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CHARACTERIZATION OF PHENOLIC CONTENT AND ANTIMICROBIAL ACTIVITIES OF FERN SPECIES USED BY MALAYS IN TRADITIONAL MEDICINE

(PENCIRIAN KANDUNGAN FENOLIK DAN AKTIVITI ANTIMIKROB OLEH SPESIS PAKU PAKIS YANG DIGUNAKAN OLEH MASYARAKAT MELAYU DALAM PERUBATAN TRADISIONAL)

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Abstract

In the past, ferns were traditionally used by the people as food, medicine, domestic use, ornamental plants and in many handicrafts. However, studies have shown that ferns produce a variety of secondary metabolites that display many bioactivities that can help treat diseases. Thus, they are popularly used in Malay traditional medicines. In this study, the researchers evaluated 3 fern species namely piai raya (Acrostichum aureum), paku midin (Stenochlaena palustris) and paku resam (Dicranopteris linearis) for their total phenolic content, individual phenolic compounds and their probable antimicrobial activities. For this purpose, the leaves of the A. aureum, S. palustris and D. linearis were extracted using the water extraction technique, followed by the solvent re-extraction of the supernatant using petroleum ether, ethyl acetate and butanol. Thereafter, these fractional extracts qualitatively and quantitatively using the HPLC analysis and also tested their antimicrobial activities against pathogenic microorganisms. The findings of the study indicated that A. aureum extracts contained the highest total phenolic content, followed by the S. palustris and D. linearis extracts. Furthermore, the HPLC analysis indicated that the A. aureum extracts contained a higher number of phenolic acid compounds compared to the S. palustris and D. linearis extracts, such as Vanillic acid and 3-Coumaric acid (0.51 µg/g DW), 4-Hydroxybenzoic acid (0.35 µg/g DW), Ferulic acid (0.27 µg/g DW), Caffeic acid (0.18 µg/g DW) and trans-p-Coumaric acid (0.06 µg/g DW). When the researchers tested the compounds for their antimicrobial activities, all 3 fern species showed active antibacterial activity than antifungal activity. The researchers concluded that the medicinal herbs that showed good bioactivity with a higher phenolic content could be introduced as a medicinal drug for improving public health. However, additional research needs to be conducted for optimising the isolation and the purification of the bioactive compounds, for any future applications.

Keywords: Ferns, *Acrostichum aureum, Stenochlaena palustris, Dicranopteris linearis*, medicine, ethnoscience

Abstrak

Sejak sekian lama, spesies paku pakis telah digunakan secara tradisional oleh masyarakat sebagai sumber makanan, ubat-ubatan, penggunaan domestik, tanaman hiasan dan *penghasilan kraftangan. Walau bagaimanapun, terdapat kajian lepas yang melaporkan bahawa paku pakis boleh menghasilkan metabolit sekunder dan menunjukkan bioaktiviti dalam membantu merawat penyakit. Selain itu, paku pakis juga banyak digunakan secara langsung sebagai perubatan tradisi masyarakat Melayu. Dalam kajian ini, penyelidik telah mengkaji jumlah kandungan fenolik, sebatian individu fenolik dan aktiviti anti-mikrob terhadap tiga spesies paku pakis iaitu piai raya (Acrostichum aureum), paku midin (Stenochlaena palustris) dan paku resam (Dicranopteris linearis). Bagi mencapai tujuan kajian ini, daun daripada A. aureum, S. palustris dan D. linearis diekstrak menggunakan teknik pengekstrakan air, kemudian hasil supernatan akan diulang ekstrak menggunakan pelarut berbeza seperti petroleum eter, etil asetat dan butanol. Seterusnya, setiap ekstrak dianalisis secara kualitatif dan kuantitatif menggunakan HPLC serta melakukan ujian anti-mikrob terhadap mikroorganisma patogen. Dapatan kajian menunjukkan jumlah kandungan fenolik dalam ekstrak A. aureum adalah tinggi diikuti oleh S. palustris dan D. linearis. Manakala analisis terhadap sebatian individu fenolik menggunakan HPLC menunjukkan ekstrak A. aureum juga adalah tinggi berbanding S. palustris dan D. linearis seperti asid vanilik dan asid 3-kumarik (0.51µg/g DW), asid ferulik (0.27 µg/g DW), asid kafeik (0.18 µg/g DW) dan trans-p-kumarik (0.06 µg/g DW). Hasil ujian terhadap aktiviti anti-mikrob terhadap ketiga-tiga spesies paku pakis tersebut menunjukkan kesan aktif aktiviti antibakteria berbanding anti-fungi. Kesimpulannya, ubatan herba yang menunjukkan bioaktiviti baik adalah tumbuhan yang mengandungi kandungan fenolik yang tinggi, ia dapat diperkenalkan sebagai ramuan ubatan dalam meningkatkan kesihatan masyarakat. Namun demikian, penyelidikan yang mendalam harus dilaksanakan bagi mengoptimumkan sebatian bioaktif melalui kaedah pengasingan dan penulenan untuk kegunaan pada masa hadapan.*

