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Comparative phytochemical profiling and mechanistic antioxidant evaluation of *Drynaria quercifolia* (L.) J.Sm. and *Curcuma aeruginosa* Roxb. rhizomes in relation to gouty arthritis

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Abstract
An inter-generic comparison of phytochemical profiles and antioxidant potential between *Drynaria quercifolia* (L.) J.Sm. (Polypodiaceae) and *Curcuma aeruginosa* Roxb. (Zingiberaceae) rhizomes were carried out in context of oxidative stress mediated Gout inflammation. This study explores two taxonomically distinct genera traditionally recognized for their traditional value in relation to their anti-inflammatory and anti-arthritic potential. Organoleptic evaluation of powdered rhizomes revealed genus-specific differences in colour, odour, texture and taste, while pH assessment indicated intrinsic variation between the two genera followed by Physiochemical characteristics. Soxhlet extraction using solvents of increasing polarity (petroleum ether, ethyl acetate, acetone, ethanol and water) enabled efficient recovery of metabolites. Qualitative screening confirmed the presence of primary and secondary metabolites, including phenolics, flavonoids, tannins, terpenoids and glycosides with varying intensities across solvents and genera. Quantitative estimation revealed that the ethanolic extract of *D. quercifolia* contained higher phenolic content (18.96 mg GAE/g extract), whereas *C. aeruginosa* showed greater flavonoid accumulation (85.26 mg QE/g extract). A range of antioxidant assays such as DPPH, ABTS, FRAP, superoxide and phosphomolybdenum were conducted to characterize their redox behavior through single electron transfer (SET) and hydrogen atom transfer (HAT) mechanisms which demonstrated significant activity in both species, with *D. quercifolia* showing prominent DPPH (IC_{50} 28.07 µg/mL), ABTS (83, 229 µg TE/g extract), FRAP (760.98 mM Fe (II)/mg extract), superoxide scavenging (72.4%) and phosphomolybdenum activity (391.3 mg AAE/g extract), while *C. aeruginosa* exhibited comparatively higher antioxidant capacity in ethanol. Since oxidative stress plays a pivotal role in triggering inflammatory disorders like gout, the strong antioxidant potential of these rhizomes may contribute to alleviating oxidative damage linked with inflammation. This highlights the mechanistic basis for the antioxidant potential and validates their traditional therapeutic relevance from an inter-generic perspective.

Keywords: *Drynaria quercifolia* (L.) J.Sm., *Curcuma aeruginosa* Roxb., phytochemical screening, antioxidant activity, oxidative stress

1. Introduction
The equilibrium between oxidative stress and antioxidant defence is essential for maintaining biological integrity. The inevitable generation of reactive oxygen species (ROS) such as superoxide anion ($O_2^{\bullet -}$), hydroxyl radical (•OH) and hydrogen peroxide (H_2O_2) during aerobic metabolism plays a dual role in physiological signalling and pathological damage [1, 2]. Oxidative stress plays a central role in driving chronic inflammation by activating transcription factors such as NF-κB and inducing pro-inflammatory enzymes like COX-2 and iNOS. Excessive generation of reactive oxygen species (ROS) disrupts redox homeostasis, amplifying inflammatory cascades which induces lipid peroxidation, protein oxidation, DNA damage and contributing to tissue injury in conditions including arthritis, gout and neuroinflammation [3, 4].
Gout is triggered by Monosodium urate (MSU) crystals which is deposited in the joint tissues during gout inflammation activate neutrophils and macrophages, leading to excessive production of mitochondrial reactive oxygen species (ROS).

This oxidative stress triggers NF-κB signalling and NLRP3 inflammasome activation, culminating in the release of pro-inflammatory cytokines such as interleukin-1β (IL-1β) and tumour necrosis factor-α (TNF-α) [5, 6]. The persistent ROS generation and inflammatory cytokine cascade together perpetuate oxidative stress, thereby sustaining and amplifying gouty inflammation.

Antioxidants are compounds that can delay or inhibit the oxidation of substrates even at low concentrations by donating electrons or hydrogen atoms to neutralize free radicals [7]. These molecules act through either the hydrogen atom transfer (HAT) or single-electron transfer (SET) mechanisms, terminating free radical chain reactions and preventing oxidative injury [8]. Antioxidants that effectively neutralize ROS can therefore interrupt the pathways which cascade inflammation and restore cellular balance.

Among natural antioxidants, plant phenolic compounds have received considerable attention due to their redox properties, which enable them to act as reducing agents, metal chelators and singlet oxygen quenchers [9]. Phenolics, originating from the shikimate and phenylpropanoid pathways, include a wide array of subgroups such as flavonoids, tannins and phenolic acids that contribute to the antioxidant potential of plants [10]. Dietary and medicinal plant phenolics significantly surpass the intake of synthetic antioxidants and vitamins in providing oxidative protection [11]. However, synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) have raised safety concerns due to their possible toxic and carcinogenic effects [12], this emphasizes the need for safer, plant-based alternatives.

Drynaria quercifolia (Family; Polypodiaceae) is an epiphytic fern commonly known as oak leaf ferns, widely distributed across tropical and subtropical regions of Asia, including India, China and Southeast Asia. It exhibits characteristic fronds with a basket-like rhizome structure that enables attachment to tree trunks, reflecting its adaptation to epiphytic habitats. This rhizome has immense ethnopharmacological properties which is used by various tribal communities across the world against inflammation and also for their antioxidant potential [13].

