands 40 were found to be polymorphic and the average number of polymorphic bands was found to be 08. OPC 11 is found to have a higher number of polymorphic bands whereas OPA 05 found 05 and OPB 17 produced to be found have 11 polymorphic bands. The level of polymorphism was found high for OPC 08 and OPC 11 (100%) minimum for OPB 17 (28.57%). The average level of polymorphism was found to be 73.474%.

PIC (polymorphism information content) values obtained from the primers ranged from 0.14 (OPB17) to 0.974 (OPC08) with an average of 0.451 for all the primers. The primer OPC08 was found to be more convenient for studies in this species as the PIC value came closest to 0.974. The effective multiplex ratio (EMR) was calculated by multiplying the of polymorphic bands and no: of bands, which is highest for the primers OPC11 (144) and lowest for the primer OPB17 (14). The marker index was calculated by multiplying EMR and PIC. RAPD primer OPC08 shows the highest Marker index with a value of 117.914 and primer OPB17 shows the lowest (1.96). (Table 8).

CLUSTER ANALYSIS

A dendrogram generated from UPGMA (Unweighted Paired Group Method using arithmetic averages) cluster analysis of RAPD primer is shown in Figure 2. The dendrogram is based on Jaccords coefficient of genetic similarity. This dendrogram separates the whole genotype into two major clusters. The first cluster containing three populations; AC1, AC2, and AC3, Second one containing two populations; AC4 and AC5. The first cluster further divided into two clades with populations AC1 and AC2.

The cluster analysis of five populations of *Acrostichum aureum Linn.* reveals that their genetical similarity coefficient (Jaccard's coefficient, J) ranges from 0.6486486 to 0.9393939. Notably, populations 1 and 2 exhibit the highest level of similarity, characterized by a Jaccard coefficient of genetic similarity of 0.9393939, suggesting a close genetic affinity between them. Similarly, population 1 demonstrates considerable genetic similarity with population 5, with a Jaccard coefficient of 0.69697, reinforcing their close genetic relationship. In contrast, populations 4 and 5 manifest distinct genetic profiles compared to the others, forming a distinct cluster in the analysis. Despite their divergence, the similarity between populations 4 and 5 remains relatively modest, with a Jaccard's coefficient of 0.8888889, indicating a lower level of genetic relatedness compared to populations 1, 2 and 3.

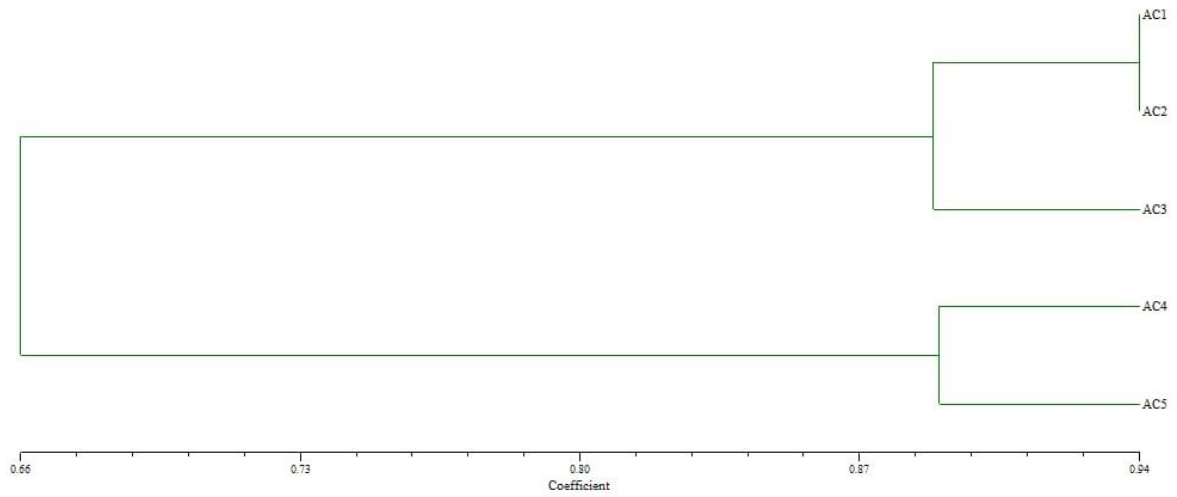


Figure 3: Dendrogram Based on Jaccards coefficient of Genetic similarity (UPGMA) Showing genetic distance

Table 9: Similarity index table showing Jaccords coefficient foe genetic similarity

Rows\Cols	AC1	AC2	AC3	AC4	AC5
AC1	1.0000000				
AC2	0.9393939	1.0000000			
AC3	0.8857143	0.8888889	1.0000000		
AC4	0.6285714	0.5945946	0.6486486	1.0000000	
AC5	0.6969697	0.6571429	0.7142857	0.8888889	1.0000000

