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Research Article

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Phytochemical evaluation and *in vitro* antioxidant potential of *Cycas circinalis* L.

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ABSTRACT

Cycas circinalis L., a gymnosperm, is traditionally used by tribal people for various medicinal and culinary purposes. This study investigates the phytoconstituents and antioxidant potential of the leaves, bark, and male cone of *C. circinalis*. Qualitative screening and quantitative analysis were conducted, followed by analysis of antioxidant potential using *in vitro* assays. High-performance thin-layer chromatography (HPTLC) analysis of phenols and flavonoids was carried out to identify active constituents. Various phytoconstituents, including alkaloids, phenols, flavonoids, glycosides, quinones, and sterols, were identified in different extracts. The ethanolic extract of the leaves exhibited the highest total phenolic content (8.169 mg GAE/g), while the male cones showed the highest flavonoid content (2.825 mg QE/g). The bark demonstrated significant radical scavenging activity, particularly in ABTS (IC_{50} 59.44 µg/mL) and DPPH (IC_{50} 358.3 µg/mL) assays. In contrast, the leaves exhibited moderate activity across all assays, and the male cones showed relatively lower antioxidant activity, except in the total antioxidant capacity assay (19.25 mg AAE/g). HPTLC analysis detected gallic acid in the bark, along with several unidentified bands. This study is the first to report presence of gallic acid in the extract of bark of *C. circinalis*. Given the plant's traditional use in treating swellings, wounds, and ulcers, along with the presence of diverse secondary metabolites and significant *in vitro* bioactivity, this study highlights the potential of *C. circinalis* as a natural antioxidant with promising applications in wound treatment.

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INTRODUCTION

Plant secondary metabolites are bioactive compounds synthesized as responses to environmental stresses, serving as a defensive strategy against potential threats which may hinder the plant's regular growth and development (Bourgaud *et al.*, 2001). These phytoconstituents possess inherent therapeutic properties, which hold promise for human well-being (Farnsworth, 1966). With the pursuit of novel pharmaceuticals and modes of disease treatments, extensive investigations have long focused on harnessing the plant derived components for disease management and overall health advancement especially focusing on the traditional uses of herbs (Balunas & Kinghorn, 2005). Despite the growing interest and extensive research efforts in this field, many plant sources remain unexplored, and there is a need to validate their traditional uses through coordinated research methods (Ganie *et al.*, 2015; Mussin & Giusiano, 2020).

The genus *Cycas* is the only accepted genus in the family Cycadaceae of Gymnosperms. It consists of about 117 species and has characteristic palm-like appearance, albeit much shorter in height, and a crown of pinnately compound leaves. Due to their appearance, many of the *Cycas* plants are used as ornamentals, their compound leaves are often used in floral decorations and flower bouquets (Afifi *et al.*, 2021). *Cycas rumphi* seeds are used to soothe and cure sores and boils. Its pollen is said to possess narcotic properties. The female cone of *Cycas revoluta* is used to relieve kidney pain whereas its male bracts have narcotic and aphrodisiac properties (Afifi *et al.*, 2021).

Cycas circinalis L., is native to Southern India, endemic to dry tropical biomes of the Western Ghats. State-wise it is found in Kerala, Karnataka, Tamil Nadu and Maharashtra (Hill, 1995; Donaldson, 2003). It is commonly known as queen sago palm and in India as *Janglimadana* or *janglisabudana*. It is employed for a number of traditional medicinal uses by the local communities in the region where it grows wild. Juice of its tender leaves is given for flatulence and to check vomiting (Ambasta, 1992; Almeida & Almeida, 2010). Powder from the endosperm of seeds is said to be beneficial for hyperplasia, burning sensation, and

general debility (Prasad *et al.*, 2007). A poultice made from bark and seed paste mixed with coconut oil is applied to sores and swellings (Chopra & Nayar, 1956; Almeida & Almeida, 2010). Sago is simple starch obtained from the pith of the trunk and has been traditionally used as a food. It is used as a diet for the sick as it is light and easily digestible. When consumed with boiled milk, it provides relief from bowel infections and internal inflammations (Whitelaw, 1826).

Owing to its noteworthy ethnobotanical uses, it is essential to conduct a thorough investigation to identify the phytoconstituents and active classes of secondary metabolites present in this plant as well as assess its antioxidant potential. Although there are scientific studies done in this regard, they are however confined in their extent. Hence, this investigation aims to fulfil the objective of decoding the phytochemistry of the plant and analysing its antioxidant property *in vitro*, depending on which examination of its therapeutic potential through *in vivo* studies and clinical trials could also be done.

MATERIALS AND METHODS

Plant Collection and Identification

Cycas circinalis L. was collected from the campus of St. Xavier's College (Autonomous) Mumbai and authenticated at Blatter Herbarium of St. Xavier's College (Autonomous), Mumbai with the specimen No 8249 of E. Blatter. The collected parts *i.e.* leaf, bark and male cone were cleaned by rinsing with water, cut into small pieces and dried in a hot air oven at 40 °C for 3 days. After complete dehydration, the pieces were grinded using a commercial grade mixer to a fine powder and stored in an airtight container at 4 °C until further use.

Chemicals and Reagents

2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) were obtained from Sisco Research Laboratories. All solvents for phytochemical screening, such as ethanol, methanol, ethyl acetate, and petroleum ether, were AR grade. HPTLC solvents were HPLC grade from Sigma-Aldrich. Standard quercetin dihydrate (98%) was purchased from SDFCL, while naringenin (95%), gallic acid, Trolox, and butylated hydroxy toluene (BHT) were procured from Merck. All other common reagents not specifically mentioned were sourced from SRL or Merck.

Extract Preparation for Qualitative Analysis

Two grams of dried powder was separately mixed in 5 mL each of aqueous, ethanol, methanol, ethyl acetate, and pet ether. The extracts were ground in a mortar and pestle for 10 min, poured in a conical flask and the final volume was made to 200 mL with the respective solvent. The extracts were then placed in a refrigerator for 24 hrs, filtered with Whatman No. 1 filter paper, centrifuged at 3000 rpm for 10-15 min until a clear extract was obtained.

Preliminary Phytochemical Screening

Phytochemical screening was carried out in triplicate according to standard procedures (Shaikh & Patil, 2020) to detect the presence of various bioactive compounds in the extract.

Lignin Test (Labat Test): 2-3 mL of the extract was mixed with 2 mL of gallic acid. The formation of an olive-green colour indicated the presence of lignin.

Resins Test: To detect resins, 1 mL of the extract was dissolved in acetone, and then poured into 5 mL of distilled water. A turbid solution confirmed the presence of resins.