In contrast, *Curcuma aeruginosa* is a rhizomatous perennial herb of the Zingiberaceae family, predominantly found in Tropical and Subtropical regions in Asia. It is commonly known as Black Turmeric. Traditionally, *C. aeruginosa* has been valued for its therapeutic potential and utilization in folk medicine for various ailments like oxidative stress related disorders and rheumatism [14].

Drynaria quercifolia and *Curcuma aeruginosa*, despite belonging to distinct taxonomic families and displaying divergent morphological traits, exhibit remarkable convergence in ethnobotanical usage and the accumulation of secondary metabolites, particularly phenolics and flavonoids. However, detailed comprehensive analyses using solvents of different polarities still remain limited, despite their crucial role in determining the solvent polarity which influences extraction efficiency and overall phytochemical composition. The investigation of their redox potential directly supports the identification of natural bio actives which are capable of disrupting urate-induced ROS cascades [15-17]. Thus, the present study focuses on quantifying total phenolic and flavonoid contents, followed by evaluating antioxidant potential using DPPH, ABTS, FRAP, superoxide and phosphomolybdenum assays, which collectively represent single-electron transfer (SET) and hydrogen atom transfer (HAT) mechanisms and correlating phytochemical concentrations with observed redox activities in rhizome extracts. Such inter-generic comparisons not only illuminate biochemical diversity but also facilitate the identification of potent bioactive compounds for subsequent pharmacological explorations.

2. Materials and Methods

2.1. Collection and Identification of Rhizomes

Rhizomes of *Drynaria quercifolia* (L.) J.Sm. were collected from Kolli Hills, Tamil Nadu, whereas rhizomes of *Curcuma aeruginosa* Roxb. were procured from the herbal market at Beltola Bazaar, Guwahati, Assam. Both plant species were authenticated by the Botanical Survey of India, Southern Circle, Coimbatore, Tamil Nadu and voucher specimens were deposited in the department herbarium for future reference. The rhizomes were thoroughly washed to remove soil and other extraneous materials, peeled, sliced and shade-dried under controlled conditions. They were subsequently pulverized into a coarse powder using a mechanical grinder. The powdered samples were stored in airtight containers until further analysis.

2.2 Organoleptic Evaluation and Physiochemical characteristics

The powdered rhizomes of *Drynaria quercifolia* and *Curcuma aeruginosa* were subjected to organoleptic analysis to assess their sensory characteristics, including colour, odour, taste and texture using standard pharmacognostic methods. The pH of 1% aqueous extract was measured using a digital pH meter. The physiochemical parameters like moisture content, Starch content, swelling index, foaming index and Water absorption capacity were determined using standard procedures [13].

2.3. Extraction

The powdered rhizomes of *Drynaria quercifolia* and *Curcuma aeruginosa* were packed separately in Soxhlet thimbles and subjected to successive extraction with solvents of increasing polarity: petroleum ether, ethyl acetate, acetone and ethanol. Between each successive extraction, the thimbles were air-dried to remove residual solvent. For the aqueous extract, the rhizome powder was prepared as a decoction by boiling in hot water with constant stirring for 30 min, followed by filtration. The solvent extracts were concentrated under reduced pressure using a rotary vacuum evaporator (Yamato BO410, Japan) and subsequently air-dried. The dried extracts obtained from each solvent were weighed and the percentage yield was calculated based on the weight of the desiccated slurry relative to the initial powdered material. Stock solutions of each extract were prepared at a concentration of 1 mg/mL in their respective solvents for further phytochemical and antioxidant analyses.

2.4. Extract recovery percentage

The amount of crude extract recovered after successive extraction was weighed and the percentage of yield was calculated by the following formula,

$$\text{Extract recovery percent} = \frac{\text{Amount of extract recovered (g)}}{\text{Amount of plant sample (g)}} \times 100$$

2.5. Qualitative phytochemical screening

The preliminary primary and secondary phytochemical analysis of the successive extracts of *Drynaria quercifolia* and *Curcuma aeruginosa* rhizomes was carried out for the screening of Carbohydrates, Proteins, Amino acids, Fixed Oils and Fats, Tannins, Saponins, Flavonoids, Steroids, Terpenoids, Coumarins, Alkaloids, Glycosides, Phytosterols, Phenols, Quinones, Anthocyanins according to standard methods [18].

2.6. Quantification of Secondary metabolites

Flavonoids and phenolic compounds were specifically quantified because they represent the primary bioactive constituents responsible for the antioxidant and anti-gout activities. This quantification is essential to justify a focused analysis prior to conducting further *in vitro* bioassays.

2.6.1. Quantification of total phenolics

The content of total phenolic compounds was determined using the Folin-Ciocalteu method [19]. The absorbance was read at 725 nm against the reagent blank. Total phenolic content was expressed as milligrams of gallic acid equivalent (GAE) per g of extract using the following equation based on the calibration curve: $y = 0.0003x + 0.0716$, $R^2 = 0.9365$, where x was the absorbance and y was the gallic acid equivalent (mg/g).

2.6.2. Quantification of total flavonoids

The flavonoid contents of all the extracts were quantified according to the method [20]. The absorbance was read at 510 nm. Total flavonoid content was calculated as quercetin (mg/g) using the following equation based on the calibration curve: $y = 0.0281x - 0.3582$, $R^2 = 0.998$, where x was the absorbance and y was the quercetin equivalent (mg/g).