Kata kunci: *Paku pakis, Acrostichum aureum, Stenochlaena palustris, Dicranopteris linearis, perubatan, etnosains.*

INTRODUCTION

The majority of the fern plants grow at lower elevations in the tropical forests; however, they can also grow in the understory region of a few temperate forests. Ferns are an interesting group of plants that are popularly studied in molecular phylogenetics owing to their diversity (>11,000 species), and a densely sampled and resolved phylogeny (Testo et al. 2019). They also display a higher regional and global variation in their rich diversity, which can be attributed to different ecoclimatic factors like precipitation regimes and edaphic specialisation (Khine et al. 2019) instead of biotic interactions, since they produce a large number of wind-borne spores (Suissa et al. 2021). In the plant kingdom, Pteridophytes (that include ferns and fern-allies) can be categorised as seedless vascular plants. They possess a body structure that is similar to other vascular plants, wherein they have roots, stem, fronds and pinnae. Ferns are cryptogamous as they do not produce any flowers, fruits or seeds, thus, differentiating them from the higher plant species. They are very similar to the Phylum Bryophyta and Algae as they reproduce using spores but differ due to the presence of vascular tissue (Yusuf 2010). Ferns play a vital role in preserving the ecology of the tropical regions as they contribute significantly to the species composition (Watkins 2011). Suissa et al. (2021) described 8 specific hotspots of fern diversity based on the species richness and endemism, i.e., tropical Andes, Mesoamerica, Greater Antilles, Guianas, Madagascar, Malesia, and East Asia (Figure 1).

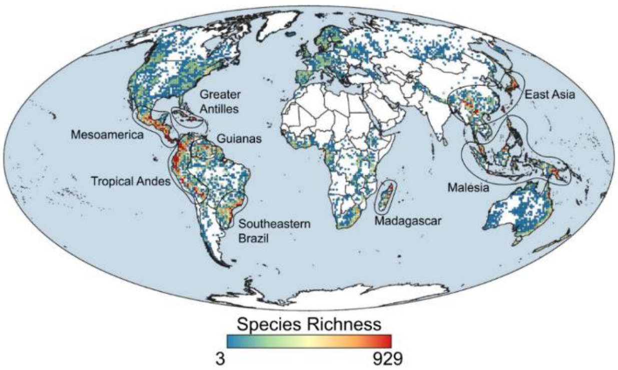

Figure 1. The species richness of ferns has been estimated using 845,878 georeferenced herbarium specimens representing nearly 8000 species. The color-coded squares indicate the estimated level of biodiversity in each 100x100km grid cell. Regions containing fern biodiversity hotspots are denoted by solid lines
Source: Suissa et al. 2021

The habitat deviation of the fern species is not certain, but there are many possibilities. The dispersibility of the spores allows many fern species to inhabit the patchily-distributed montane habitats, thus allowing appropriate gene flow between various populations and avoiding isolation of the fern species on the 'sky islands' (Barrington 1993). Furthermore, many researchers have presented evidence to indicate that the fern species show a stronger affinity to specific soil types since the species distribution at a local level is based on their edaphic characteristics (Lehtonen et al. 2017). It was also hypothesised that a dynamic relationship existed between the climatic variations and the seasonal stability within the elevational transects in the tropical and subtropical mountains, which decreased the range size of various organism groups, leading to higher allopatric speciation (Polato et al. 2018). The fern species can be used for phytoremediation where they help in cleaning the environment that is contaminated by heavy metals (Rahmad & Akomolafe 2018).

As shown in Figure 1, Malaysia is located in the Malesia region as stated by Suissa et al. (2021). Malaysia is seen to be one of the 12 megadiverse countries in the world and possesses a diverse variety of flora and fauna that are endemic to the region. Malaysia has >15,000 plant species (like ferns, fern allies, lichens, bryophytes, and fungi), 372 mammalian species, 742 resident bird species, 267 amphibian species, 397 reptilian species, more than 449 freshwater fish species, >1619 marine fish species, >150,000 species of invertebrates, where the insects formed the largest species amongst the invertebrates (Latiff 2018). Very few researchers have attempted to document the diversity patterns in the Pteridophytes group in Malaysia and across the world, compared to the angiosperm or other higher plant group diversity (Rahmad & Akomolafe 2018).

Ethnobotany refers to how the indigenous people in a specific region use plants for fulfilling their basic needs like shelter, clothing, food and medicine (Aiyeloja & Bello 2006). In the past, many people have used ferns as ornamental plants, handicrafts, ingredients in foodstuff or cosmetics, domestic utensils and medicines (Morais-Braga et al. 2012; Rahmad & Akomolafe 2018). Many of them have been used in traditional medicines for treating different diseases. However, the wild fern plant species in the world are facing a serious threat owing to environmental changes, which can lead to losses of their habitat and number, thereby negatively affecting the biodiversity (Cao et al. 2017).

