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Research

Solvent-Dependent Extraction and comparative Antioxidant Profiling of *Drynaria quercifolia* and *Curcuma aeruginosa* rhizomes using Multivariate Statistical Approach

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ABSTRACT

Oxidative stress is the major factor responsible for chronic diseases, which creates a need for safety and effective natural antioxidants. The study investigates the phytochemical composition and antioxidant activities of *Drynaria quercifolia* and *Curcuma aeruginosa* rhizomes extracts using different solvents of increasing order of polarity which include Petroleum ether, Ethyl acetate, Acetone, Ethanol and Water. Preliminary phytochemical screening revealed the presence of bioactive compounds such as tannins, phenols, glycosides, steroids and flavonoids. The Ethanol extracts shows the broadest spectrum of secondary metabolites in both species. Antioxidant potential was evaluated through five standard *in vitro* assays such as DPPH, ABTS, FRAP, Superoxide radical scavenging and Phosphomolybdenum assays. Ethanol and aqueous extracts exhibited the highest antioxidant activity across assays, especially in *Curcuma aeruginosa*, which showed superior performance in FRAP (263.76 µmol/g) and Phosphomolybdenum (46.08 mg AAE/g) in contrast with *Drynaria quercifolia* where ethanol extracts showed higher performance in ABTS (64840.00 µmol/g). Multivariate statistical tools, including Principal Component Analysis (PCA), Hierarchical Clustering Analysis (HCA) and KMeans clustering, were applied to discern patterns and extract potency groupings. MANOVA revealed a statistically significant difference in antioxidant profiles between species (Wilks' $\lambda = 0.0374$, $p = 0.0079$). KMeans clustering further distinguished high-potency in the solvents such as ethanol and aqueous from low-potency non-polar extracts. These findings indicates that the polarity-dependent extractability of antioxidant compounds and support the potential of *C. aeruginosa* and *D. quercifolia* as a significant source of multifunctional antioxidants.

Keywords: *Drynaria quercifolia*, *Curcuma aeruginosa*, Antioxidant assays, Phyto-chemical screening, Multivariate analysis.

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1. INTRODUCTION

The phenomenon known as oxidative stress arises from an imbalance between the cellular production of reactive oxygen species (ROS) and the cell's capacity to scavenge them. This imbalance results in cellular damage, including damage to nucleic acids and DNA. Such damage may become more extensive due to compromised cellular antioxidant defence systems. All biological systems possess antioxidant defence mechanisms that protect against oxidative damage and include repair enzymes to eliminate damaged molecules. However, these natural antioxidant mechanisms can be inefficient, thereby necessitating the dietary intake of antioxidant compounds [1, 2]. Scientific data on the antioxidant properties of endemic plants were restricted to specific regions and often known only through traditional or local knowledge and it

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remains limited. As a result, exploring these underutilized plants offers a valuable opportunity to discover novel and promising sources of natural antioxidants, with potential applications in functional foods and nutraceutical development [2,3,4]. Natural antioxidants derived from medicinal plants are garnering increased interest due to their efficacy and lower toxicity compared to synthetic alternatives. Among these, *Drynaria quercifolia* Linn commonly known as Attukal Kilangu, belongs to the family Polypodiaceae, which is a fern with various traditional applications in Indian medicine [5]. *Curcuma aeruginosa* Roxb. belonging to the family Zingiberaceae ,known as Pink and Blue ginger have garnered attention for their ethno pharmacological significance by various tribes over the world [6].

C. aeruginosa and *D. quercifolia* rhizomes from two different groups was recognized for various phytochemical composition, Anti-oxidant properties, Anti-inflammatory activities which were used against various rheumatic related diseases as per various literatures [5-8], yet their comparative analyses of its antioxidant behaviour were limited. This study addresses this gap by conducting solvent-dependent extraction and evaluating both plants using multiple antioxidant assays, alongside a preliminary phytochemical screening.

A multivariate statistical approach was employed to analyze and interpret the data. Techniques such as Principal Component Analysis (PCA), Hierarchical Clustering Analysis (HCA), and KMeans clustering were applied to classify extracts based on antioxidant efficacy. Additionally, a multivariate analysis of variance (MANOVA) was used to determine the statistical significance of species-specific antioxidant profiles [9]. This integrated evaluation provides insight into the phytochemical composition and antioxidant behaviour of these two rhizomes in statistical approach.

2. MATERIALS AND METHODS

2.1 Plant Collection and Authentication

Fresh rhizomes of *Drynaria quercifolia* were collected from Yercaud, Salem District of Tamilnadu and *Curcuma aeruginosa* rhizomes were collected from Guntur district, Telengana during the months of August 2023 – November 2023. Both the rhizomes were authenticated by Botanical Survey of India, Southern Circle, Coimbatore, Tamil nadu and voucher specimens were deposited for further reference.