Alkaloids Test: Alkaloids were detected by performing Dragendorff's test, Mayer's test, and Wagner's test. Two to three drops of Dragendorff's reagent produced an orange-brown precipitate, Mayer's reagent resulted in a yellow precipitate, and Wagner's reagent formed a reddish-brown precipitate, confirming the presence of alkaloids in the extract.

Flavonoids Test: The presence of flavonoids was confirmed through several tests: Shinoda test (a few Mg turnings and concentrated HCl were added to 1 mL of extract, resulting in a pink/scarlet colour), alkaline reagent test (NaOH solution formed a yellow colour that disappeared with the addition of diluted HCl), ammonia test (addition of diluted ammonia and concentrated H_2SO_4 formed a yellow solution), and lead acetate test (10% lead acetate formed a white precipitate).

Phenolic Compounds Test: Iodine solution was added to 1 mL extract, producing a transient red colour. $FeCl_3$ (5%) solution formed a dark green/bluish-black colour, and potassium dichromate solution produced a dark colour, confirming the presence of phenolic compounds.

Tannins Test: The presence of tannins was confirmed using $FeCl_3$ (5%) solution, which formed a blue-green colour for simple tannins or a green colour for condensed tannins. Lead acetate (2%) formed a white precipitate, gelatin (1%) and NaCl (10%) formed a white precipitate, and phlobatannins formed a red precipitate when boiled with HCl.

Terpenes Test: Terpenoids were detected by adding concentrated H_2SO_4 to a mixture of chloroform and extract, resulting in a grey colour. Diterpenes were confirmed by adding copper acetate solution, which produced an emerald green colour.

Triterpenoids and Sterols Test: The addition of concentrated H_2SO_4 to the extract formed a yellow colour for triterpenoids and a red colour for sterols. For Hesse's test, a mixture of extract and chloroform was treated with concentrated H_2SO_4, forming a pink or red ring in the lower layer in the presence of sterols. Sulphur powder confirmed sterols by sinking to the bottom of the extract.

Saponins Test: Saponins were detected by the formation of stable froth when 1 mL of extract was vigorously shaken with water.

The addition of sodium bicarbonate ($NaHCO_3$) also produced stable foam, confirming the presence of saponins.

Cardiac Glycosides Test: The presence of cardiac glycosides was confirmed by Baljet's test using picric acid, which produced an orange colour. In the Keller Kilani test, a mixture of glacial acetic acid, $FeCl_3$, and concentrated H_2SO_4 formed a blue colour in the acetic acid layer, confirming the presence of glycosides.

Coumarins Test: The presence of coumarins was confirmed by NaOH (1%) test, which formed a cloudy solution upon addition of HCl (2%), and chloroform (10%) test, which produced a yellow colour upon mixing with NaOH and extract.

Quinones Test: Quinones were detected by adding concentrated HCl, which formed a green colour, and by using alcoholic KOH, which formed a red to blue colour.

Anthraquinones Test: The presence of anthraquinones was confirmed by adding 10% ammonia solution to 1 mL of extract, which resulted in a pink/violet or red colour.

Determination of Extractive Yield

One gram of sample was taken in 5 mL of each solvent in a test tube. Each tube was vortexed for 10 min and kept overnight at 4°C. It was then centrifuged at 3500 rpm for 5 min, supernatant was collected in an evaporating dish. To the residue, 5 mL of solvent was added, vortexed again for 10 min, centrifuged and supernatant collected. The extraction was repeated four times. The resulting supernatants were pooled in a previously weighed evaporating dish and evaporated to dryness in a digital water bath at 50 °C and weighed again. The weight of extract was obtained by getting the difference between the weight of evaporating dish after drying and weight of empty dish (Zimila *et al*., 2021). The percentage yield was calculated as Wt. (g) of extract/Wt. (g) of sample powder taken×100.

Determination of Total Phenolics

The test was performed with slight modifications according to the standard method (Singleton *et al*., 1999). 1 mL of ethanolic plant extract (conc;100 mg/mL) dissolved in distilled water was mixed with folin-ciocalteu's reagent (0.1 mL, 1 N) and allowed to stand for 15 min. 5 mL of saturated Na_2CO_3 was added and allowed to stand for 30 min at room temperature. After 30 min, absorbance was measured at 760 nm on Shimadzu Double monochromator UV-2700i UV-Visible spectrophotometer. The calibration curve was prepared using a series of gallic acid concentrations 10, 20, 30- 90 µg/mL as standard (Figure 1a). Total phenols are expressed in terms of gallic acid equivalent (mg GAE/g).

Determination of Total Flavonoids

The assay was carried out as per the standard method for estimation of flavonoids (Chang *et al*., 2020). 1 mL of ethanolic plant extract (conc: 50 mg/mL) was mixed with 75 µL of $NaNO_2$. After 5 min, 150 µL of 10% $AlCl_3$ was added and allowed to stand