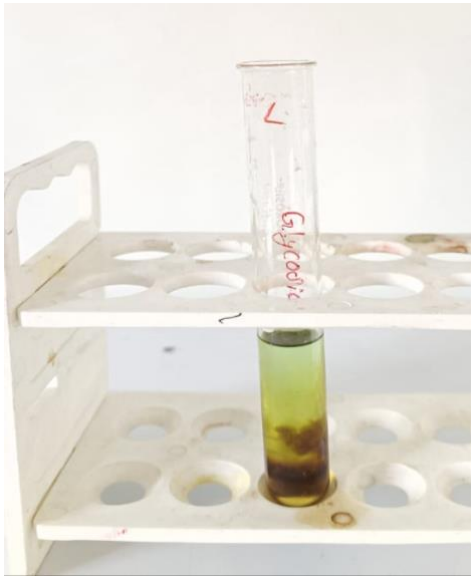
Phytochemical Analysis of *Acrostichum aureum*

Table 10: Phytochemical Screening of Ethanolic Extract of *Acrostichum aureum L.*			
Sl. No.	**Name of the Phytochemical**	**Name of the Test**	**Result**
01	ALKALOIDS	WAGNER'S TEST	-
02	FLAVANOIDS	SODIUM HYDROXIDE TEST	+
03	GLYCOSIDES	LIBERMANN'S TEST	-

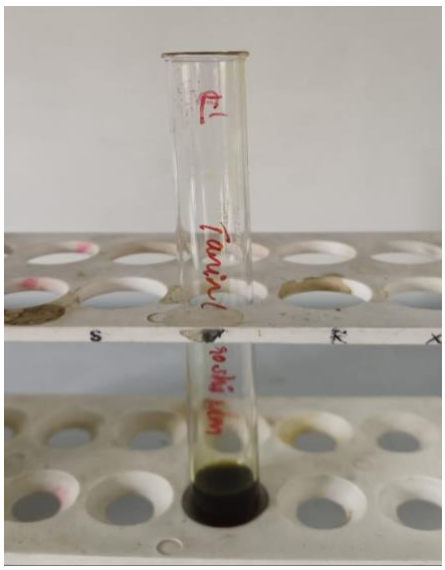
04	TANNINS	FERRIC CHLORIDE TEST	+
05	TERPENOIDS	SALKOWSKI'S TEST	+
06	STEROIDS	SALKOWSKI'S TEST	-
07	SAPONINS	FROTH TEST	+
08	PHENOL	FERRIC CHLORIDE TEST	-

TEST FOR ALKALOID

TEST FOR FLAVANOID



TEST FOR GLYCOSIDE

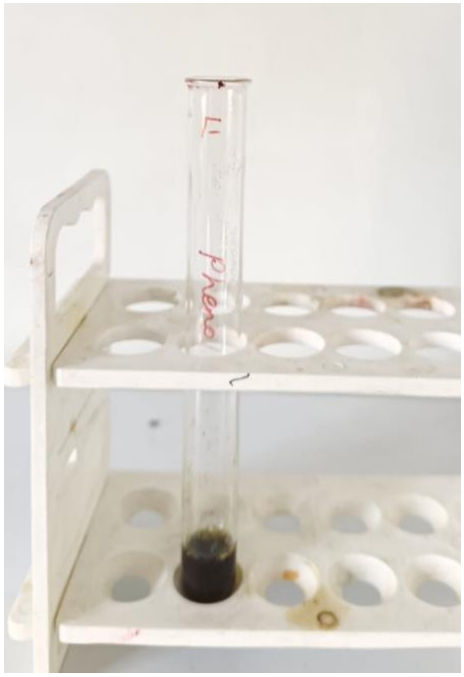


TEST FOR TANNIN

TEST FOR TERPENOID

TEST FOR STEROID

TEST FOR SAPONIN



TEST FOR PHENOL

Figure 4: Represents the presence of Phytochemicals Present in the Extract of *Acrostichum aureum.*

Antibacterial Analysis of *Acrostichum aureum*

Table 11: Antibacterial Activity of Ethanolic Extract of *Acrostichum aureum Linn.*by Disc Diffusion Method.			
SL. NO.	**BACTERA LSTRAIN**	**ZONE OF INHIBITION (mm) *Acrostichum aureum***	
		Extract (100µl)	**Control DMSO**

1	*Escherichia coli*	12mm	R
2	*Staphylococcus aureus*	11mm	R

Bacteria: ***Staphylococcus aureus***

Bacteria: ***Escherichia coli***

Plate 4: Antibacterial Activity *Acrostichum aureum Linn.* by Disc Diffusion Method.