2.2 Preparation of Extracts

The Fresh rhizomes were cleaned, shade-dried and ground to a coarse powder. About 100 grams of each rhizome powder were taken for extraction. Sequential extraction was performed using Petroleum ether, Ethyl acetate, Acetone and Ethanol using Soxhlet apparatus. Aqueous extracts were obtained through infusion using distilled water. All extracts were filtered, concentrated using a distillation method and were stored at 4 °C for further analysis.

2.3 Extract Recovery Percentage

The amount of crude extract recovered after sequential extraction was weighed and the percentage yield of the extracts were calculated using the formula,

Extract recovery percentage=

$$\frac{Amount\ of\ Extract\ recovered(g)}{Amount\ of\ the\ Rhizome\ sample\ taken\ (g)}\ X\ 100$$

2.4 Preliminary Phytochemical Screening

Standard qualitative phytochemical screening procedures following Raaman, 2006 [10] method were employed to detect phytochemical groups including tannins, flavonoids, saponins, phenols, alkaloids, steroids, glycosides, terpenoids, coumarins, carbohydrates, phytosterols, proteins, quinones, and anthocyanins. Each test was performed across all extracts and results were recorded based on intensity (++/+/−).

2.5 *In vitro* Antioxidant Assays

The antioxidant potential of the extracts was evaluated using using five standard *in vitro* antioxidant assays namely DPPH radical scavenging assay, ABTS radical cation decolorization assay, Ferric Reducing Antioxidant Power(FRAP) assay, Superoxide radical scavenging assay and Phosphomolybdenum Total antioxidant capacity assay. All the experiments were conducted in triplicates and the results were expressed as mean ± standard deviation.

2.5.1 DPPH Radical scavenging Assay

The antioxidant activity of the extracts was evaluated based on their ability to donate hydrogen atoms or scavenge free radicals, using the stable DPPH radical, following the procedure described by Gursoy *et al.,* (2009)[11] with slight modifications. Different concentrations of the extracts (ranging from 20–100 µL) were used, and each was made up to a final volume of 100 µL with ethanol. To these, 3 mL of 0.004% DPPH solution prepared in ethanol

was added, and the mixture was shaken thoroughly. All reaction mixtures were incubated in the dark at 27 °C for 30 minutes. The absorbance was recorded at 517 nm against a ethanol blank. The radical scavenging activity was quantified by calculating the IC_{50} value, defined as the concentration of extract required to reduce the initial DPPH concentration by 50%.

2.5.2 ABTS Radical Cation Decolorization Assay

The antioxidant activity was evaluated using the ABTS radical cation scavenging assay, as described by Re *et al.,* (1999) [12]. $ABTS^{\bullet+}$ was generated by reacting ABTS with 2.4 mM potassium persulfate and incubating the mixture in the dark for 12–16 hours. Before analysis, the ABTS solution was diluted with ethanol (1:89, v/v) to achieve an absorbance of 0.70 ± 0.02 at 734 nm. To each test tube, 1 mL of the diluted ABTS solution was added, followed by 30 µL of the sample and 10 µL of Trolox (final concentrations 0–15 µM). A control was prepared by adding 30 µL of ethanol instead of the sample. The mixtures were vortexed and incubated for 30 minutes at room temperature, after which absorbance was recorded at 734 nm, using ethanol as a blank. Antioxidant activity was expressed as µM Trolox equivalents per gram of extract.

2.5.3 Phosphomolybdenum Assay

The antioxidant capacity was measured using the phosphomolybdenum complex method, according to Prieto *et al.,* (1999) [13]. In each test tube, 100 µL of sample was mixed with 200 µL of ethanol, and the total volume was brought up to 300 µL with ethanol. The blank consisted of 300 µL ethanol. To each tube, 3 mL of the reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate) was added, and the tubes were vortexed. The samples were then incubated in a water bath at 95°C for 90 minutes. After cooling to room temperature, absorbance was measured at 695 nm. The results were expressed as milligrams of ascorbic acid equivalents (AAE) per gram of extract**.**

2.5.4 Ferric Reducing Antioxidant Power (FRAP) Assay

The FRAP assay was conducted to assess the ferric reducing ability of the extracts, following the method described by Pulido *et al.,* (2000) [14]. The FRAP reagent was freshly prepared by combining 2.5 mL of 20 mM TPTZ in 40 mM HCl, 2.5 mL of 20 mM $FeCl_3 \cdot 6H_2O$, and 25 mL of 0.3 M acetate buffer (pH 3.6). In each test tube, 900 µL of FRAP reagent was mixed with 90 µL of distilled water and 30 µL of sample. The blank was prepared by adding ethanol. The test tubes were incubated at 37°C for 30 minutes, after which the absorbance was measured at 593 nm. A calibration curve was constructed using $FeSO_4 \cdot 7H_2O$ solutions ranging from 500 to 4000 µM. Antioxidant capacity was expressed as the concentration of antioxidant capable of reducing ferric ions, equivalent to the reducing ability of 1 mM $FeSO_4 \cdot 7H_2O$