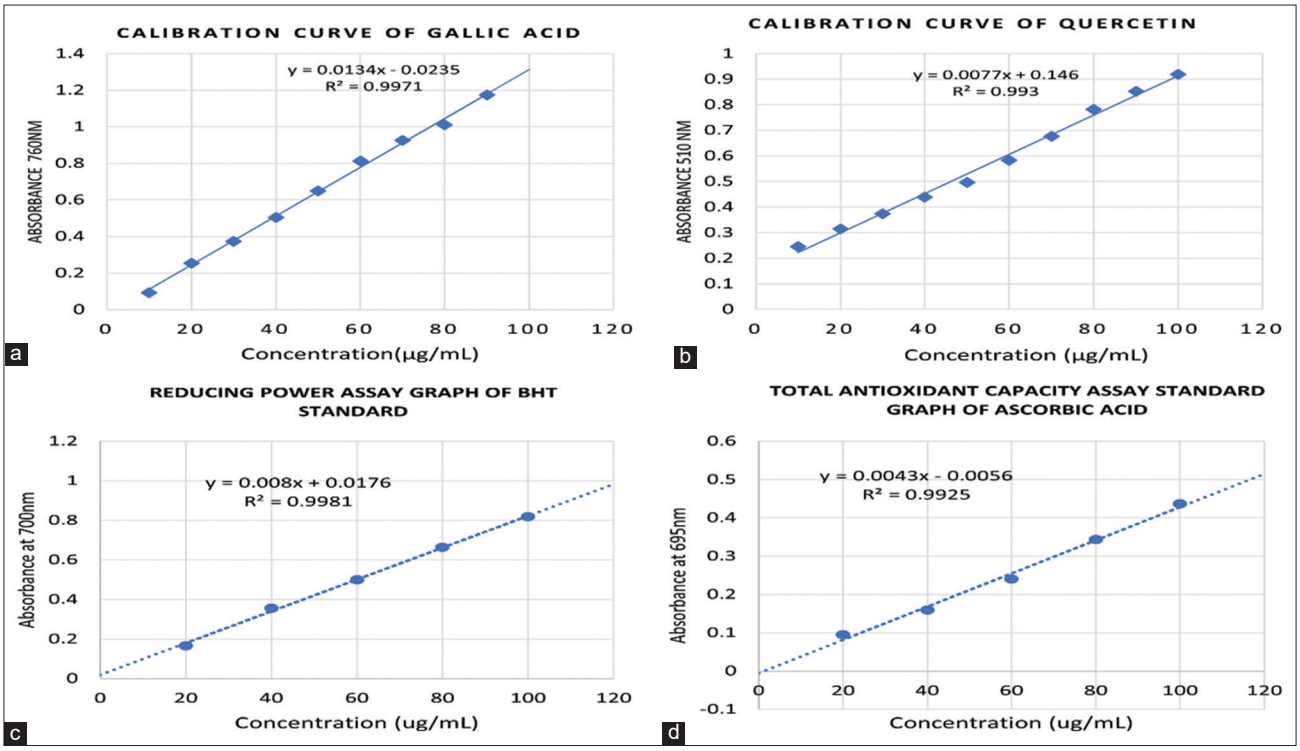

Figure 1: a) Standard curve of gallic acid for quantification of total phenols, b) Standard curve of quercetin for quantification of total flavonoids, c) Standard curve of BHT for quantification of reducing power of activity and d) Standard curve of ascorbic acid for quantification of total antioxidant capacity

for 6 min. The volume was then made to 2.5 mL with distilled water and 0.5 mL of NaOH was added and the absorbance was read at 510 nm. The calibration curve was prepared using Quercetin (series of 10, 20, 30-100 µg/mL) as the standard (Figure 1b). Total flavonoids are expressed as mg QE/g.

In vitro Antioxidant Assays

Total antioxidant capacity assay by phosphomolybdenum method

The phosphomolybdenum method was used with some modifications (Prieto *et al.*, 1999). First, 100 µL of extract was pipetted into a micro-centrifuge tube. Next, 1 mL of a reagent solution comprising of 0.6 M H_2SO_4, 4 mM ammonium molybdate, and 28 mM sodium phosphate was added. After being tightly sealed, tubes were incubated for 90 min at 95 °C. The tubes were then cooled down to 25 °C. Relative absorbance was recorded at 695 nm using an Elisa Plate Reader (SpectraMax iD3) against a blank. Ascorbic acid was employed as standard for analysis (Figure 1c).

Reducing power assay

Reducing power was assessed following the method described (Oyaizu, 1986). One mL of the extract or standard at varying concentrations was combined with 2.5 mL of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide. After thorough vortexing, mixture was incubated at 50 °C for 20 min, followed by cooling. Subsequently, 2.5 mL of 10% Trichloroacetic acid was added, and mixture was centrifuged at 3000 rpm for 10 minutes. Upon centrifugation, 2.5 mL of upper supernatant layer was mixed with an equal volume of distilled water and 0.5 mL of 0.1% $FeCl_3$. Absorbance was then recorded at 700 nm against blank with BHT serving as the standard (Figure 1d).

DPPH radical scavenging assay

The DPPH assay was conducted following the microplate method (Prieto, 2012). In each well, 100 µL of the sample at various concentrations was combined with 100 µL of 0.2 mM DPPH solution. The plate was then covered with aluminium foil and incubated in darkness at 25 °C for 30 min. Absorbance was subsequently recorded at 517 nm. The control well contained 100 µL of 70% ethanol instead of sample. Ascorbic acid served as the standard. DPPH scavenging activity was determined as follows-

$$\text{Scavenging \%} = \text{Absorbance control} - \text{Absorbance sample} \times 100/\text{Absorbance control}.$$

ABTS radical scavenging assay

Initially, an ABTS reagent was made by combining 5 mL of 7 mM ABTS solution with 88 µL of 140 mM potassium persulfate solution. This mixture was incubated at 25 °C in darkness for 16 hrs to allow free radicals to generate. Subsequently, the solution was diluted with water (1:44, v/v). In the microplate method, 100 µL of the extract or standard at varying concentrations was combined with 100 µL of ABTS reagent in each well. The plate was then placed in the plate reader set at 25 °C and the absorbance was recorded at 734 nm after 6 min. Control well had 100 µL of 70% ethanol instead of sample (Re *et al.*, 1999). Trolox served as standard. ABTS scavenging was calculated as given before.

Nitric-oxide scavenging activity

First, 0.5 mL of the plant extract or standard (BHT) at different concentrations was mixed with 1 mL of the sodium nitroprusside solution (10 mM sodium nitroprusside in phosphate-buffered saline of 0.1M, pH 7.4). This mixture was then incubated at 25 °C for 150 min. After the incubation, 0.5 mL of Griess reagent (1% sulphanilamide, 2% phosphoric acid, 0.1% N-(1-naphthyl) ethylene diamine dihydrochloride) was added to the mixture. A control was set up using the same reaction mixture without the sample, while phosphate-buffered saline served as the blank. Optical density readings were taken at 546 nm. The scavenging activity percentage was calculated (Sreejayan & Rao, 1997).

High Performance Thin Layer Chromatography (HPTLC) of Phenols and Flavonoids

Sample and standard preparations

Standard solutions of quercetin dihydrate, gallic acid, and naringenin (0.1 mg/mL) and sample extracts of *C. circinalis* leaves, bark and male cone were individually prepared in methanol and were stored in Eppendorf tubes at 4 °C.

Optimization and selection of mobile phase

For maximum resolution and separation of the standard and sample bands, a number of mobile phases were tried and tested. The list of mobile phases tested are listed in Table 1. Finally, toluene: ethyl acetate: formic acid (5:4:1) was used as it gave efficient separation of bands in the standards and samples tested.