It was reported that approximately 70-80% of the global population relies on forest plants as a source of therapeutic plants for health (Dossou-Yovo et al. 2017). This type of indigenous knowledge regarding the traditional medicinal plants is transferred from one generation to the next, especially within one cultural group. However, this traditional knowledge is not passed to any outsider (Majumdar & Datta 2007). Herbal medicines can be made using different plant parts; however, leaves are more frequently used (46%), followed by roots (17%), fruits (8%), stem (7%), whole plant (7%), bark (6%), shoots (4%), rhizome (2%), bulb (1%), sap (1%), and hair (1%) (Nuneza et al. 2021). The photosynthesis process helps the leaves store a large concentration of chemical compounds that act as active ingredients in many herbal preparations (Guevara & Garcia 2018). Thus, the leaves synthesise secondary metabolites that contain many chemical groups (Hamel et al. 2018). Due to the higher concentration of phenolic compounds (Farràs et al. 2019), ferns are used as conventional medicinal plants. They contain potent antioxidants that help in preventing and fighting many diseases (Cao et al. 2017). The traditional medicinal herbs are commonly used in the form of decoction, poultice, heating on heat, in the form of extracts, raw leaves and infusion for treating ulcers, boils, fever or external wounds (Nuneza et al. 2021; Ponnusamy et al. 2013). For instance, in Malaysia, people used young leaves of the *L. circinnatum* plant as a postpartum remedy, while the exudate from its rhizome was used as a common insect repellent, and for treating an aquatic animal or snake bites (Rahayu et al. 2020). Furthermore, *P. vulgare* and *A. adiantum-nigrum* plants have anti-inflammatory and expectorant properties and are usually used in traditional medicine for treating colds (Ho et al. 2010).

Acrostichum aureum L., which is locally called "piai raya" is a member of the Pteridaceae family. It is a mangrove fern that grows in the subtropical and tropical regions in the world, particularly in tropical countries like India, Taiwan, Japan, Indonesia, Fiji, China and Malaysia (Uddin et al. 2013). The leaves and roots of the mangrove fern are commonly used in traditional herbal medicines for treating ulcers, wounds, and preventing bleeding (Hossan et al. 2010). The leaves and rhizomes of the *A. aureum* plant are also used for treating ulcers, wounds, boils and for stopping bleeding (Uddin et al. 2013). A few researchers phytochemically analysed the extracts of this mangrove fern species and noted that it contained many compounds like antioxidants, phenolics, phenols, steroids, saponins, flavonoids and active flavonoid chemicals (like kaemferol and quercetin). Furthermore, this species also contained many compounds possessing the phthalic acid ester bond (like 2- methoxycarbonyl- 5-methyl pentyl-2 methyl hexyl phthalates) that can be used in pharmacology and display many biological properties like anti-oxidation, cytotoxicity, anti-inflammation, anti-tumour, antiviral, antimicrobial and anti-parasitic activities (Ultari et al. 2021).

The second fern species included in this study was the *Stenochlaena palustris*, which is locally called "*lemidin*" or "*paku midin*" and belonging to the Blechnaceae family. A few other common habitats of this fern species are rubber plantations, riverbanks, secondary forests, disturbed forests, oil palm plantations and roadside vegetation (Chai 2016). The younger fronds of the *S. palustris* are edible and generally used as a vegetable and in the traditional herbal medicine for treating fever (Chai et al. 2012; Ponnusamy et al. 2013). This fern is commonly found in the Indian subcontinent, from the Southeast Asian region to Polynesia and Australia. This plant species produces many fertile fronds that contain spores and some non-spore bearing sterile fronds. The younger sterile fronds of this plant are reddish and are sold in the local markets and consumed in Malaysia as a vegetable (Giesen et al. 2006). Chai et al. (2012) determined the nutrient composition of this fern species and observed that it is a good source of potassium and phosphorous, while the leaves of this plant are used in traditional medicine for treating ulcers, skin diseases, fever and stomach ailments. Furthermore, the mothers from the Penan community usually consume '*midin*' after childbirth for regaining their energy (Chai 2016).

Dicranopteris linearis or fork fern is generally called "*paku resam*" in the Malaysian community. It is a member of the Gleicheniaceae family and is seen to be a major succession species that is observed under critical conditions like road cutting, landslides or degraded forest lands (Mai et al. 2019). *D. linearis* is regarded as a noxious weed that is commonly present in disturbed sites, while its light-loving nature helps it adapt to the degraded areas with poor soil resources (Liyanage et al. 2021). *D. linearis* is a terrestrial pteridophyte, which is covered with hair and scales, and possesses monomorphic leaves with large scrambling and are forked multiple times. This fern species has a higher flavonoid content and display high antioxidation, anti-inflammatory, antimicrobial and anthelmintic activities (Rajesh et al. 2016). It is used for its pharmacological properties in Malaysian traditional medicine. However, very few researchers have characterised the phenolic compounds and antimicrobial activities of the *D. linearis* fern species. In this study, the researchers have attempted to investigate the antifungal and antimicrobial effects of the phenolic compounds extracted from the *Acrostichum aureum*, *Stenochlaena palustris* and *Dicranopteris linearis* fern species.