2.7. *In vitro* antioxidant assays

The antioxidant potential was evaluated through DPPH, ABTS, FRAP, and phosphomolybdenum assays to assess their ability to scavenge free radicals and mitigate oxidative stress relevant to gout-related inflammation.

2.7.1. DPPH scavenging assay

The antioxidant activity of the extract was determined in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH**,** according to the method [21]. The absorbance of the samples and control were measured at 517 nm against the methanol blank. Radical scavenging activity of the samples was expressed as IC_{50} which is the concentration of the sample required to inhibit 50% of $DPPH^{\cdot}$ concentration.

2.7.2. ABTS Assay

The total antioxidant activity was determined by ABTS radical cation scavenging assay by the method [18]. After incubation the absorbance of samples and standards (BHT and Rutin) were measured at 734 nm against the ethanol blank. The results were expressed as the concentration of Trolox having equivalent antioxidant activity expressed as µg/ g sample extracts.

2.7.3. Ferric reducing antioxidant power assay

The antioxidant capacities of different extracts of samples were estimated according to the procedure described [22]. At the end of incubation, the absorbance of the blue colour developed was read immediately at 593 nm against the reagent blank. Methanolic solutions of known $FeSO_4.7H_2O$ concentration ranging from 500 to 4000 µM were used for the preparation of the calibration curve. The parameter Equivalent Concentration was expressed as the concentration of antioxidant having a ferric-TPTZ reducing ability equivalent to that of 1 mM $FeSO_4{\cdot}7H_2O$.

2.7.4. Superoxide radical scavenging activity

The assay was based on the capacity of various extracts to inhibit formazan formation by scavenging the superoxide radicals generated in riboflavin-light-NBT system [23]. The absorbance was measured at 590 nm against the blank (unilluminated reaction mixture without plant sample). The scavenging activity on superoxide anion generation was calculated as:

Scavenging activity (%) = [(Control OD - Sample OD)/Control OD] X 100

2.7.5. Phosphomolybdenum assay

The antioxidant activity of samples was determined by the green phosphomolybdenum complex formation according to the method [24]. The absorbance of the mixture was measured at 695 nm against the reagent blank. Ascorbic acid was used as the reference standard and the results were expressed as milligrams of ascorbic acid equivalents (AAE)/g extract.

2.8 Statistical Analysis

The statistical analysis was performed by one-way ANOVA. The results were expressed as means±SE. to show variations in the various experiments. Differences are considered significant when $P < 0.05$.

3. Results and Discussion

3.1. Organoleptic evaluation

The powdered rhizomes of *Drynaria quercifolia* and *Curcuma aeruginosa* exhibited distinct organoleptic characteristics (Table 1& Fig.1A, 1B). *D. quercifolia* was brownish-yellow with a mild woody and earthy odour, slightly bitter-astringent taste and showed near neutral pH of 6.2±0.2. In contrast, *C. aeruginosa* was pale orange, aromatic and camphoraceous, bitter with a mild pungency and showed a slightly acidic pH of 5.8±0.3.

The differences reflect their taxonomic and phytochemical variations such characteristics were useful for preliminary identification and may influence the extraction efficiency and antioxidant potential of the rhizomes. They also suggest the presence of terpenoids and essential oil components previously associated with XO inhibition and anti-inflammatory activity [25]. These sensory traits indirectly indicate the presence of bioactive constituents relevant to gout therapy.

Table 1: Organoleptic characteristics of powdered rhizomes of *Drynaria quercifolia* and *Curcuma aeruginosa*

Parameter	*Drynaria quercifolia*	*Curcuma aeruginosa*
Colour	Brownish-yellow	Pale Orange
Odour	Mild woody and Earthy	Aromatic, camphoraceous.
Taste	Slightly Bitter-astringent	Bitter, mild pungent
pH (1% aqueous)	6.2±0.2	5.8±0.3

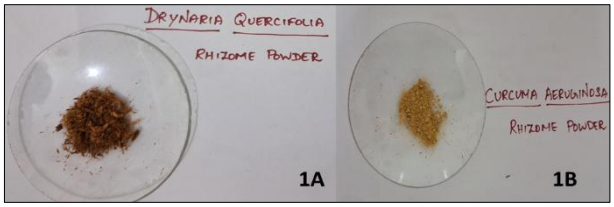


Fig 1A &B: Powdered Rhizomes of *Drynaria quercifolia* and *Curcuma aeruginosa*

3.2. Physiochemical and functional Characteristics of powdered rhizomes

The powdered rhizomes of *Drynaria quercifolia* and *Curcuma aeruginosa* were analyzed for key physicochemical and functional parameters (Table 2). These parameters are important indicators of quality, purity and stability of plant materials and are commonly used in the standardization of herbal drugs. They can influence both extraction efficiency and antioxidant potential. The observed optimal pH of the powdered rhizomes resembles physiological synovial fluid, suggesting that phenolic compounds remain active in joint environments affected by gout [26].