Table 1: Developing solvents for separation of phenolic acids tested in this study

Developing solvent (v/v)	References
Chloroform: ethanol: glacial acetic acid (9.4:0.5:0.1)	(Syarifah *et al.*, 2019)
Acetone: methanol (1:1)	This paper
Ethyl Acetate: formic acid: glacial acetic acid: water (10:0.5:0.5:1.3)	(Das *et al.*, 2013)
Toluene: pyridine: formic acid (100:20:7)	(Gocan & Cimpan, 2004)
Ethyl acetate: glacial acetic acid: methanol (5.5:0.5:4)	This paper
Ethyl acetate: methanol: water (8.1:1.1:0.8)	(Syarifah *et al.*, 2019)
Hexane: ethyl acetate (1:1)	This paper
Acetone: ethanol: glacial acetic acid (8.4:0.1:1)	This paper
Ethyl acetate–toluene–formic acid (4:5:1)	(Patel *et al.*, 2017)
Ethyl acetate: methanol: glacial acetic acid (0.7:0.1:0.2)	This paper

HPTLC Analysis

The final HPTLC run with the selected mobile phase was performed on 20x10 cm Merck TLC Silica gel 60 F254 plates. Plates were pre-washed with chloroform: methanol (1:1), dried at 100 °C for 10 min. Bands of sample and standard solutions were applied in triplicates on the plates using Camag Linomat 5 Applicator as 8 mm bands, on 15 distinct tracks with the first position being 19 mm and track distance 11.5 mm. Applied bands were heated at 90 °C for 2 min following which the plates were developed in twin trough chamber (20x10 cm) pre-saturated with the mobile phase (10 min) till the solvent front reached 70 mm. The developed plate was then dried at 100 °C for 2-3 min and visualized in Camag TLC Visualizer 2. Images were taken at 254 nm and 366 nm and bands of interest were marked with their relative front (Rf). The plates were subsequently scanned in slit-scanning densitometer Camag TLC Scanner 3. Absorption spectra of the standard bands were recorded which were then compared with sample bands at the same Rf values to ascertain their identity.

Statistical Analysis

All the tests for phytochemical screening and estimation of phenols and flavonoids antioxidant assays were performed in triplicates. Results are expressed as mean±standard deviation. For HPTLC analysis, bands of both sample and standard were spotted on the TLC plates in triplicates. One-way ANOVA (PSPP Ver 2.0.1) was performed to test significance difference between leaf, bark and male cone in antioxidant assays. Values at $p \leq 0.05$ were considered statistically significant.

RESULTS

Preliminary Phytochemical Screening

The phytochemical screening of various extracts of *C. circinalis* leaves, bark and male cone showed presence of alkaloids, flavonoids, tannins, terpenoids, sterols, coumarins, etc. Saponin was not found in any of the extracts. Lignin was present in only the ethanolic extract of all the parts. Leaves gave positive results for coumarins in all solvents except for ethyl acetate extract. Sterols were also detected in all extracts of leaves but were weak in ethyl acetate and methanolic extracts. Flavonoids, terpenes and cardiac glycosides were found strongly in only two extracts each. Sterols and coumarin were found in all extracts of bark. In male cones, sterol was only absent in aqueous extracts while coumarins was absent from ethyl acetate extract. Table 2 summarizes the results obtained in different parts and extracts.

Extractive Yield

The extractive yield was highest in ethanolic extracts for both leaves and bark, with yields of 14.31% and 7.72%, respectively (Table 3). This suggests that ethanol is the most effective solvent for extracting phytochemicals from *C. circinalis*, because of its polarity and ability to dissolve multiple compounds. Consequently, only ethanolic extracts were utilized for the study of total phenols and flavonoids and antioxidant assays due to their maximal yield.

Total Phenols, Flavonoids and Antioxidant Activity Assays

The total amount of phenols and flavonoids in ethanolic extracts of leaves, bark and male cone was investigated by spectrophotometric assays. Results are expressed as means±standard deviation. Leaves showed the highest amount of phenols (8.169±0.1204 mg GAE/g), whereas male-cone showed higher amounts of flavonoids (2.82535±0.1128 mg QE/g) as compared to leaves and bark (Table 4).

The antioxidant capacity of the bark, leaves, and male cone was evaluated using five different assays. The results for the RPA and TAC assays are given in Table 4, while IC_{50} values for the DPPH, ABTS, and nitric oxide scavenging assays are provided in Table 5.

Table 2: Results of phytochemical screening of various extracts and parts of *C. circinalis*

Extracts	Lignin	Alkaloid	Flavonoid	Tannin	Phenol	Diterpenoid	Triterpenoid	Sterol	Saponin	Cardiac glycoside	Coumarin	Quinone
LPE	-	-	+	-	-	++	-	++	-	+	++	+
LEA	-	-	++	+	+	-	-	+	-	++	-	+
LME	-	+	++	++	-	-	+	+	-	+	++	+
LEE	++	+	+	+	-	-	-	++	-	++	++	+
LAE	-	+	+	-	-	++	-	++	-	+	++	-
BPE	-	-	+	-	-	++	-	++	-	+	++	+
BEA	-	-	-	-	-	-	-	+	-	-	+	-
BME	-	+	++	+	-	-	-	++	-	-	++	-
BEE	++	++	++	+	-	++	–	++	-	-	+	+
BAE	-	-	+	+	-	-	-	+	-	-	+	+
MPE	-	-	-	-	-	-	+	+	-	-	+	+
MEA	-	-	-	-	+	-	-	+	-	-	-	-
MME	-	-	+	+	-	++	-	++	-	-	++	-
MEE	++	-	-	+	+	+	+	++	-	+	+	-
MAE	-	+	+	+	-	++	-	-	-	-	+	-
MAE	-	+	+	+	-	++	-	-	-	-	+	-

Each+values correspond to the test employed for detection which was positive; - values indicate all the tests performed for the class of compound showed no results. LPE, LEA, LME, LEE, LAE- Leaf pet ether extract, ethyl acetate extract, methanolic extract, ethanolic extract, aqueous extract respectively; BPE, BEA, BME, BEE, BAE- Bark pet ether extract, ethyl acetate extract, methanolic extract, ethanolic extract, aqueous extract respectively; MPE, MEA, MME, MEE, MAE- Male cone pet ether extract, ethyl acetate extract, methanolic extract, ethanolic extract, aqueous extract respectively

Compared to the samples, the standards (ascorbic acid, BHT, and Trolox) exhibited greater activity in their respective assays. The bark demonstrated the highest potential across all three scavenging assays (Figure 2) as well as in RPA, with IC_{50} values of 358.3 µg/mL, 59.44 µg/mL, and 4173.68 µg/mL in DPPH, ABTS, and Nitric-oxide scavenging assays, respectively, and an RPA activity equivalent to 22.546±0.212 mg BE/g. The leaf exhibited moderate scavenging effects, with an IC_{50} of 531.84 µg/mL in the ABTS scavenging assay. However, in the DPPH and Nitric oxide scavenging assays, it achieved only 34% and 13% inhibition at a concentration of 5000 µg/mL, respectively. Additionally, it demonstrated moderate activity with 5.972±0.472 mg BE/g and 18.712±0.495 mg AAE/g in RPA and TAC assays, respectively. The male cone showed relatively lower activity in all assays except for the TAC assay, where it exhibited the highest activity compared to the leaf and bark, at 19.252±0.393 mg AAE/g. When percentage inhibition of radicals by leaf, bark and male cone were compared using one-way ANOVA, the resultant p values were 0.0090, 0.0001 and 0.031 for DPPH, ABTS and nitric-oxide radicals respectively. Since all the three values are below p 0.05, the differences can be considered significant.