MATERIALS AND METHODS

Plant materials and sample preparation

The *Acrostichum aureum* (Pteridaceae) and *Stenochlaena palustris* (Blechnaceae) species were collected from Bagan Lalang, Selangor; while the *Dicranopteris linearis (Gleicheniaceae) species* was acquired from Sungai Pusu, Gombak, Selangor. Then, the fern species were authenticated at the Herbarium Unit, Department of Landscape Architecture, Kulliyyah of Architecture and Environmental Design, International Islamic University, Malaysia. The phenolic acid standard compounds (Ferulic acid, Caffeic acid, trans-p Coumaric acid, 2-Coumaric acid, 4-Coumaric acid, Hydroxybenzoic acid and Vanillic acid) were purchased from Sigma Aldrich (USA).

Table 1. Taxonomy classification of ferns species

Classification	*Acrostichum aureum*	*Stenochlaena palustris*	*Dicranopteris linearis*
Kingdom	Plantae	Plantae	Plantae
Subkingdom	Tracheobionta	Tracheophytes	Tracheophytes
Division	Pteridophyta	Polypodiophyta	Polypodiophyta
Class	Filicopsida	Polypodiopsida	Polypodiopsida
Order	Polypodiales	Polypodiales	Gleicheniales
Family	Pteridaceae	Blechnaceae	Gleicheniaceae
Genus	Acrostichum	Stenochlaena	Dicranopteris
Species	aureum	palustris	linearis
Habitat	Mangrove forest	Lowland forest, swampy land	Terrestrial
Common name	Leather Fern, Sea Fern, Mangrove Fern, Swamp Fern	Climbing fern	Fork fern
Malay	Piai raya	Paku midin	Paku resam

Source: Chai 2016; Thomas et al. 2007 & Ultari et al. 2021

Extraction of phenolic compounds from the different fern species

The phenolic compounds were extracted from the fern species using the technique described by Aarabi et al. (2016) with a few modifications. Here, for every sample, the researchers were mixed the powdered freeze-dried plant material (10 g) with distilled water (100 ml) and incubated this mixture in the oven at 60°C for 30 mins. Then, this mixture was allowed to stand at room temperature overnight in a dark place. The next day, the researchers collected the supernatant and sequentially re-extracted it with different solvents of varying polarities, such as petroleum ether, ethyl acetate and butanol. The petroleum ether, ethyl acetate and butanol extracts were collected using the funnel separator. For analysing the crude solvent extracts, the researchers resuspended the dried crude material into methanol (5 ml) in glass tubes, which were capped and sealed with a parafilm strip to prevent oxidation. These extracts were stored at -20°C until further analysis. The researchers analysed these extracts using the High-Performance Liquid Chromatography (HPLC) technique. They also isolated the phenolic compounds from the aqueous extracts of *A. aureum*, *S. palustris* and *D. linearis* plants using the method described earlier (Bertin et al. 2003).

Determination of Total Phenolic Content (TPC) analysis

In this study, the researchers determined the TPC of the extracts using the Folin-Ciocalteau as mentioned earlier (Singleton & Rossi 1965). For this purpose, they diluted the Folin-Ciocalteau reagent with deionised water (final concentration of 20% v/v) in every well of the flat-bottomed 96-well clear microtiter plate. Thereafter, they added the aqueous extract of each plant sample (1.0 mg/g DW of each plant sample diluted using distilled water at a final concentration of 1000 μg/mL) in each well and incubate the microtiter plate for 5 mins at room temperature. After incubation, they added the sodium carbonate solution (90 μL of 7.5% w/v concentration) in every well of the plate, mixed it and incubated the plate for 2 h at room temperature. Finally, the researchers carried a spectrophotometric analysis of the extracts/ standards in the microplate at λmax = 725 nm, against a blank sample (only deionised water) with the help of a TECAN microplate reader. The TPC concentrations of the extracts were determined after plotting the standard graph.