2.5.5 Superoxide Radical Scavenging Assay

Superoxide radical scavenging activity was determined using the method of Beauchamp and Fridovich (1971)[15], based on the inhibition of formazan formation in the riboflavin-light-NBT system. The reaction mixture (3 mL) consisted of 50 mM sodium phosphate buffer (pH 7.6), 20 µg riboflavin, 12 mM EDTA, and 0.1 mg NBT. To this, 100 µL of sample was added. The reaction was initiated by illuminating the mixture for 90 seconds. A control, without the sample, was exposed to light under identical conditions, while the blank was kept in the dark. The absorbance was recorded at 590 nm. Superoxide scavenging activity was calculated using the following formula:

$$\%\text{Scavenging Activity} = \frac{Abscontrol - Abssample}{Abscontrol} \text{ X } 100$$

2.6 Statistical and Multivariate Analysis

All quantitative results from antioxidant assays are presented as mean ± standard deviation (SD), derived from three independent experimental replicates. Each assay operates on a different biochemical principle and reports in different units, making direct comparisons difficult. Therefore, to place all assay results on a uniform scale for multivariate statistical analysis, Z-score standardization was applied.

One important consideration was that not all antioxidant assays are interpreted the same way. Specifically, the DPPH assay measures IC_{50} values, where lower values indicate higher antioxidant activity (i.e., less extract is needed to neutralize free radicals). In contrast, assays like ABTS, FRAP, Superoxide, and Phosphomolybdenum interpret higher values as better activity. To ensure consistency across all assays, the Z-score values for DPPH were reversed by multiplying them by –1. This way, in all assays, a higher Z-score reflects stronger antioxidant performance, regardless of the original unit or assay direction.

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Z-scores were calculated using the standard formula:

This standardization made the data unitless and comparable, and it allowed for fair interpretation through multivariate analysis. The method aligns with the best practices outlined by Granato *et al.,* (2018) [16] and Sridar & Charles (2018) [9].The Z-score for each data point was calculated using the formula:

$$Z=\frac{X-\mu}{\sigma}$$

All statistical analyses were conducted using Python 3.11. Principal Component Analysis (PCA), KMeans clustering and Hierarchical Clustering Analysis (HCA) using Ward's method and Euclidean distance were performed using the scikit-learn package (Pedregosa *et al.,* , 2011)[17]. The optimal number of clusters was determined using Silhouette score analysis (Rousseeuw, 1987) [18]. Multiple Correspondence Analysis (MCA**)** were also performed. The Multivariate Analysis of Variance (MANOVA) was carried out using the statsmodels library (Seabold & Perktold, 2010) [19], with the model set as:

DPPH + ABTS + FRAP + Superoxide + Phosphomolybdenum~Species

All graphical visualizations, including PCA plots, heat maps and dendrograms were generated using Python visualization libraries including matplotlib and seaborn.

3. RESULTS AND DISCUSSION

3.1 Extract Recovery Percentage

The rhizomes of *Drynaria quercifolia* and *Curcuma aeruginosa* from different solvent extracts with percentage yields were presented in the table-1. The maximum yield were obtained in the aqueous extracts of *C. aeruginosa* rhizome (15.18%). *D. quercifolia* aqueous extracts also yielded about 13.60%. The second highest yield was from Ethanol extracts in *C.aeruginosa* yielded 10.70% and *D .quercifolia* yielded about 09.51%, suggesting its dual polarity nature that facilitates the extraction of both polar and semi-polar compounds, aligning with literature emphasizing ethanol as a versatile and effective solvent for phytoconstituent recovery (Miliauskas *et al.,* , 2004)[20].

The Ethyl acetate extracts were yield about 08.21% in *C. aeruginosa* and 06.10% in *D.quercifolia.* This is indicative of water's ability to solubilize a broad range of hydrophilic phytochemicals such as carbohydrates, glycosides, and phenolics. Petroleum ether and acetone extracts were found to have the lowest yield percentages. These findings are consistent with earlier reports that non-polar solvents primarily extract lipophilic components and may not yield significant amounts of antioxidant-related bioactives (Balasundram *et al.,* , 2006)[21]. Therefore, the high recovery percentages with aqueous and ethanol solvents reflect their efficiency in solubilizing compounds of pharmacological relevance.

Table 1. Percentage yield of different solvent extracts of *Drynaria quercifolia* and *Curcuma aeruginosa* rhizomes.