Table 2: Physicochemical and Functional Properties of Powdered Rhizomes

Characteristics	*Drynaria quercifolia*	*Curcuma aeruginosa*
Moisture Content (%)	9.03±0.22	8.47±0.17%
Starch content (%)	4.0%	14.0%
Swelling index (mL/g)	2.45±0.15	3.68±0.23
Foaming index (mL)	50±3	43±2
Water Absorption Capacity (mL/g)	2.67±0.25	4.56±0.31

The moisture content of *D. quercifolia* (9.03±0.22%) and *C.aeruginosa* (8.47±0.17%) remained within the pharmacopoeial limit (<10%), indicating effective drying, reduced microbial risk and minimal oxidative degradation of sensitive phytoconstituents [27, 28]. This low moisture content helps limit oxidation and preserves polyphenols, which can mitigate urate-driven ROS generation.

The starch content differed significantly, being higher in *C.aeruginosa* (14.0%) than *D. quercifolia* (4.0%), suggesting enhanced carbohydrate deposition in *Curcuma* species. Such starch enrichment may increase solvent penetration and the extraction of hydrophilic compounds, particularly phenolic acids and curcuminoids, during aqueous and hydroalcoholic extraction [29]. Conversely, the lower starch and higher lignified matrix in *D.quercifolia* may favor extraction of polyphenolic compounds with strong radical scavenging activity [30].

The swelling index and water absorption capacity (WAC) further support this difference which exhibited higher swelling index in *C.aeruginosa* 3.68±0.23 mL/g with WAC of about 4.56±0.31 mL/g when compared to *D. quercifolia* swelling index of about 2.45±0.15 and 2.67±0.25 mL/g, respectively, indicating the presence of hydrophilic polysaccharides that enhance solvent interaction and antioxidant recovery [31]. The foaming index which reflects the presence of saponins and surface-active constituents, was slightly higher in *D.quercifolia* (50±3 mL) than *C.aeruginosa* (43±2 mL). Such amphiphilic compounds may enhance solubility and stability of lipophilic antioxidants [32].

Collectively, *D. quercifolia* has a moderately hydrophilic profile, while *C.aeruginosa* shows starch-dominant, polysaccharide-enriched hydrophilic behaviour, this may affect distinct solvent-dependent extraction and antioxidant dynamics, potentially impacting the efficacy of these rhizomes in gout therapy

3.3 Extract Recovery Percentage

The rhizomes of *Drynaria quercifolia* and *Curcuma aeruginosa* were extracted using successive solvents of increasing polarity and the percentage yields are presented in Table 3. The maximum yield was obtained from the aqueous extract of *C. aeruginosa* (15.18%), followed by *D. quercifolia* (13.60%). Ethanol extracts also showed comparatively high yields of about 10.70% and 13.51% for *C. aeruginosa* and *D. quercifolia*, respectively, while ethyl acetate and acetone extracts gave moderate yields. Petroleum ether extracts yielded the least, reflecting their limited capacity to extract polar phytoconstituents.

The higher recovery in aqueous and ethanolic extracts indicates their effectiveness in solubilizing a wide spectrum of bioactive compounds, particularly phenolics, flavonoids, tannins, and glycosides as their compounds are generally polar in nature [33, 34]. The solvent polarity plays a pivotal role in phytochemical extraction efficiency and polar solvents like ethanol and water are particularly efficient in recovering antioxidant-active molecules [35].

Table 3: Percentage yield of different solvent extracts of *D. quercifolia* and *C. aeruginosa* rhizomes

S. No.	Solvent	*Drynaria quercifolia* (%)	*Curcuma aeruginosa* (%)
1	Petroleum Ether	4.87	6.09
2	Ethyl Acetate	6.10	8.21
3	Acetone	3.75	4.90
4	Ethanol	10.70	13.51
5	Aqueous	13.60	15.18

3.4 Preliminary Qualitative Phytochemical Screening

Preliminary phytochemical analyses revealed that the distribution of metabolites varied across solvents (Tables 4 and 5). Petroleum ether extracts were rich in lipophilic compounds such as fixed oils, steroids and terpenoids, whereas ethyl acetate and acetone extract predominantly contained phenols, flavonoids and alkaloids. Ethanol, being amphiphilic, extracted the broadest range of phytochemicals including tannins, flavonoids, phenolics, glycosides and alkaloids, while aqueous extracts were rich in carbohydrates, proteins, glycosides and saponins.

The intensity of colour change during chemical tests indicated the relative abundance of each metabolite, confirming solvent-dependent extraction patterns. These findings are consistent with previous studies reporting that solvent polarity governs the recovery of both hydrophilic and lipophilic compounds [36, 37]. The ethanol extracts of both species showed immense presence of phenolics and flavonoids, which aligns with their known pharmacological roles in antioxidant and anti-inflammatory mechanisms [38, 39].

The detection of phenolics, flavonoids, tannins, terpenoids and saponins substantiates the anti-gout relevance because Phenolics and flavonoids will scavenge ROS and inhibit XO [40]. The presence of Tannins and saponins will suppress neutrophil infiltration during MSU-induced inflammation

[41] and Terpenoids block NLRP3 inflammasome activation (12). Thus, the chemical profile of both rhizomes supports the modulation of oxidative-inflammatory pathways central to gout. These results also support the taxonomic and chemotaxonomic value of these rhizomes, as both species were belonging to different genera that is *Drynaria* is a fern; and *Curcuma*, a is monocotyledon which show convergence in secondary metabolite biosynthesis, particularly in polyphenolic and terpenoid content, reflecting adaptive chemical strategies in rhizomatous species [35].