HPTLC Analysis of Phenols and Flavonoids

After visualization in the white light, UV 254 and 366 nm lights (Figure 3), it was observed that several bands in the samples corresponded to the established standard bands. Notably, specific bands in the leaf (Tracks 7, 8, 9) exhibited identical relative front-Rf values when compared to all the standards. In contrast, bark and male cone samples indicated the presence of gallic acid and naringenin respectively. However subsequent scanning of the absorption spectra of the standards unveiled that only gallic acid was discernible in the sample tracks located at positions 13, 14, and 15 within the *C. circinalis* bark. This was corroborated by the resemblance between the peak pattern of the band observed in the *C. circinalis* bark sample and the characteristic peak pattern of gallic acid (Figure 4).

Table 3: Extractive yields of various parts of C. circinalis

S. No.	Extracts	Leaf (% yield)	Bark (% yield)
1	Aq.	8.32	7.23
2	EtOH.	14.31	7.72
3	P.E.	2.63	0.73
4	E.A.	3.82	1.4

Aq.-aqueous; EtOH.-ethanol; P.E.-petroleum ether; E.A.-ethyl acetate

DISCUSSION

Plant-derived medicinal compounds have been employed as therapeutic agents throughout history, marking their distinctive and enduring role in healthcare There has been an active pursuit in the exploration of plant sources in the last few decades to elucidate their unique phytoconstituents with promising pharmacological applications (Dahanukar *et al.*, 2000; Gohil *et al.*, 2010). Studies on different species of *Cycas* have shown bioflavonoids to be the most reported class apart from other classes like phenols, terpenoids, glycosides, sterol etc. (Afifi *et al.*, 2021). Our preliminary investigation of *C. circinalis* also showed similar results with leaves and bark both indicating the presence of flavonoids, phenols, coumarins, and sterols, while the male cone gave the most positive results when tested for the presence of terpenoids (Table 2). The compounds identified belong to major classes of secondary metabolites that are well-documented for their therapeutic properties and have already been employed in the mainstream pharmaceutical industry as standard therapeutics. For instance, flavonoids and phenolic compounds, found abundantly in leaves, have shown significant antioxidant, antibacterial, and anti-inflammatory properties, which align with their established roles in healthcare (Hollman & Katan, 1997; Shahidi & Ambigaipalan, 2015).

The maximum extractive yield was obtained in ethanolic extracts (Table 3) hence only ethanolic extracts were used for estimation of total phenols and flavonoids as well as antioxidant activity assays. Maximum phenols in leaves (8.169 mg GAE/g) and highest flavonoid content were found in the male cone (2.825 mg QE/g). The substantial presence of phenols and flavonoids in different parts of *Cycas circinalis* highlights its potential as a natural source of these bioactive compounds. To further understand the bioactive potential of the plant, antioxidant capacity was evaluated using multiple assays. It was observed that the bark had highest antioxidant potential as compared to leaves and male cone, especially for ABTS assay (IC_{50} value-59.44 µg/mL) and TAC (22.55±0.212 BE mg/g).

To gain deeper insights into the specific phenolic and flavonoid compounds potentially present in *C. circinalis*, a modern sophisticated approach of analysing the extracts using high performance thin layer chromatography (HPTLC) was undertaken. Previously, high pressure liquid chromatography (HPLC) studies on *C. circinalis* have

Table 4: Results of total phenols, flavonoids, reducing power assay and total anti-oxidant capacity assay

S. No.	Plant part	TP (mg GAE/g)	TF (mg QE/g)	RPA (mg BE/g)	TAC (mg AAE/g)
1	Leaves	8.169±0.120	2.825±0.318	5.97±0.472	18.71±0.495
2	Bark	5.979±0.165	1.068±0.276	22.55±0.212	14. 024±0.171
3	Male cone	6.045±0.058	2.825±0.113	2.747±0.224	19.25±0.393

GAE-Gallic acid equivalent; QE-Quercetin equivalent; BE-BHT equivalent; AAE-Ascorbic acid equivalent; TP-Total phenols; TF-Total flavonoids; RPA-Reducing power activity; TAC-Total antioxidant capacity; Results expressed as means of triplicates±standard deviation