S.No	Solvents	*Drynaria quercifolia* Rhizome (%)	*Curcuma aeruginosa* Rhizome (%)
1.	Petroleum Ether	04.87	06.09
2.	Ethyl acetate	06.10	08.21
3.	Acetone	03.75	04.90
4.	Ethanol	10.70	09.51
5.	Aqueous	13.60	15.18

3.2 Preliminary Phytochemical Screening

The preliminary phytochemical analysis revealed the presence of a variety of bioactive compounds across both *Drynaria quercifolia* and *Curcuma aeruginosa* extracts. Ethanol extracts from both species showed the widest spectrum of secondary metabolites, including tannins, flavonoids, glycosides, steroids and terpenoids. Aqueous and acetone extracts exhibited moderate presence of these compounds, while petroleum ether and ethyl acetate extracts showed more limited phytochemical diversity. These secondary metabolites are reported to neutralize free radicals and chelate metal ions, contributing significantly to the antioxidant capacity of plant extracts (Balasundram *et al.,* , 2006; Gursoy *et al.,* , 2009)[21,11]. From the Table-2, it suggests that ethanol is the most efficient solvent for extracting a broad range of bioactive compounds from both rhizome species, while non-polar solvents are less effective at extracting such compounds.

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Table-2 Preliminary phytochemical screening of *Drynaria quercifolia* and *Curcuma aeruginosa* Rhizomes

Phytochemical	*Drynaria quercifolia*	*Curcuma aeruginosa*
Tannins	++	++
Saponins	++	+
Flavonoids	+	+
Steroids	+	+
Terpenoids	+	++
Coumarins	+	+
Alkaloids	+	++
Glycosides	++	++
Carbohydrates	++	++
Phytosterols	+	+
Fixed oils and fats	+	+
Phenols	+	+
Proteins/Aminoacids	+	+
Quinones	-	-
Anthocyanins	-	-

Results were recorded based on intensity (++/+/–).

3.3 Antioxidant Assays and Z-score Standardization

Antioxidant assays revealed significant variability in activity based on both the solvent and species. Ethanol and aqueous extracts, particularly from *Curcuma aeruginosa*, exhibited the highest antioxidant capacity. The ethanol extract of *C. aeruginosa* showed superior performance in FRAP (263.76 µmol/g) and Phosphomolybdenum (46.08 mg AAE/g), while *D. quercifolia* ethanol extract was most effective in ABTS (64840.00 µmol/g). The lowest antioxidant activity was consistently recorded in petroleum ether and ethyl acetate extracts.

Z-score standardization enabled a unitless comparison across all assays. Ethanol and aqueous extracts showed high positive Z-scores across the board, indicating their superior antioxidant potential. The ethanol extract of *C. aeruginosa* had the highest Z-scores in DPPH (+1.58), FRAP (+1.40), and Phosphomolybdenum (+1.92). These findings validates the efficiency of polar solvents in extracting bioactive antioxidant compounds (Granato *et al.*, , 2018; Sridar & Charles, 2018)[16,9].The reversal of Z-scores for DPPH (IC50 based) ensured interpretive consistency across assays. The aligned scale allowed multivariate comparisons and statistical analysis, supporting the hypothesis that ethanol and aqueous extracts are most potent.

Table-3 Quantitative results of in vitro antioxidant assays for *D. quercifolia* and *C. aeruginosa* extracts.

Species	Extracts	DPPH IC_{50} in µg/mL	ABTS µmol TE/g	FRAP µmol Fe(II)/g	Superoxide % inhibition	Phosphomoly-bdenum mg AAE/g
D. quercifolia	Pet.Ether	37.32±0.45	16333.33±375	83.45±1.80	11.08±0.72	10.1±0.70
	E.acetate aceacetate	21.57±0.70	28347.22±236	76.41±4.31	12.68±1.58	9±1.07
	Acetone	22.80±0.98	43034.73±967	217.89±4.54	28.09±1.22	3.90±2.00
	Ethanol	21.50±0.72	64840.00±264	240.79±3.00	56.87±0.31	32.46±2.90
	Aqueous	34.76±0.47	63486.10±880	187.46±1.11	15.61±1.3	9.53±0.70
C.aeruginosa	Pet.ether	28.05±0.73	16784.72±513	132.28±2.40	10.37±0.61	10±1.05
	E.acetate	37.33±0.46	23416.67±908	153.89±4.55	13.40±0.63	11±1.72
	Acetone	20.59±0.70	32340.28±1661	235.18±1.05	27.01±0.81	40.6±20.5
	Ethanol	15.00±0.47	63486.11±885	263.76±1.05	36.02±0.97	46.08±3.22
	Aqueous	37.56±0.16	43034.72±967	183.42±1.09	17.20±1.09	9.13±0.99

Pet.Ether- Petroleum ether; E.acetate- Ethyl acetate

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Table-4 Z-score normalized antioxidant assay values of both rhizomes for multivariate comparison.