Table 4: Preliminary Phytochemical Screening of *Drynaria quercifolia* Rhizome extracts

Constituents Tested	Petroleum Ether	Ethyl Acetate	Acetone	Ethanol	Aqueous
Carbohydrates	+	+	+	++	++
Proteins / Amino acids	-	-	-	+	+
Fixed oils and fats	++	+	-	+	-
Phytosterols	+	+	+	+	-
Tannins	++	+	+	+	+
Flavonoids	-	+	+	++	+
Phenols	-	+	+	++	+
Alkaloids	-	-	-	+	+
Saponins	-	+	-	+	+
Steroids	+	+	-	+	-
Terpenoids	-	-	-	+	+
Coumarins	-	-	-	+	+
Glycosides	-	-	-	+	+
Quinones	-	-	-	+	-
Anthocyanins	-	-	-	-	-

(+) Presence of chemical compound, (-) Absence of chemical compound. (+) < (++): Based on the intensity of characteristic colour.

Table 5: Preliminary Phytochemical Screening of *Curcuma aeruginosa* Rhizome extracts

Constituent Tested	Petroleum Ether	Ethyl Acetate	Acetone	Ethanol	Aqueous
Carbohydrates	+	+	+	++	++
Proteins / Amino acids	-	-	-	+	+
Fixed oils and fats	++	+	-	+	-
Phytosterols	+	+	+	+	-
Tannins	+	+	+	++	+
Flavonoids	-	+	+	++	+
Phenols	-	+	-	++	+
Alkaloids	-	-	-	+	+
Saponins	-	+	-	+	+
Steroids	+	+	-	+	-
Terpenoids	-	-	-	+	+
Coumarins	-	-	-	+	+
Glycosides	-	-	-	+	+
Quinones	-	-	-	-	-
Anthocyanins	-	-	-	-	-

(+) Presence of chemical compound, (-) Absence of chemical compound. (+) < (++): Based on the intensity of characteristic colour

3.5 Quantification of Secondary Metabolites

3.5.1 Total Phenolic Content (TPC)

The total phenolic content (TPC) was highest in ethanol extracts of *D. quercifolia* (118.96 mg GAE/g extract) and *C. aeruginosa* (106.22 mg GAE/g extract), followed by aqueous, ethyl acetate, acetone and petroleum ether extracts (Fig.2). The phenolic redox cycle plays a key biochemical role in preventing lipid peroxidation and protecting cellular biomolecules. Phenolic antioxidants scavenge peroxyl radicals (ROO•) via hydrogen atom donation (HAT) and regenerate through intra/intermolecular electron transfer (SET) [36]. Thus, the elevated phenolic content in ethanol extracts indicates strong solubility of polyphenols in polar solvents, which may be linked to enhanced antioxidant potential [37, 38]. The observed results correlate with studies on *Curcuma longa* and *Drynaria fortunei*, where ethanol extracts similarly exhibited the highest TPC [39, 40]. As observed earlier that the Phenolics such as gallic and ferulic acids are known as XO inhibitors that lower serum urate and attenuate gouty inflammation.

3.5.2 Total Flavonoid Content (TFC)

The total flavonoid content followed a similar pattern as phenolics, with ethanol extracts recording the highest values of about 83.42 mg QE/g for *D. quercifolia* and 85.26 mg QE/g for *C. aeruginosa* (Fig.3) The ethyl acetate extracts also showed considerable flavonoid content, emphasizing the semi-polar nature of these metabolites. Flavonoids are potent antioxidants known for their radical scavenging, metal chelating and anti-inflammatory activities [41]. Structurally, flavonoids possess conjugated π-electron systems and hydroxyl groups capable of stabilizing radicals via electron delocalization and metal chelation, effectively participating in both SET and HAT pathways [42, 43]. High flavonoid (TFC) content indicates the potential to reduce uric acid formation and oxidative load. Flavonoids such as quercetin, are known as potential XO inhibitors that correlates with inflammation associated with gout [41, 42].

These findings suggest that the ethanol extracts from both rhizomes are rich sources of bioactive polyphenols, justifying their traditional use in oxidative and inflammatory conditions.

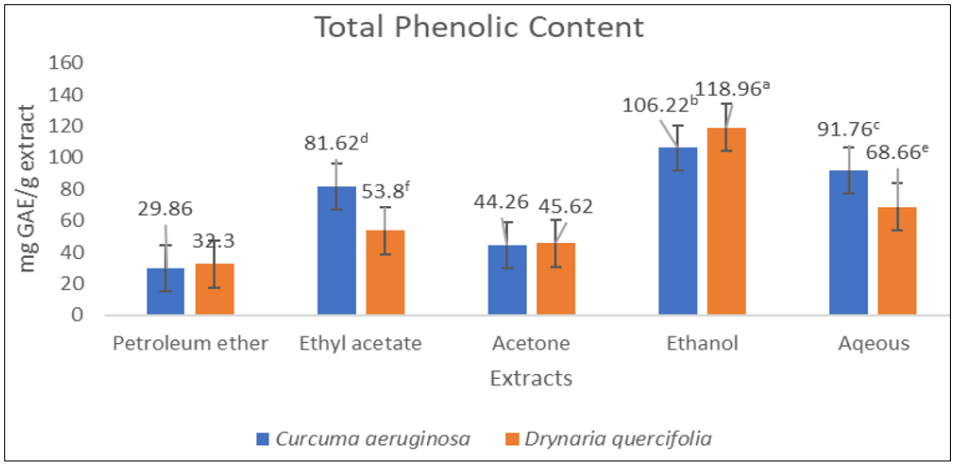


Fig 3: Total phenolic content of *D. quercifolia* and *C. aeruginosa* rhizome extracts

Values are mean of triplicates determination(n=3) ±standard deviation, GAE-Gallic acid Equivalent Statistically significant at $p<0.05$ where $^{a}>^{b}>^{c}>^{d}>^{e}>^{f}$.