DISCUSSION

Pteridophytes are well known for their adaptation to grow in wet and marshy areas. They play a significant role in their ecosystems. However, most ferns are understudied, and their potential uses are not yet well understood. *Acrostichum aureum Linn.* is a fern belonging to the family Pteridaceae, which thrives in various soil salinity and sunlight conditions (Medina *et al.,* 1990). *Acrostichum aureum* is primarily found in mangrove forests and coastal regions in tropical and subtropical climates. This plant has been utilised in many traditional medicines in these regions and it have many has a variety of biological benefits (Giesen *et al.*,2007; Ragavan *et al.,* 2014).

Many conditions, including non-healing ulcers, boils, wounds, snakebite, bleeding, worm infections, asthma, sore throats, constipation, and elephantiasis, are traditionally treated with *A. aureum* throughout the world. Nevertheless, it is still unknown the chemical composition of *A. aureum* which causes pharmacologically effects.

The degree of genetic variability found in individuals of a variation or a population within a species is known as genetic diversity. A healthy population depends on genetic diversity to preserve the variety of genes that may provide resistance against illnesses, pests, or other stressful situations. It also makes it possible for people to adjust to different biotic and abiotic stressors. It also makes it possible for people to adjust to different biotic and abiotic stressors (Salgotra & Chauhan 2023). Phenotypic qualities are the foundation of genetic diversity research traditionally because they are simple, inexpensive, and do not require complex instruments or methods. However, due to the environment's influence on gene expression, phenotypic assessments of genetic diversity could not be reliable or accurate. Therefore, more molecular analysis of the genetic diversity of germplasm accessions is needed (Fonseca R. M *et al*., 2008).

Studies on population genetics are crucial for conservation efforts and the recovery of endangered populations. The foundation for genomics has been established by advances in molecular genetics. Plant demography and population ecology are related topics of study that give information about how well plant populations are doing in their surroundings.

Understanding the dynamics of plant populations is crucial for conservation biology. The ability to create thorough and successful conservation plans for threatened plant species is impacted by this knowledge. However, studies of population ecology provide light on the factors influencing the survival and fertility of individual plants and evaluate the fitness of all the various varieties within a population, as evolutionary change starts at the population level.

The dependability of plant genetic research and their advancement has grown with the advent of new types of molecular markers. These markers contribute to a more comprehensive and accurate understanding of plant genetic resources by offering additional information. Since the 1990s, PCR-generated markers that provide information regarding intraspecific genetic variation at the nuclear level have been in use. Among these, the random amplified polymorphic DNA, or RAPD, approach has garnered a lot of attention. The incomplete genotypic information arising from dominance complicates the analysis of population structure with RAPD data because it increases the sampling variance associated with individual loci and introduces bias in parameter estimation (Lynch & Milligan 1994).

When compared to traditional approaches like hybridization-based protocols, the RAPD test and its associated techniques provide many advantages. Specifically, no prior understanding of the genome being studied is needed. Very little source material is needed for these tests, and in some cases, non-destructive analysis is possible, which is helpful for screening uncommon or precious items (Atienzar & Jha 2006).

populations of A, aureum were gathered for the current investigation, and DNA isolation was carried out largely utilising the appropriate methodology. Different workers employ different protocols. All DNA alteration experiments must start with high-quality DNA. This is caused by the tissue being disrupted in a mortar and pestle with the use of liquid nitrogen, different homogenization or extraction buffer components, and the precipitating and purification process that is used. The protocol adopted in this work is ideal to isolate a considerable amount of DNA which was quantified in a spectrophotometer. The DNA isolated are with high purity and concentration of about 1.7 purity and 100 to 500 concentration.

The isolated DNA was treated with 5 RAPD primers i.e., OPA05, OPB17, OPC08, OPG03, OPC11. In a PCR thermocycler with appropriate conditions amplified fragments of DNA produced by the PCR were subjected to gel electrophoresis to separate the bands according to their size and charge. A clear banding pattern was obtained by viewing the gel under the gel imager. The genetic analyzed these scoring these bands. The data of scored bands from amplified products of PCR revealed the polymorphism exhibited by these populations. The binary scored data used NTSYS software and it generated the genetic distance and genetic similarity. A dendrogram was generated from UPGMA (Unweighted Pair Group Method with Arithmetic Mean) cluster analysis of RAPD and ISSR primers in which plant populations were grouped into certain clusters.