Species	Extracts	DPPH	ABTS	FRAP	Superoxide	Phosphomolybdenum
D. quercifolia	Pet.Ether	-1.20	−1.27	−1.52	−0.84	−0.56
	E.acetate aceacetate	+0.76	−0.61	−1.63	−0.73	−0.63
	Acetone	+0.60	+0.19	+0.65	+0.38	−0.98
	Ethanol	+0.77	+1.39	+1.02	+2.44	+0.98
	Aqueous	-0.89	+1.32	+0.16	−0.52	−0.59
C.aeruginosa	Pet.Ether	-0.05	−1.25	−0.73	−0.89	−0.56
	E.acetate	-1.21	−0.89	−0.38	−0.68	−0.49
	Acetone	+0.88	−0.39	+0.93	+0.30	+1.54
	Ethanol	+1.58	+1.32	+1.40	+0.95	+1.92
	Aqueous	-1.23	+0.19	+0.10	−0.40	−0.62

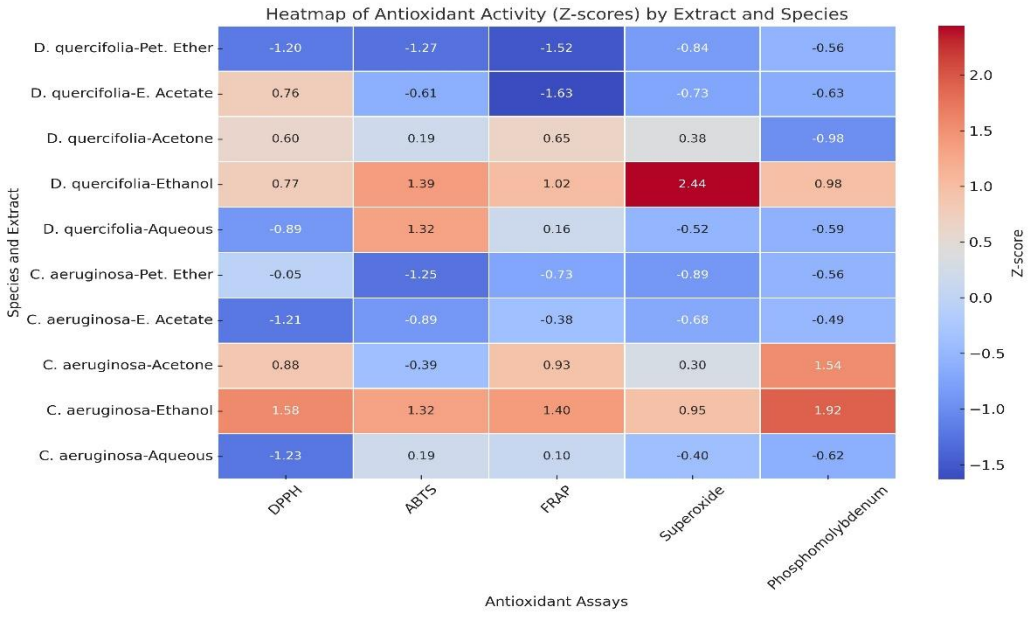
Pet.Ether- Petroleum ether; E.acetate- Ethyl acetate

3.3 Heatmap of Antioxidant Assays

The heatmap (Fig. 1) provides a visual summary of antioxidant assay results for both rhizomes. The extracts clustered by solvent polarity show consistent trends: ethanol and aqueous extracts of both *D. quercifolia* and *C. aeruginosa* exhibited strong antioxidant responses which are indicated by darker shades, particularly in ABTS, FRAP, and Phosphomolybdenum assays. In contrast, petroleum ether and ethyl acetate extracts showed lighter shading, correlating with lower assay responses.

This visualization confirms the numerical assay findings and supports the interpretation that polar solvents are more effective at extracting antioxidant-rich phytochemicals. The prominent performance of ethanol and aqueous extracts, especially in *C. aeruginosa*, suggests that these solvents extract a diverse profile of hydrophilic and moderately polar antioxidants. These results are in agreement with previous studies which noted strong visual clustering in heatmaps for ethanol-extracted phenolic-rich samples (Re *et al.*, 1999; Miliauskas *et al.*, 2004)[12,20].

Fig.1 Heatmap of antioxidant activity levels across different solvent extracts.