HPLC analysis of the extracts

The researchers carried out an HPLC analysis of the phenolic acids using an Agilent 1200 series rapid resolution LC system (Agilent Technologies, Palo Alto, CA, USA), comprising of a binary pump, micro vacuum degassers, autosampler injector, thermostatic column compartment and also a Diode Array Detector (DAD) (Zhao et al. 2008). The extracts were analysed using the Zorbax Eclipse XDB-C 18 end-capped 5 μm×4.6×150 mm Reverse Phase column (Agilent Technologies, USA). For analysing the extracts, the researchers used a linear gradient elution technique having 2 mobile phases, where Phase A consisted of 1% formic acid dissolved in water/ acetonitrile (90:10 v/v); while Phase B was Acetonitrile (100%). The following elution gradient was used for analysis: a linear gradient was used from 0-20 mins, i.e., 0% B to 40% B; linear gradient for 20-25 mins from 40% B to 60% B; linear gradient from 25.10-35 mins for 100% B to 100% B; and an isocratic gradient was used from 35.10-40 mins consisting of 0% B. The column was operated at 25°C throughout the HPLC run. 20 μl sample was injected for every run and the elution was carried out at a flow rate of 1.0 ml/min. The researchers also carried out the HPLC analysis for different phenolic acid standard compounds like Caffeic acid, trans-p Coumaric acid, 2-Coumaric acid, 4-Coumaric acid, Ferulic acid, Hydroxybenzoic acid and Vanillic acid. All individual phenolic acid standards were detected at the maximum absorption wavelength in a mobile phase, i.e., 280 nm with the help of a Photodiode array. The concentrations of different phenolic acids were expressed as micrograms per one gram of dry weight of the freeze-dried matter (μg/g DW).

Determination of the antibacterial activity of different fern extracts

The researchers tested the antibacterial activity of the different fern samples against 5 Gram-negative bacterial species, i.e., *Staphylococcus epidermidis, S. aureus, Pseudomonas aeruginosa, Methicillin-resistant S. aureus* (MRSA), and *Escherichia coli*. The different bacterial strains were collected from the Microbiology Lab, International Islamic University, Malaysia. In this assay, the researchers mixed the Muller Hinton (MH) and broth medium, which acted as the nutrient source for the bacterial species. This mixture was also used for preparing the inoculum that aided in bacterial growth. Then, the agar well diffusion technique was used for delicately swiping the bacterial strains into the MH agar plates (Biruhalem et al. 2011). The extracts that displayed a microbial growth inhibition zone of >7 mm diameter were selected and their Minimum Inhibitory Concentration (MIC) was determined. Thereafter, the researchers incubated the medium at 37°C for 24 h, and again determined the MIC value. The minimal concentration of the extracts that inhibited the bacterial growth was selected as the MIC value for that extract.

Determination of the antifungal activity of different fern extracts

The researchers also tested the antifungal activity of the different fern extracts using the pathogenic fungal species of *Fusarium* sp., *Aspergillus niger, Phanerochaete chrysosporium, Microsporum gypseum,* and *Candida albicans*. For this purpose, all the fungal species were cultivated on the Potato Dextrose Agar (PDA) plates and the antifungal activities of the extracts were tested as mentioned above. All materials needed for these assays were procured from the Microbiology Lab, International Islamic University, Malaysia.

Statistical analysis

The researchers carried out the statistical analysis of all the results noted in the study. The values of the phenolic acid extracts that are reported here, were based on the Mean ± standard deviation values, after conducting the experiments in triplicates. The researchers also carried out the One-way VAriance assessment (ANOVA) and the Tukey's test with the help of the XLSTAT-Pro (2014) software (Addinsoft, France). These tests showed that the average values were significant at a confidence level of 99% ($p<0.001$).

RESULTS AND DISCUSSION

The researchers believed that it was necessary to collect all information regarding the chemical composition and properties of the phenolic compounds present in the fern species (*Acrostichum aureum, Stenochlaena palustris* and *Dicranopteris linearis*) as this information could improve their use in traditional medicine. Hence, they used bioassay techniques for investigating the antimicrobial characteristics of the crude extracts derived from the fern species.

Analysis of the phenolic compounds extracted from the fern plants

The researchers used the Folin-Ciocalteau assay for assessing the total phenolic content of the plant extracts, which was then expressed as gallic acid equivalent (μg GAE/g DW) (Table 2). As shown in the Table 2, the TPC values ranged between 6.99.83 and 1129.52 μg GAE/g DW for the *Acrostichum aureum, Stenochlaena palustris* and *Dicranopteris linearis* species. *A. aureum* showed the maximal TPC value (1129.52±10.70 μg GAE/g DW), followed by the *S. palustris* (938.68±7.22 μg GAE/g DW) and *D. linearis* (699.83±6.26 μg GAE/g DW) species.

Table 2. Total phenolic content of *A. aureum*, *S. palustris* and *D. linearis* (μg GAE/g DW)

Fern species	Total phenolic Content (μg GAE/g DW)
A. aureum	1129.52±10.70
S. palustris	938.68±7.22
D. linearis	699.83±6.26

The researchers also analysed these fern species for their phenolic compounds using the HPLC analysis. Figure 2 presents the graph depicting the extracted phenolic acids from the 3 species. Out of the 7 standard phenolic acids that were analysed by HPLC in the different fractional extracts (i.e., petroleum ether, ethyl acetate and butanol), the researchers could detect only 6 phenolic acids. As shown in the results, ferulic acid was primarily present in the fern extracts and its concentration ranged between 0.23-0.52 μg/G DW. The butanol and ethyl acetate extracts of *D. linearis* showed the maximal ferulic acid content, i.e., 0.52 μg/G DW and 0.32 μg/G DW, respectively.