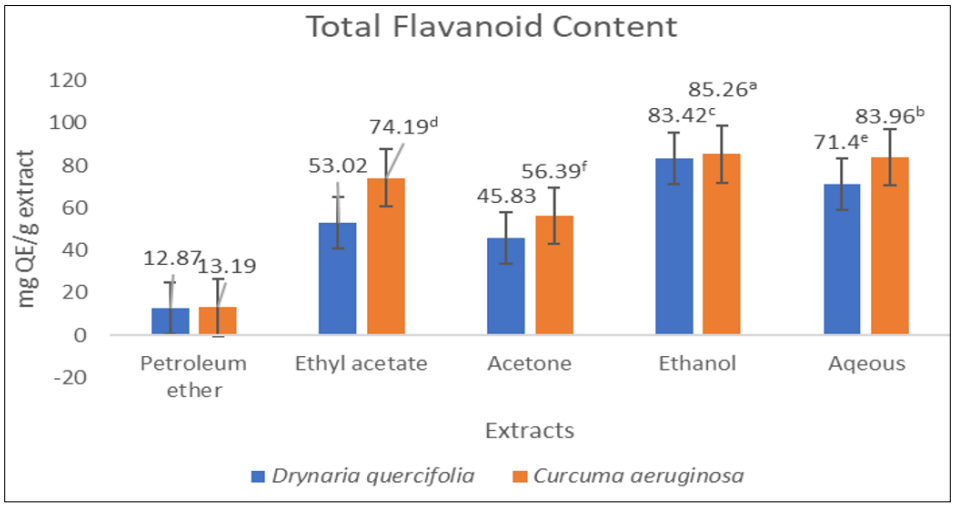


Fig 4: Total Flavonoid content of *D. quercifolia* and *C. aeruginosa* rhizome extracts

Values are mean of triplicates determination(n=3) ±standard deviation, QE-Quercitin Equivalent. Statistically significant at $p<0.05$ where $^{a}>^{b}>^{c}>^{d}>^{e}>^{f}$.

3.6 Antioxidant Activities

3.5.1 DPPH Radical Scavenging Activity

The DPPH assay primarily reflects both HAT and SET-based mechanisms since DPPH• can be reduced either by hydrogen atom transfer or by electron donation. The DPPH assay revealed concentration-dependent radical scavenging activity across all extracts (Table-6). Ethanol extracts of *D. quercifolia* and *C. aeruginosa* displayed IC_{50} values of 28.07 µg/mL and 64.83 µg/mL, respectively, indicating high antioxidant potential. The strong scavenging efficiency of *D. quercifolia* may be attributed to its higher phenolic and flavonoid content. These results are comparable with those of standard antioxidants Rutin (23.09 µg/mL) and BHT (21.99 µg/mL). This mechanistic behaviour mirrors the radical neutralization capacity of antioxidants in biological membranes [44, 45]. High DPPH activity reflects the ability of the extracts to neutralize the free radicals generated during gout flares. MSU-stimulated leukocytes produce ROS that amplify joint damage, and hydrogen-donating antioxidants suppress this oxidative burst [5, 6]. Thus, DPPH performance predicts the mitigation of ROS-driven inflammatory injury.

Table 6: DPPH radical scavenging activity of *Drynaria quercifolia* and *Curcuma aeruginosa* rhizome extracts.

Samples	Extract	DPPH% scavenging activity IC_{50} (µg/mL)
C. aeruginosa	Petroleum ether	121.61
	Ethyl acetate	75
	Acetone	93.07
	Ethanol	64.83
	Aqueous	41.85
D. quercifolia	Petroleum ether	112.5
	Ethyl acetate	31.73
	Acetone	38.03
	Ethanol	28.07
	Aqueous	98.82
Standard	Rutin	23.09
	BHT	21.99

3.6.2 ABTS Radical Cation Scavenging Activity

The ABTS assay involves reduction of the ABTS radical, which occurs predominantly through electron transfer. The ABTS assay (Table-7) showed that *D. quercifolia* ethanol extract exhibited the highest radical scavenging capacity (83, 229±625 µg TE/g), followed by *C. aeruginosa* (69, 764±833 µg TE/g). Although slightly lower than standards Rutin and BHT, the extracts demonstrated significant activity, reflecting their efficient hydrogen and electron-

donating ability. The high SET capacity indicates efficient electron donation to reactive nitrogen and oxygen species (ROS/RNS). Mechanistically, these antioxidants disrupt superoxide nitric oxide coupling, reducing formation of peroxynitrite ($ONOO^-$), which is a major pro-inflammatory oxidant in inflammation related diseases [46]. An efficient electron-transfer capacity indicates the potential to counter xanthine oxidase-derived superoxide and peroxynitrite formation [16, 17]. Similar redox behaviour underlies the clinical antioxidant benefits of febuxostat therapy [16].