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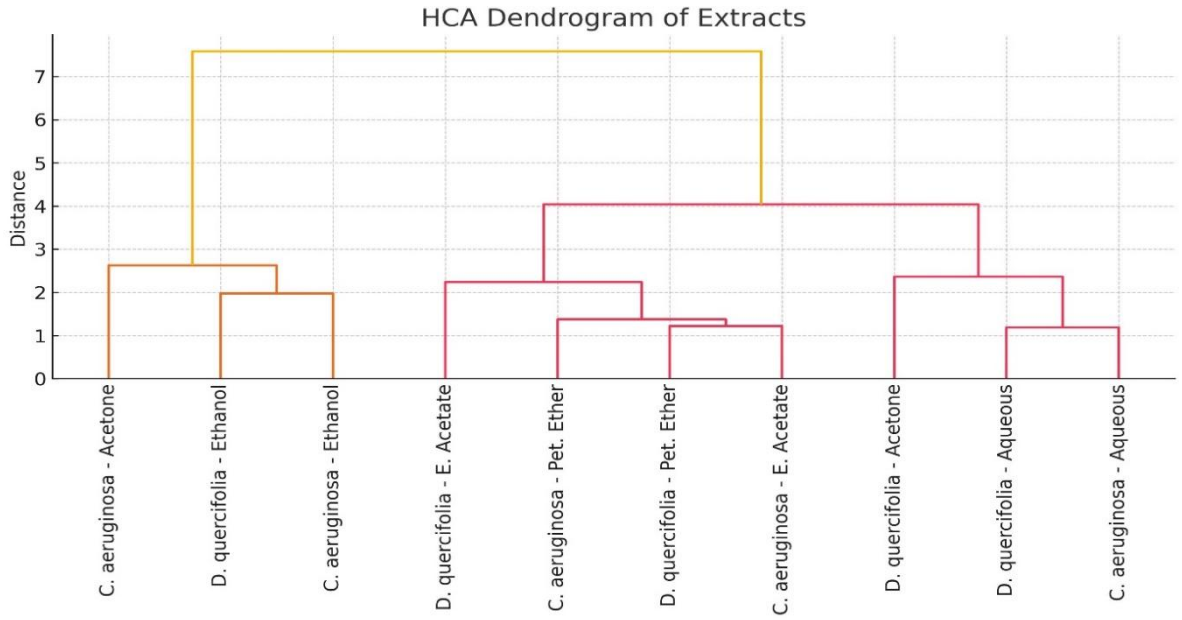
3.4 Hierarchical Clustering Analysis (HCA)

Hierarchical Clustering Analysis (HCA) was conducted to better understand the relationships among the various extracts based on their antioxidant activity profiles. As illustrated in the dendrogram (Fig. 2), ethanol and aqueous extracts from both *Drynaria quercifolia* and *Curcuma aeruginosa* grouped closely together, suggesting that these extracts exhibit similar antioxidant behaviours across the different assays. In contrast, the petroleum ether and ethyl acetate extracts formed distinct clusters, highlighting their unique antioxidant characteristics—typically associated with lower activity levels.HCA has been widely recognised as a reliable tool for classifying plant extracts based on their bioactivity and is particularly useful for interpreting complex biochemical data (Granato *et al.*, 2018) [16].

These clustering patterns aligned well with the trends observed in the heatmap and Z-score analysis, both of which showed that ethanol and aqueous extracts consistently demonstrated the strongest antioxidant potential, while non-polar extracts like petroleum ether and ethyl acetate generally exhibited weaker activity.

Fig. 2 HCA dendrogram showing clustering of extracts based on antioxidant activity



3.5 Principal Component Analysis (PCA) and KMeans Clustering

Principal Component Analysis (PCA), illustrated in Fig. 3, was carried out to simplify the dataset and uncover underlying patterns in the antioxidant profiles of the plant extracts. The first two principal components (PC1 and PC2) accounted for over 90% of the total variance, capturing the most relevant differences among the extracts. The PCA scatter plot showed a clear clustering of ethanol and aqueous extracts, suggesting that these polar solvents tend to extract similar antioxidant compounds from both *Drynaria quercifolia* and *Curcuma aeruginosa*. In contrast, petroleum ether and ethyl acetate extracts were more dispersed, reflecting the distinct profiles of non-polar extracts.

These findings emphasize the important role of solvent polarity in determining antioxidant potential, with polar solvents generally yielding more active extracts. PCA has been widely used in food and plant sciences to interpret complex datasets and highlight multivariate relationships (Jolliffe & Cadima, 2016) [22].

To delve deeper into the extract groupings, KMeans clustering was also applied (see Figure 3). This technique grouped the extracts based on similarities in their antioxidant activity. The ideal number of clusters was determined using silhouette scores (Figure 4), which measure how well each extract fits within its assigned group. The analysis revealed two primary clusters: Cluster 0, composed mainly of high-activity extracts—particularly those from ethanol and aqueous extractions—and Cluster 1, which included low-activity extracts such as those obtained with petroleum ether and ethyl acetate.This clustering approach further reinforced the PCA

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findings, distinguishing extracts into high and low antioxidant activity groups. The use of silhouette scores supported the two-cluster solution, a method known for improving the clarity and reliability of group classification in phytochemical research (Rousseeuw, 1987) [23]. Together, the PCA, KMeans and HCA analyses consistently demonstrated the influence of solvent polarity on antioxidant outcomes.