The findings showed that the *A. aureum* extracts contained a higher phenolic acid content compared to the *S. palustris* and *D. linearis* fern species. Thus, it was noted that the *A. aureum* fractional extracts consisted of 6 types of phenolic acids, i.e., Vanillic acid and 3-Coumaric acid (0.51 μg/G DW), 4-Hydroxybenzoic acid (0.35 μg/G DW), Ferulic acid (0.27 μg/G DW), Caffeic acid (0.18 μg/G DW) and trans-p-Coumaric acid (0.06 μg/G DW). On the other hand, the *D. linearis* plant contained 4 types of phenolic acids, i.e., Vanillic acid, Ferulic acid, 4-Hydroxybenzoic acid and trans-p-Coumaric acid; while the *S. palustris* extracts showed the presence of 2 phenolic acids, i.e., Caffeic acid and Ferulic acid. Thus, the HPLC analysis indicated a similarity between the 3 fern species, since 2-Coumaric acid was not detected in any of the fractional extracts. Also, the petroleum ether extracts of the 3 fern species did not show the presence of any phenolic acid. The *A. aureum* extracts contained the highest TPC value and number of phenolic acids.

Based on the above results, the researchers concluded that the type of phenolic acid that was detected in the fractional extracts was based on the polarity of the solvent that was used for extraction. As the phenol compounds are more soluble in polar solvents, a higher concentration of phenolic acids was noted if polar solvents were used for extraction compared to the non-polar solvents (Stankovi 2011). Chai et al. (2012) observed that 2 types of phenolic acids i.e., flavonoids and hydroxycinnamic acids were more nutritionally important. Plants contain a variety of essential and non-essential compounds that help in preventing diseases and improving health (Andarwulan et al. 2012). These plants have been used in the traditional Malay formulations due to their medicinal significance. In this study, the researchers noted that the total phenolic content of the 3 fern species ranged between 6.99.83 and 1129.52 μg GAE/g DW. Out of the 3 fern species that were analysed, the *A. aureum* extracts contained the highest TPC value and the number of phenolic acids compared to the other species. Even though Ultari et al. (2021) had detected the presence of phthalic acid and flavonoids in the *A. aureum* extracts, in this study, the researchers noted the presence of a few novel active compounds, i.e., phenolic acids. Phenolic acid is seen to be a commonly ingested antioxidant compound that significantly affects methyl linoleate oxidation, free radical scavenging, and also affects human LDL cholesterol oxidation (Abdel-Aal & Rabalski 2013). Furthermore, it was reported that an anaerobic environment decreases the degradation of the phenolic acids, which increases the average concentration of phenolic acids in the soil during anaerobic conditions compared to upland (aerobic) conditions (Olofsdotter et al. 2002). Ferulic acid is commonly detected in the fern species and it displays many physiological properties, such as antibacterial, antioxidation, anti-inflammatory, and anti-cancer (Boz 2015). It is also present in the cell walls of the fern plants as a free molecule, or in the form of a dimer or is esterified with the proteins and polysaccharide molecules, which highlights its significance in many food applications (Boz 2015).