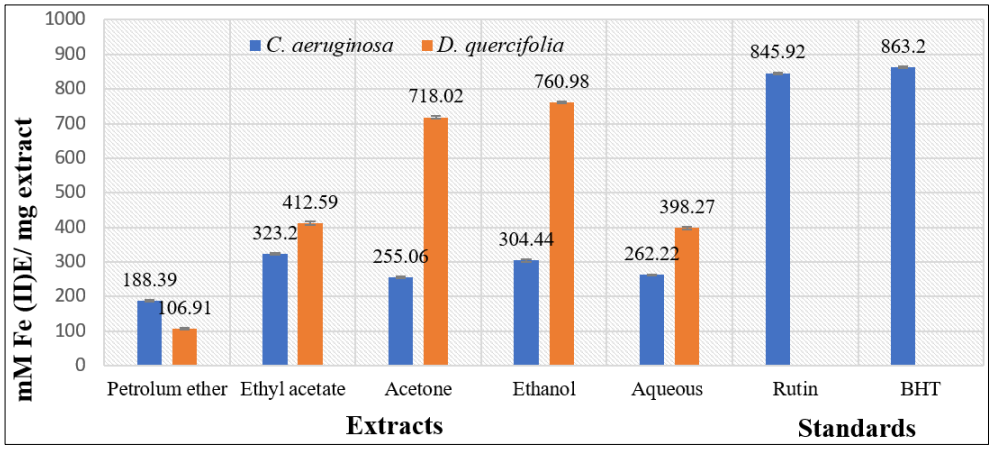
Table 7: ABTS radical cation scavenging activity of *Drynaria quercifolia* and *Curcuma aeruginosa* rhizome extracts

Samples	Extracts	ABTS (µg TE/g extract)
Curcuma aeruginosa	Petroleum ether	38090.3±420.98
	Ethyl acetate	48090.3±573.7
	Acetone	55763.9±469.71
	Ethanol	69763.9±833.33^d
	Aqueous	59375±416.6
Drynaria quercifolia	Petroleum ether	45173.6±1242.75
	Ethyl acetate	82048.6±693.57^b
	Acetone	78576.4±469.71^c
	Ethanol	83229.2±625^b
	Aqueous	59965.3±669.69
Standard	Rutin	94166.7±416.6^a
	BHT	95347.2±636.4^a

Values are mean of triplicate determination (n=3) ± standard deviation, TE - Trolox Equivalents
Statistically significant at $p<0.05$ where $^a > ^b > ^c > ^d$

3.6.3 Ferric Reducing Antioxidant Power (FRAP)

Ethanol extracts of *D. quercifolia* (760.98±2.99 mM Fe (II)/mg) and *C. aeruginosa* (304.44±4.07 mM Fe (II)/mg) demonstrated potent reducing capabilities (Fig.6). This assay measures electron-donating potential, confirming the presence of strong reductants in these extracts [47]. In FRAP, the ability of the compounds present in the outperformed extracts may donate an electron to neutralize free radicals or reduce ferric ions which demonstrates SET mechanism [48, 49]. The superior FRAP values of *D. quercifolia* are consistent with its high phenolic content, which plays a dominant role in ferric ion reduction. This confirms SET-driven redox capacity. This strong reducing power suggests the ability to interrupt Fe^{3+}/Fe^{2+} which are catalysed during ROS cycles in the synovial fluid [50]. This mechanism also stabilizes molybdenum in its reduced form within the XO, thereby inhibiting uric acid generation [51].



Fe (II) - Ferric Equivalents

Fig 6: Ferric reducing antioxidant power assay of *D. quercifolia* and *C. aeruginosa* rhizome extracts

3.6.4 Superoxide Radical Scavenging Activity

Superoxide inhibition (Fig.7.) was significantly higher in ethanol extracts of *D. quercifolia* (72.4±0.42%) and *C. aeruginosa* (69.35±1.82%) comparable to Rutin and BHT. Superoxide radicals, being precursors to other reactive oxygen species, are efficiently neutralized by polyphenolic antioxidants [52]. These results substantiate the extract's potential in oxidative stress regulation. This mimics the biological action of endogenous superoxide dismutase (SOD), converting $O_2^{\bullet -}$ radicals into less harmful species. Phenolic antioxidants act as redox modulators by interacting with NADPH oxidase pathway responsible for ROS overproduction during oxidative stress [53, 54]. This parallels towards the inhibition of XO and NADPH oxidase activity implicated in gouty arthritis [3, 17]. By mimicking superoxide dismutase, these extracts may lower the oxidative pressure in chondrocytes [40].