Fig.3 Principal Component Analysis (PCA) and Kmeans clustering plot of antioxidant profiles of both the rhizomes

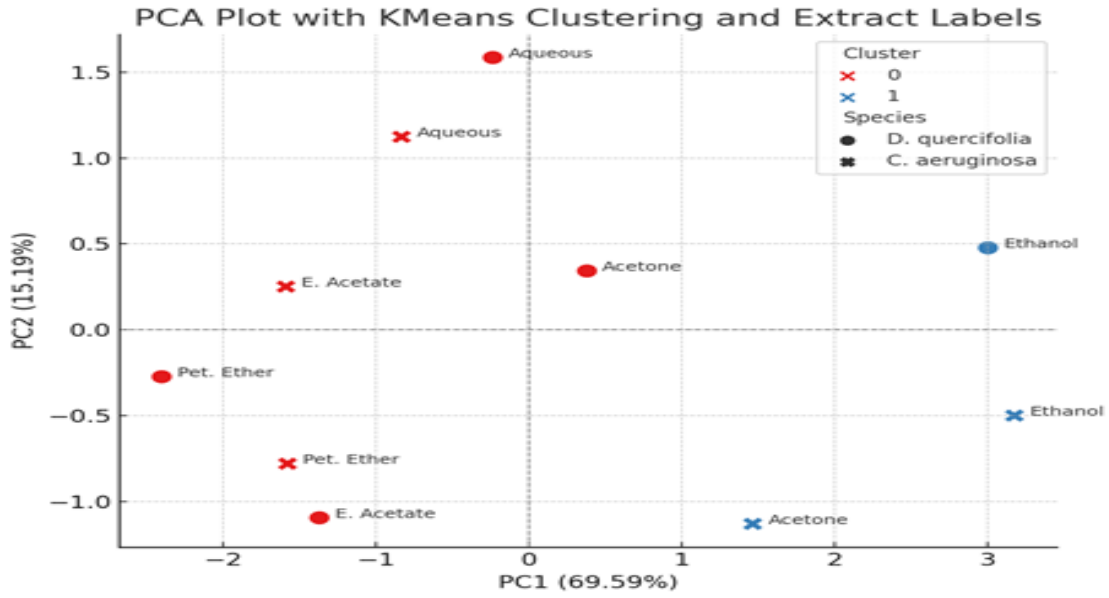
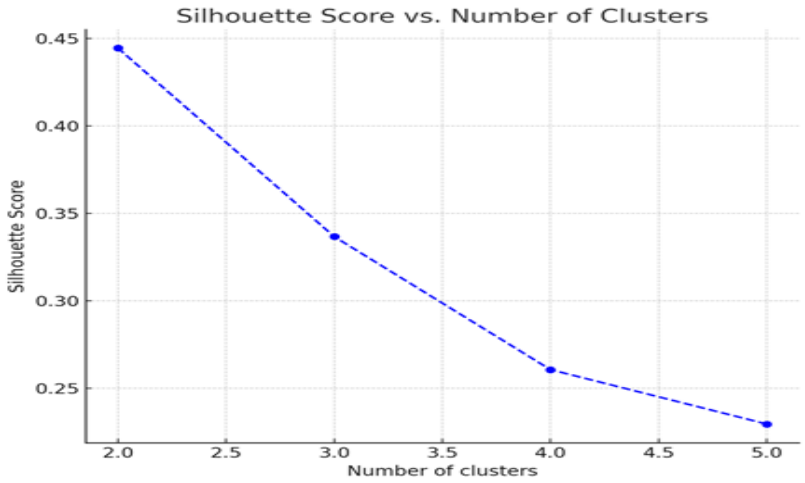


Fig.4 Silhouette score plot indicating optimal cluster count.



3.7 Multivariate Analysis of Variance (MANOVA)

Multivariate Analysis of Variance (MANOVA) was employed to assess whether the plant species—*Drynaria quercifolia* and *Curcuma aeruginosa*—significantly influenced antioxidant activity across the five different assays. The analysis revealed clear differences between the species' antioxidant profiles. Specifically, the Wilks' lambda value was 0.0374, accompanied by a p-value of 0.0079, indicating a statistically significant effect of species on the overall antioxidant performance of the extracts.