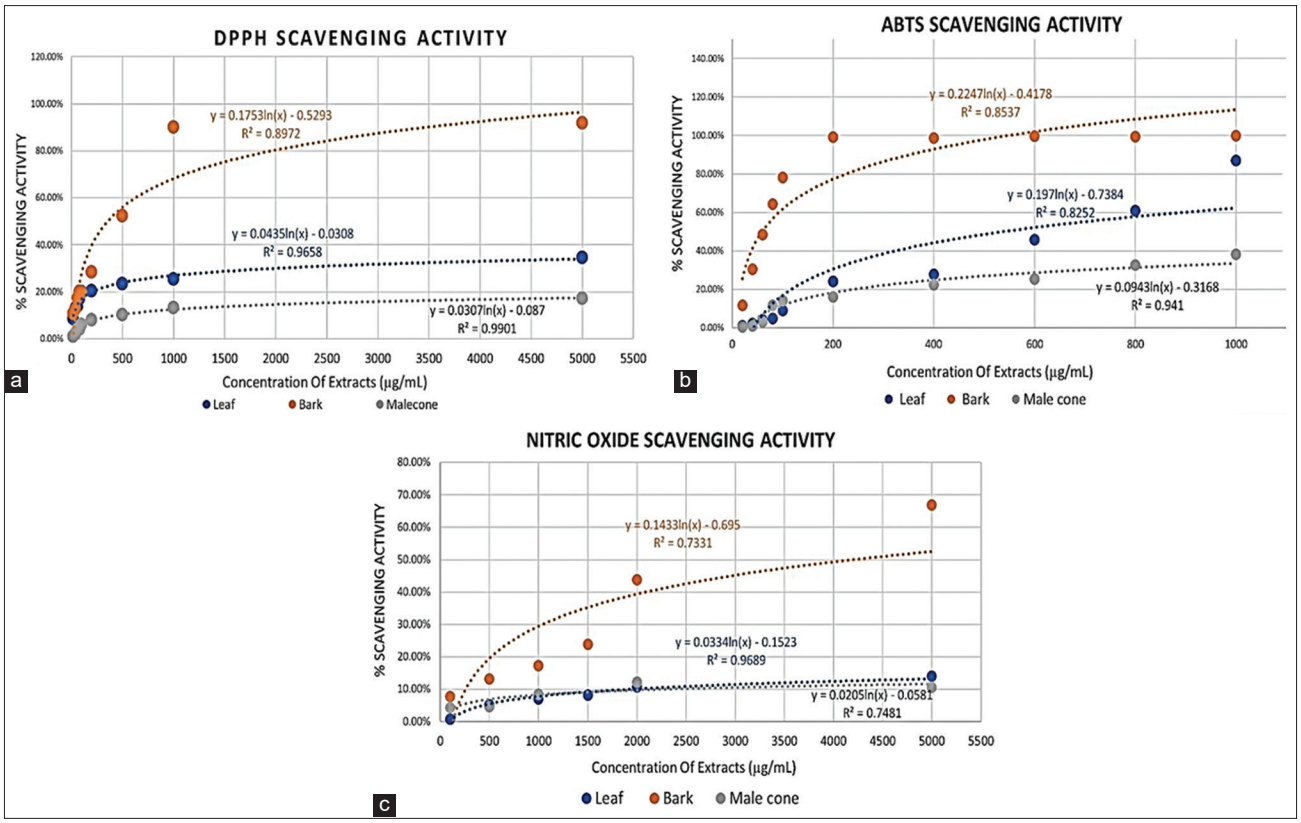

Figure 2: Radical scavenging activity of *C. circinalis* leaf, bark and male cone. a) Percentage scavenging activity of DPPH radical, b) Percentage scavenging activity of ABTS radical and c) Percentage scavenging activity of nitric-oxide radical

Table 5: IC_{50} values of samples and standards for scavenging assays

Sample	IC_{50} Values		
	DPPH (µg/mL)	ABTS (µg/mL)	NO (µg/mL)
Standard	21.12	3.07	67.01
Leaf	NA	531.84	NA
Bark	358.35	59.44	4173.68
Male cone	NA	NA	NA

DPPH radical scavenging activity; ABTS scavenging activity; NO-Nitric-oxide scavenging activity; NA-Not applicable (inhibition did not reach 50%).

identified several phenols and biflavonoids like quercetin, ellagic acid, gallic acid, etc., from ethanolic extracts of leaves (Arshad *et al.*, 2021), naringenin, epicatechin, isoginkgetin from methanolic extracts of leaves (Moawad *et al.*, 2010). However, our analysis did not yield similar results, except for presence of gallic acid in the bark extract. No prior studies have identified constituents in the bark of *C. circinalis*, making this the first study to do so. The bark extracts offer therapeutic benefits, as gallic acid is known for its antioxidant and anti-inflammatory properties (Badhani *et al.*, 2015). In a previous study, extracts of *C. circinalis* bark has shown significant antibacterial activity against *Escherichia coli*, *Salmonella typhi*, *Enterobacter aerogenes*, and *Agrobacterium tumefaciens* (Bissa & Bohra, 2008). Furthermore, the presence of bioflavonoid content, specifically 2,3-dihydroamentoflavone, 2,3,2",3"-tetrahydrobilobetin, and 2,3,2",3"-tetrahydroisoginkgetin, contribute to the plant's strong antibacterial activity. With IC_{50} values ranging from 3.8 to 12.5 µM, these compounds demonstrated moderate antibacterial activity against *S. aureus* and MRSA, confirming their potential as antimicrobial agents (Moawad *et al.*, 2010).

Beyond these properties, *C. circinalis* has also demonstrated diverse pharmacological activities (El-Seadawy *et al.*, 2023). The methanolic extract significantly improved sexual performance and behaviour in animal models when compared to sildenafil citrate (Kumar *et al.*, 2013). In a recent study, treatment with male cone extracts led to a significant increase in spermatogonia, spermatocytes, and sperm motility in alcohol-induced sterile male *Wistar* rats, further supporting the reproductive benefits of *C. circinalis* (Mane *et al.*, 2023). A study conducted on rabbit and guinea pig ileum revealed that aqueous, chloroform, and ethyl acetate extracts of *C. circinalis* leaves inhibited acetylcholine-induced colic and intestinal muscle spasms, indicating antispasmodic activity (Ali *et al.*, 2011). Larvicidal assays demonstrated that the chloroform: methanol (1:2) extract was effective against *Aedes aegypti* larvae, while the hexane extract exhibited activity against *Culex quinquefasciatus* larvae (Sethi, 2014). These findings highlight the potential of *C. circinalis* as a source of varied pharmacological effect.

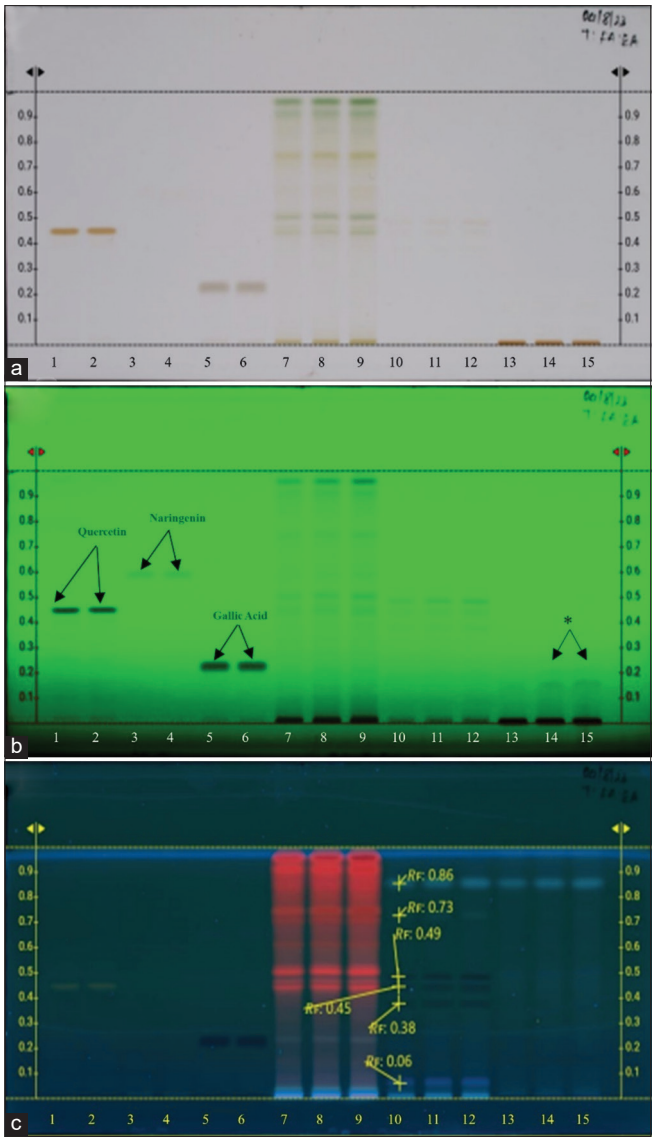

Figure 3: a) Plate after development under white light, b) Plate visualized under UV 254 nm and c) Plate visualized under UV 366 nm. quercetin (tracks 1, 2), naringenin standard (tracks 3, 4), gallic Acid (tracks 5, 6), *C. circinalis* leaf (tracks 7, 8, 9), *C. circinalis* male cone (10, 11, 12), *C. circinalis* bark (tracks 13, 14, 15), *highlighted band with same Rf as gallic acid std