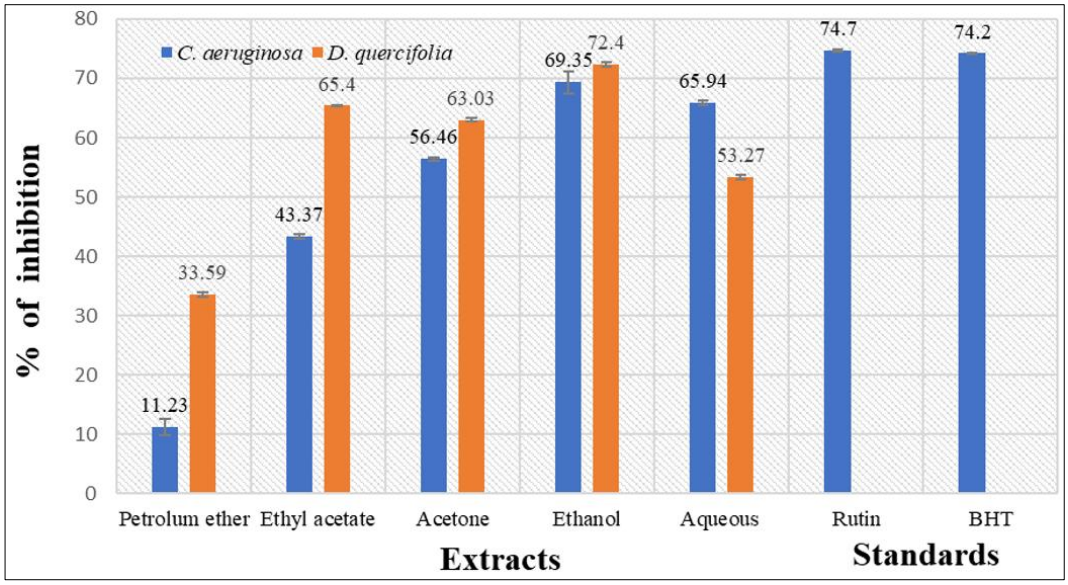


Fig 8: Superoxide Radical Scavenging Activity of *D. quercifolia* and *C. aeruginosa* rhizome extracts.

3.6.5 Phosphomolybdenum Assay

The total antioxidant capacity (TAC) as measured by the phosphomolybdenum assay reflects cumulative HAT and SET capacities. The total antioxidant capacity (Table-8) was higher in ethanol extracts of *D. quercifolia* (391.3±2.02 mg AAE/g extract) and *C. aeruginosa* (366.77±3.22 mg AAE/g extract). The phosphomolybdenum assay primarily detects compounds capable of reducing Mo (VI) to Mo(V), reflecting cumulative antioxidant potential [55]. Ethanol extracts displayed high redox potential, indicative of multiple reaction pathways involving Mo (VI)→Mo(V) reduction through both hydrogen and electron transfer. This redox versatility supports their therapeutic potential in oxidative pathologies, particularly in urate-mediated inflammation, where oxidative stress drives activation of NF-κB, NLRP3 inflammasome and COX-2 pathways [56]. This also signifies the ability of the extracts to counter cumulative oxidative stress during MSU-induced inflammasome activation.

Overall, the antioxidant assays collectively revealed that ethanol extracts of both species possess strong radical scavenging and reducing abilities, attributed to the synergy of polyphenols, flavonoids and terpenoids.

Table 8: Phosphomolybdenum Assay of *D. quercifolia* and *C. aeruginosa* rhizome extracts.

Samples	Sample	mg AAE/g (extract)
Curcuma aeruginosa	Petroleum ether	159.01±1.3
	Ethyl acetate	207.86±0.62
	Acetone	244.13±2.38^{d}
	Ethanol	366.77±3.22^{b}
	Aqueous	114.78±2.26
Drynaria quercifolia	Petroleum ether	154.4±3.32
	Ethyl acetate	151.67±4.41
	Acetone	348.95±3.46^{c}
	Ethanol	391.3±2.02^{a}
	Aqueous	347.9±2.2^{c}

Values are mean of triplicate determination (n=3) ± standard deviation

Statistically significant at $p < 0.05$ where $^{a} > ^{b} > ^{c} > ^{d}$

4. Conclusion

The present study provides a comprehensive evaluation of the phytochemical profiles and antioxidant potential of *Drynaria quercifolia* and *Curcuma aeruginosa* rhizomes extracted using solvents of varying polarity. Both species exhibited distinct organoleptic and physicochemical characteristics that correspond to their taxonomic and metabolic diversity. Qualitative and quantitative analyses revealed the presence of diverse secondary metabolites, with ethanol emerging as the most effective solvent for recovering phenolics and flavonoids. The ethanolic extracts of both rhizomes exhibited significantly higher antioxidant activity across DPPH, ABTS, FRAP, superoxide and phosphomolybdenum assays, confirming their strong single electron transfer (SET) and hydrogen atom transfer (HAT) capacities. The enhanced redox potential of these extracts can be attributed to the synergistic action of phenolics, flavonoids and terpenoids, which effectively neutralize reactive oxygen species and prevent oxidative damage. These findings validate the traditional use of both rhizomes as potent natural antioxidants and highlight their pharmacological relevance in managing oxidative stress-related inflammation in gout. The phenolic and flavonoid rich ethanolic extract of *both* the rhizomes demonstrated potent free-radical scavenging and ferric-reducing abilities, aligning with mechanistic pathways involved in oxidative stress and gout. These findings provide a rationale for further studies to validate its anti-gout efficacy.

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7. Competing interests

Authors have declared that no competing interests exist.

8. Authors' Contributions: Author Narthanaa S, designed the study, performed the analysis and Statistics, wrote the protocol and wrote the first draft of the manuscript. Author Deepa M.A and Pugalenthi M approved the analyses of the study and approved. And other cross checked, read and help for approval of the final manuscript.

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