These findings support the view that the antioxidant activity is not solely dependent on the extraction solvent, but also significantly influenced by the inherent biochemical composition of the plant species. The use of MANOVA in this context is particularly valuable, as it allows for the simultaneous comparison of multiple dependent

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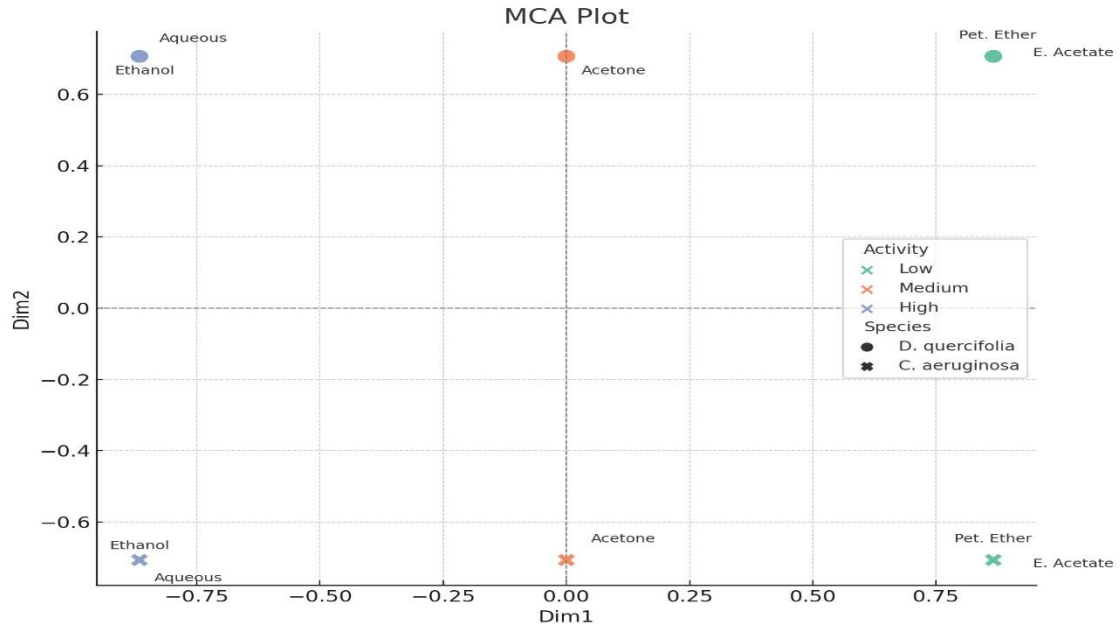
variables—making it a powerful tool in nutritional and phytochemical research (Tabachnick & Fidell, 2013)[24].Overall, this result validates the conclusion that *D. quercifolia* and *C. aeruginosa* possess distinct antioxidant profiles, regardless of the extraction solvent used.

3.8 Multiple Correspondence Analysis (MCA)

To understand the better relationships among solvent type, plant species and antioxidant activity levels, Multiple Correspondence Analysis (MCA) was employed. This method helps visualize how categorical variables are related by projecting them into a simplified, low-dimensional space. In this study, MCA was used to explore the interactions between three key factors: the plant species (*Drynaria quercifolia* and *Curcuma aeruginosa*), the solvents used for extraction (petroleum ether, ethyl acetate, acetone, ethanol, and aqueous), and the antioxidant activity level (categorized as high or low).

As given in Fig. 5, the ethanol and aqueous extracts from both *C. aeruginosa* and *D. quercifolia* tend to cluster in the quadrant representing high antioxidant activity. This observation supports earlier findings from PCA and clustering analyses. On the other hand, extracts obtained using petroleum ether and ethyl acetate are more closely associated with the low antioxidant activity group.

Fig.5 MCA biplot associating solvents, species and antioxidant activity groups.



This analysis reinforces the influence of both species-specific phytochemistry and solvent polarity on antioxidant capacity, illustrating how these variables interact categorically within the dataset.

4. CONCLUSION

This study offers a comprehensive evaluation of the antioxidant potential of *Drynaria quercifolia* and *Curcuma aeruginosa* rhizome extracts, evaluated through preliminary phytochemical screening and a series of *in vitro* antioxidant assays. The findings clearly indicates that solvent polarity significantly influences the extraction efficiency of antioxidant compounds. Based on the results of the antioxidant assays, multivariate analyses and clustering, it is evident that the ethanol and aqueous extracts of both species exhibit the highest antioxidant potential, particularly in assays such as FRAP, ABTS, and Phosphomolybdenum. *Curcuma aeruginosa* was identified as the most potent antioxidant source, especially in FRAP and Phosphomolybdenum assays, while *Drynaria quercifolia* demonstrated notable efficacy in Superoxide radical scavenging, suggesting its potential for targeted oxidative stress applications. Furthermore, MANOVA results revealed significant differences in antioxidant potential between *D. quercifolia* and *C. aeruginosa*, suggesting the influence of species-specific phytochemistry, as both belong to different plant groups. These findings support the potential of ethanol and aqueous extracts of these plants in nutraceuticals, functional foods and as therapeutic agents targeting diseases related to oxidative stress. The study also reinforces the importance of employing a multivariate approach to capture the complexity of plant-based antioxidant systems,

facilitating more informed decisions in extract selection and drug formulation design.

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