The five Random Amplified Polymorphic DNA markers used in the study generated 51 scorable bands. An average of 10 bands per primer was generated. Among the 5 RAPD markers, OPC11 and OPG03 produced the maximum number of bands 12 bands and OPB17 produced a minimum number of bands 07. Out of the 51 bands, 40 bands were found polymorphic. They show 73.474% polymorphism and the average number of the polymorphic band per primer was 8. All the primers produced the same levels of polymorphism among the different genotypes. The primers exhibited 73.474% polymorphism. The PIC value for OPB 17 is have minimum value of 0.14 and OPC 08 is have maximum PIC value of 0.974. The EMR value is the highest for the OPC 11 marker and the lowest for OPB17 marker. The Marker Index is highest for OPC08, with a value of 117.914 and it is lowest for OPB 17, with a value of 1.96.

A. aureum has been shown to contain sterols, glycosides, saponins, alkaloids, tannins, flavonoids, phthalates, and terpenoids, among other secondary metabolites (Akinwumi et al.,2022). Kazeem et al., 2022 are discussed *Acrostichum aureum* has been traditionally used to cure a wide range of illnesses because it contains advantageous phytochemicals.Strong anti-inflammatory, anti-ulcer, and antioxidant qualities have been discovered in the plant, among other therapeutic benefits. Only a small number of the bioactive chemicals found in *Acrostichum aureum* have been isolated or found thus far, and their reports have been few.

A mangrove species found in the Niger Delta called Acrostichum aureum showed antibacterial properties against Staphylococcus aureus and Escherichia coli. At larger extract concentrations, *Acrostichum aureum* was more successful in suppressing the bacteria.

According to the study, *Acrostichum aureum* may be used in the manufacturing of medications to treat prevalent illnesses in the area.

CONCLUSION

The present study was aimed at developing RAPD molecular markers for studying genetic diversity among the different accessions of *Acrostichum aureum Linn.* plant populations collected from various geographical regions of Kerala. The *Acrostichum aureum Linn.*is a mangrove plant also known as the golden leather fern mangrove fern and swamp fern are two other frequent names. Large species of fern that thrives in mangrove swamps and other damp places.

With the growing urban population, the significance of plant genetic diversity (PGD) is now acknowledged as a distinct field. Several plant species natural habitats, including common marshlands, are under danger of disappearing. Diversity increases an ecosystem's ability to withstand adverse weather conditions. The loss of species may reduce the worth of ecosystems if the reasons for their value are that they benefit humans and that ecosystems work better when they include a greater number of species. To effectively utilize and conserve these plants, it is imperative to understand the variety that now exists among the many behaviors of *Acrostichum* plant species.

In this work, genomic DNA from 05 populations of *Acrostichum aureum Linn.*is isolated, and the DNA is subsequently amplified in PCR using random polymorphic DNA markers. 5 primers of RAPD were used in this study and they are OPA 05, OPB17, OPC08, OPC11and OPG03. Following gel electrophoresis to visualize the amplified products, a qualitative score band was created using gel photos.

NTSYSpc version 2.2 software was used to evaluate the binary data that was retrieved from the marker system. Finding structures and trends in multivariate data can be accomplished with NTSYSpc. To create dendrograms, apply the UPGMA algorithm. The same program was used to calculate the genetic characteristics, namely the polymorphism among the population (PIC), the effective multiplex ratio (EMR), and the marker index (MI). The genetic

makeup of every population may be disclosed using RAPD markers. Furthermore, the study compared the genomic diversity of *Acrostichum aureum Linn.*from various geographic areas. Through the utilization of distinct genomic areas, RAPD markers have the potential to identify non-coding and consequently more polymorphic DNA.

Since DNA technologies consistently yield results regardless of sample age, tissue, origin, physiological circumstances, environmental factors, harvest, storage, or processing, they are dependable and effective instruments for taxonomic identification at a variety of taxonomic levels.

The growing demand for premium herbs will accelerate the need for DNA identification in order to guarantee medicine efficacy, promote fair trade, and boost consumer confidence. However, building a comprehensive database for all studied medicinal taxa with reference data on nomenclature, phylogenetic relationships, macroscopic and microscopic features, chemical constituents, and profiling, toxicity, and voucher specimens in herbaria or museums is unavoidable in the future for the modernization of traditional medicines. This study establishes the usefulness of RAPD markers in comparison to other molecular markers for determining *Acrostichum aureum Linn.* plant genetic diversity.

On the chosen plant sample, a phytochemical screening was conducted. Plants are an excellent source of phytochemicals that may be used to treat a variety of illnesses, according to the study's finding's numerous Phytoconstituents were found abundant in plant extract of *Acrostichum aureum Linn.* which include Tannins, Terpenoids, Steroids, Flavanoids etc…
Additionally, this study demonstrated the tremendous potential of plant extracts as antibacterial agents against specific strains of *Staphylococcus aureus* and *Escherichia coli* bacteria. As a result, they can be applied to treat infectious disorders brought on by microorganisms with resistance.

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