Traditionally, bark paste mixed with coconut oil has been used for sores, swellings, ulcers, and wounds (Chopra & Nayar, 1956; Almeida & Almeida, 2010). The significant antibacterial properties as supported by previous studies and the antioxidant potential of its extracts align with this traditional knowledge, making it a potential candidate for natural wound-healing formulations. With the rising interest in herbal-based products in the Indian cosmetic and skincare industry (Sharma *et al.*, 2022), further studies on the bark are essential to confirm its efficacy as a wound-healing agent and to explore its potential for broader therapeutic applications.

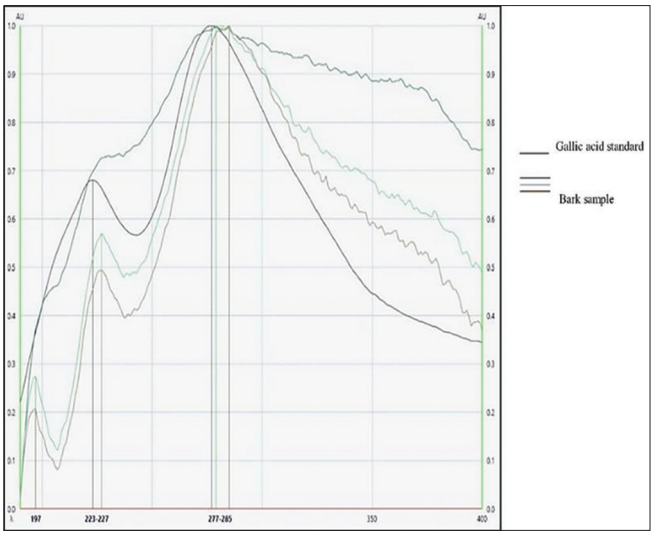

Figure 4: Gallic acid spectrum with respect to sample of *C. circinalis* bark

CONCLUSION

C. circinalis, with its reported presence of a wide array of secondary metabolites like terpenoids, sterols, along with phenols and gallic acid offers a deeper understanding of its therapeutic implications. This study is the first to report gallic acid in the bark, known for its antioxidant and anti-inflammatory properties. This attributes to the significant antioxidant activity observed in the bark extract. Given its traditional use for healing ulcers, wounds, swellings etc., along with its reported antibacterial activity against various bacteria, make it a good candidate to be studied further, with implications as natural therapeutic agent for varied skin conditions. There is also significant opportunity for research on the impact of extracts on enzymatic antioxidants, utilizing specific cell lines for assays followed by *in vivo* studies. The complex phytochemicals present in the plants also need thorough identification and characterization. The effect-directed *in vitro* and *in vivo* assays hold the promise of unveiling valuable insights into the plant's prospective contributions to pharmaceutical and other industrial applications.

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REFERENCES

Afifi, N., Moawad, A., Hassan, M., El Amir, D., Elwekeel, A., & Amin, E. (2021). Phytochemical content and biological activity of the genus *Cycas*, Family Cycadaceae: A review. *Pharmaceutical Sciences Asia, 48*(4), 300-319. https://doi.org/10.29090/psa.2021.04.20.174

Ali, S. A., Sule, I. M., Ilyas, M., Haruna, A. K., Abdulraheem, O. R., & Sikira, A. S. (2011). Ethnopharmacological activities of the leaves of *Cycas circinalis* (Cycadaceae). *Research Journal of Pharmaceutical, Biological and Chemical Sciences, 2*(1), 616-628.

Almeida, S. M., & Almeida, M. R. (2010). *Hand book of diseases and their herbal remedies in Maharashtra*. (Vol. 2). Mumbai, India: Orient Press Ltd.

Ambasta, S. P. (1992). *The Useful Plants of India: National Institute of Science Communication*. New Delhi, India: Council of Scientific & Industrial Research.

Arshad, M., Chaudhary, A. R., Mumtaz, M. W., Raza, S. A., Ahmad, M., Mukhtar, H., & Bashir, R. (2021). Polyphenol fingerprinting and hypoglycemic attributes of optimized *Cycas circinalis* leaf extracts. *Journal of the Science of Food and Agriculture, 101*(4), 1530-1537. https://doi.org/10.1002/jsfa.10771

Badhani, B., Sharma, N., & Kakkar, R. (2015). Gallic acid: A versatile antioxidant with promising therapeutic and industrial applications. *RSC Advances, 5*(35), 27540-27557. https://doi.org/10.1039/C5RA01911G

Balunas, M. J., & Kinghorn, A. D. (2005). Drug discovery from medicinal plants. *Life Sciences, 78*(5), 431-441. https://doi.org/10.1016/j.lfs.2005.09.012

Bissa, S., & Bohra, A. (2008). Antibacterial potential of three naked-seeded (Gymnosperm) plants. *Indian Journal of Natural Products and Resources, 7*(5), 420-425.

Bourgaud, F., Gravot, A., Milesi, S., & Gontier, E. (2001). Production of plant secondary metabolites: A historical perspective. *Plant Science, 161*(5), 839-851. https://doi.org/10.1016/S0168-9452(01)00490-3

Chang, C.-C., Yang, M.-H., Wen, H.-M., & Chern, J.-C. (2020). Estimation of total flavonoid content in propolis by two complementary colometric methods. *Journal of Food and Drug Analysis, 10*(3), 3. https://doi.org/10.38212/2224-6614.2748

Chopra, R. N., & Nayar, S. L. (1956). *Glossary of Indian medicinal plants*. New Delhi, India: Council of Scientific and Industrial Research.

Dahanukar, S. A., Kulkarni, R. A., & Rege, N. N. (2000). Pharmacology of medicinal plants and natural products. *Indian Journal of Pharmacology, 32*(4), 81-118.