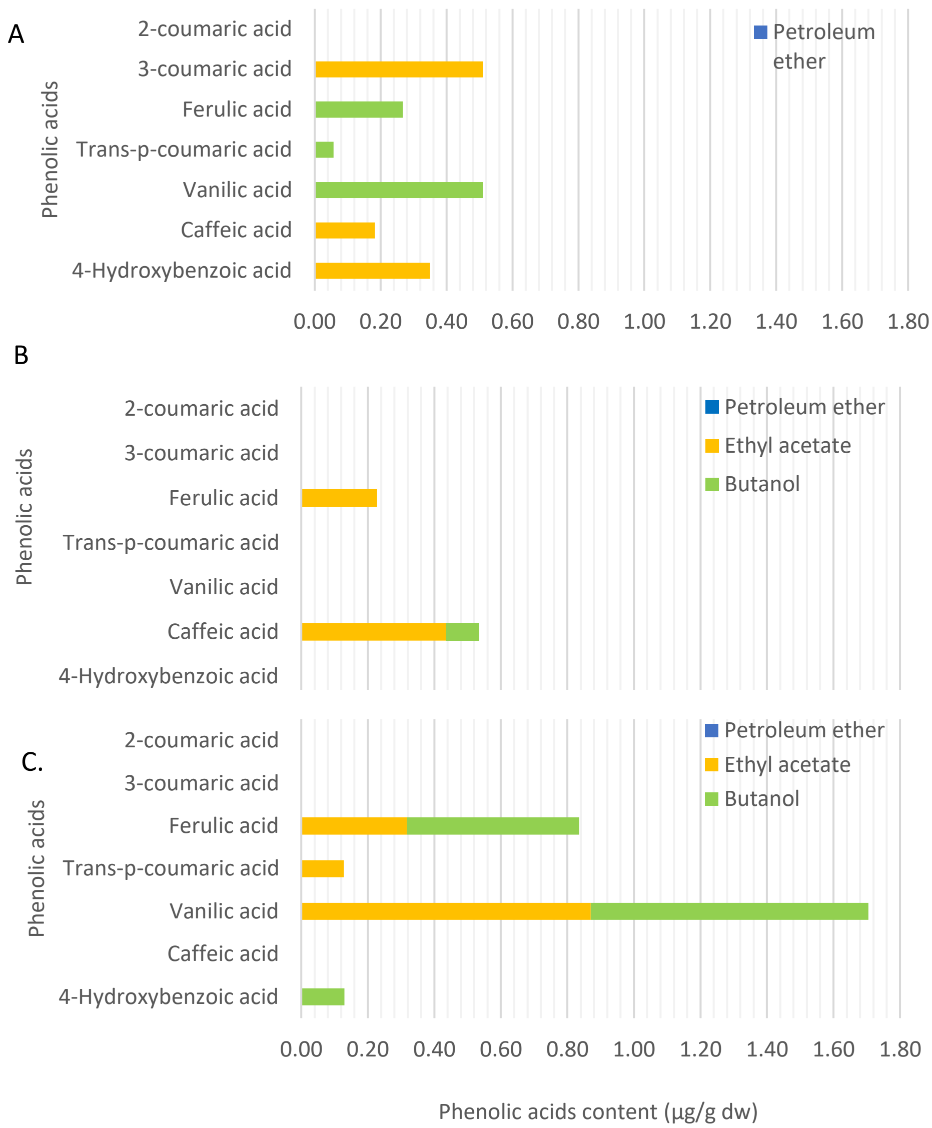

Figure 2. Phenolic acids content of A) *A. aureum*, B) *S. palustris* and C) *D. linearis*

Analysis of the antimicrobial activity of the three fern species

In this study, the researchers analysed the antibacterial activities of the fractional extracts derived from the *A. aureum*, *S. palustris* and *D. linearis* fern species, against 5 known pathogenic organisms, using the agar well diffusion technique. The antibacterial activities displayed by the 3 fern species have been summarised in Table 3, in addition to the antibacterial activities displayed by a few other plants for comparison (Rauha et al. 2000). The extracts displayed either a strong antibacterial activity (if the diameter of the inhibition zone >10 mm); a clear strong activity (if the inhibition zone ranged 4-10 mm), moderate antibacterial activity (if the inhibition zone ranged 3-4 mm), slight antibacterial activity (if the inhibition zone ranged 1-3 mm) or no antibacterial activity (if the inhibition zone <1 mm). In this study, the researchers noted that the fractional extracts of *D. linearis* displayed a wide spectrum of antimicrobial activity, and they were more active against the 5 pathogenic microbes (*S. aureus*, *S. epidermis*, *E. coli*, MRSA and *P. aeruginosa*). On the other hand, the *A. aureum* fractional extracts were active against all the pathogenic microorganisms except MRSA. Meanwhile, the *S. palustris* extracts were not very effective against the pathogenic microbes, except *P. aeruginosa*. After comparing the bacterial resistance, it was concluded that *P. aeruginosa* was the most sensitive pathogenic microbe, followed by *S. aureus, S. epidermis, E. coli* and MRSA. A comparison of the fractional extracts of the 3 fern species did not show any consistency with regards to their antibacterial activity.

Table 3. Result of antibacterial activities of *A. aureum*, *S. palustris* and *D. linearis*

Fern species	Solvents	*S. aureus*	*S. epidermidis*	*E. coli*	MRSA	*P. aeruginosa*
A. aureum	PE	~	~	-	-	-
	EA	++	+	~	-	~
	B	++	+	-	-	~
S. palustris	PE	-	-	-	-	++
	EA	-	-	-	-	~
	B	-	-	-	-	++
D. linearis	PE	++	~	++	+	+
	EA	++	+	++	+	~
	B	++	++	++	+	~

Note as referred to Rauha et al. (2000):
a) - : No antimicrobial activity, inhibition zone of sample < inhibition zone of ethanol +1 mm
b) ~ : Slight antimicrobial activity, inhibition zone of sample 1-3 mm > inhibition zone of ethanol
c) + : Moderate antimicrobial activity, inhibition zone of sample 3-4 mm > inhibition zone of methanol
d) ++ : Clear antimicrobial activity, inhibition zone of sample 4-10 mm > inhibition zone of ethanol
e) +++ : Strong antimicrobial activity, inhibition zone of sample +10 mm > inhibition zone of ethanol

In another experiment, the researchers also evaluated the antifungal activities of the fractional extracts of the 3 fern species (*A. aureum*, *S. palustris* and *D. linearis*) against 5 pathogenic fungal species, using the agar well diffusion technique. Table 4 presents a summary of the results and also includes the antifungal activities of a few other species for comparison (Rauha et al. 2000). As shown in the Table 4, the fractional extracts of the fern species displayed good antifungal activity against only the *C. albicans* and *Fusarium sp.*; while no antifungal activity was detected against *M. gypseum*, *P. chrysosporium* and *A. niger*. The *S. palustris* fractional extracts did not show any antifungal activity against the selected fungal species. On the other hand, the *A. aureum* and *D. linearis* species displayed antifungal activity against *Fusarium* sp. and *C. albicans*.

Table 4. Result of antifungal activities of *A. aureum*, *S. palustris* and *D. linearis*

Fern species	Solvents	*C. albicans*	*Fusarium* sp.	*M. gypseum*	*P. chrysosporium*	*A. niger*
A. aureum	PE	~	++	-	-	-
	EA	~	++	-	-	-
	B	-	~	-	-	-
S. palustris	PE	-	-	-	-	-
	EA	-	-	-	-	-
	B	+	-	-	-	-
D. linearis	PE	~	~	-	-	-

EA	-	-	-	-	-
B	-	-	-	-	-

Note as referred to Rauha et al. (2000):
a) - : No antimicrobial activity, inhibition zone of sample < inhibition zone of ethanol +1 mm
b) ~ : Slight antimicrobial activity, inhibition zone of sample 1-3 mm > inhibition zone of ethanol
c) + : Moderate antimicrobial activity, inhibition zone of sample 3-4 mm > inhibition zone of methanol
d) ++ : Clear antimicrobial activity, inhibition zone of sample 4-10 mm > inhibition zone of ethanol
e) +++ : Strong antimicrobial activity, inhibition zone of sample +10 mm > inhibition zone of ethanol

Appalaraju et al. (2013) mentioned that the disc diffusion technique could be effectively used for testing the antimicrobial and antifungal properties of the chloroform, ethanol and aqueous extracts of the dries *Pereskia bleo* leaves. They noted that only the aqueous extracts displayed an antifungal activity compared to the other extracts. In one study, the researchers noted a difference in the phytochemical composition and antibacterial activities when they used fresh and dried parts of the same plant (Alabi et al. 2012). A similar difference was noted in this study, where the researchers noted a variation in the antimicrobial activities and phenolic compounds even when they used the same dried leaf samples. Thomas et al. (2016) described the therapeutic significance of the *A. aureum* fern species, based on the presence of bioactive compounds that were detected during the *in vitro* and *in vivo* assays. Phenolic acids, such as hydroxybenzoic acids and hydroxycinnamic acids, are important types of phenolic compounds (Bohm 1968). In the past, many researchers identified >70 medicinal plants that contained phenolic acids. A few of these phenolic compounds, like phenolic acids, quinones, flavonoids, stilbenes, coumarins, curcuminoids and lignans, displayed potent anti-oxidation activity, in addition to anti-carcinogenic, antimicrobial, anticancer, and anti-mutagenic activities (Cai et al. 2004). Furthermore, when Cai et al. (2003) tested 20 pteridophytes species for their bacteriostatic activity against the pathogenic organisms like *E. coli, S. aureus, S. lutea, B. subtilis*, *P. vulgari,* and *S. cerevisiae*, they noted that the *P. ensiformis* Burm. and *P. semipinnata* species displayed strong antibacterial activities against the pathogenic microbes.

CONCLUSIONS

In this study, the researchers assessed the antibacterial and antifungal properties of the fractional extracts derived from 3 fern species, i.e., *A. aureum*, *S. palustris* and *D. linearis.* The results indicated that the *A. aureum* extract contained a higher total phenolic content, followed by the *S. palustris* and *D. linearis* extracts. HPLC analysis of the extracts showed that the *A. aureum* extract contained the highest number of phenolic acids, compared to the *S. palustris* and *D. linearis* samples. The *A. aureum* extract contained 6 phenolic acids, such as Vanillic acid and 3-Coumaric acid (0.51 μg/g DW), 4-Hydroxybenzoic acid (0.35 μg/g DW), Ferulic acid (0.27 μg/g DW), Caffeic acid (0.18 μg/g DW) and trans-p-Coumaric acid (0.06 μg/g DW). When the fractional extracts were tested for their antimicrobial and antifungal activities, it was noted that all 3 fern species displayed better antibacterial activities than antifungal activity. The high antibacterial activity of the 3 medicinal fern species could be attributed to their high phenolic content. Thus, these ferns could be used as a potential candidate for preparing herbal drug formulations. However, additional research needs to be conducted for optimising the technique for isolating and purifying the bioactive components present in the fern species. For this purpose, the researchers need to focus more on the aqueous leaf extracts of the *A. aureum*, *S. palustris* and *D. linearis* fern species, as they showed a higher selectivity and potency.

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