Das, B., Paul, T., Apte, K. G., Chauhan, R., & Saxena, R. C. (2013). Evaluation of antioxidant potential & quantification of polyphenols of *Diplazium esculentum* Retz. With emphasis on its HPTLC chromatography. *Journal of Pharmacy Research, 6*(1), 93-100. https://doi.org/10.1016/j.jopr.2012.11.020

Donaldson, J. (2003). *Cycads: Status survey and conservation action plan*. Gland, Switzerland: IUCN–the World Conservation Union. Retrieved from https://portals.iucn.org/library/sites/library/files/documents/2003-010.pdf

El-Seadawy, H. M., Abo El-Seoud, K. A., El-Aasr, M., & Ragab, A. E. (2023). Cycadaceae: An Important Source for Biflavonoids and Various Pharmacological Effects of Different *Cycas* Species. *Journal of Advanced Medical and Pharmaceutical Research, 4*(2), 35-41. https://doi.org/10.21608/jampr.2023.202016.1051

Farnsworth, N. R. (1966). Biological and Phytochemical Screening of Plants. *Journal of Pharmaceutical Sciences, 55*(3), 225-276. https://doi.org/10.1002/jps.2600550302

Ganie, S. H., Upadhyay, P., Das, S., & Sharma, M. P. (2015). Authentication of medicinal plants by DNA markers. *Plant Gene, 4*, 83-99. https://doi.org/10.1016/j.plgene.2015.10.002

Gocan, S., & Cimpan, G. (2004). Review of the Analysis of Medicinal Plants by TLC: Modern Approaches. *Journal of Liquid Chromatography & Related Technologies, 27*(7-9), 1377-1411. https://doi.org/10.1081/JLC-120030607

Gohil, K. J., Patel, J. A., & Gajjar, A. K. (2010). Pharmacological Review on *Centella asiatica*: A Potential Herbal Cure-all. *Indian Journal of Pharmaceutical Sciences, 72*(5), 546-556. https://doi.org/10.4103/0250-474X.78519

Hill, K. D. (1995). The genus *Cycas* (Cycadaceae) in the Indian region, with notes on the application and typification of the name *Cycas circinalis*. *Taxon, 44*(1), 23-31. https://doi.org/10.2307/1222674

Hollman, P. C. H., & Katan, M. B. (1997). Absorption, metabolism and health effects of dietary flavonoids in man. *Biomedicine & Pharmacotherapy, 51*(8), 305-310. https://doi.org/10.1016/s0753-3322(97)88045-6

Kumar, B. S., Kumar, J. V., & Selvaraj, R. (2013). Aphrodisiac activity of *Cycas circinalis*. L and *Ionidium suffruticosum*. Ging on male wister albino rats. *Asian Journal of Pharmaceutical and Clinical Research, 6*(3), 215-217.

Mane, S. B., Ambali, M. P., & Doshi, M. A. (2023). Effects of *Cycas circinalis* and *Ionidium suffruticosum* on Male Wistar Rats that Have Been Made Sterile by Alcohol. *Journal of Coastal Life Medicine, 11*, 2703-2711.

Moawad, A., Hetta, M., Zjawiony, J. K., Jacob, M. R., Hifnawy, M., Marais, J. P. J., & Ferreira, D. (2010). Phytochemical Investigation of *Cycas circinalis* and *Cycas revoluta* Leaflets: Moderately Active Antibacterial Biflavonoids. *Planta Medica, 76*(8), 796-802. https://doi.org/10.1055/s-0029-1240743

Mussin, J., & Giusiano, G. (2020). Ethno–Phytopharmacology: Product Validation Process Based on Traditional Knowledge of Medicinal Plants. In P. A. Chong, D. J. Newman & D. A. Steinmacher (Eds.), *Agricultural, Forestry and Bioindustry Biotechnology and Biodiscovery* (pp. 331-353) Cham, Switzerland: Springer International Publishing. https://doi.org/10.1007/978-3-030-51358-0_17

Oyaizu, M. (1986). Studies on products of browning reaction. Antioxidative activities of products of browning reaction prepared from glucosamine. *The Japanese Journal of Nutrition and Dietetics, 44*(6), 307-315. https://doi.org/10.5264/eiyogakuzashi.44.307

Patel, A. A., Amin, A. A., Patwari, A. H., & Shah, M. B. (2017). Validated high performance thin layer chromatography method for simultaneous determination of quercetin and gallic acid in Leea indica. *Revista Brasileira de Farmacognosia, 27*(1), 50-53. https://doi.org/10.1016/j.bjp.2016.05.017

Prasad, M. N. V., Padmalatha, K., Jayaram, K., Raju, N. L., & da Silva, J. A. T. (2007). Medicinal plants from Deccan Ecoregion, India: Traditional knowledge, ethnopharmacology, cultivation, utilization, conservation and biotechnology–opportunities and impediments. *Medicinal and Aromatic Plant Science and Biotechnology, 1*(2), 155-208.

Prieto, J. M. (2012). Procedure: Preparation of DPPH Radical, and Antioxidant Scavenging Assay. *Dr Prieto's DPPH Microplate Protocol,* 1-3.

Prieto, P., Pineda, M., & Aguilar, M. (1999). Spectrophotometric Quantitation of Antioxidant Capacity through the Formation of a Phosphomolybdenum Complex: Specific Application to the Determination of Vitamin E. *Analytical Biochemistry, 269*(2), 337-341. https://doi.org/10.1006/abio.1999.4019

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology & Medicine, 26*(9-10), 1231-1237. https://doi.org/10.1016/s0891-5849(98)00315-3

Sethi, P. (2014). Larvicidal activity of a living fossil! *Journal of Entomology and Zoology Studies, 2*(6), 246-248.

Shahidi, F., & Ambigaipalan, P. (2015). Phenolics and polyphenolics in foods, beverages and spices: Antioxidant activity and health effects – A review. *Journal of Functional Foods, 18*, 820-897. https://doi.org/10.1016/j.jff.2015.06.018

Shaikh, J. R., & Patil, M. K. (2020). Qualitative tests for preliminary phytochemical screening: An overview. *International Journal of Chemical Studies*, *8*(2), 603-608. https://doi.org/10.22271/chemi.2020.v8.i2i.8834

Sharma, R. R., Deep, A., & Abdullah, S. T. (2022). Herbal products as skincare therapeutic agents against ultraviolet radiation-induced skin disorders. *Journal of Ayurveda and Integrative Medicine, 13*(1), 100500. https://doi.org/10.1016/j.jaim.2021.07.016

Singleton, V. L., Orthofer, R., & Lamuela-Raventós, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. *Methods in Enzymology*, 299, 152-178. https://doi.org/10.1016/S0076-6879(99)99017-1

Sreejayan, & Rao, M. N. A. (1997). Nitric Oxide Scavenging by Curcuminoids. *Journal of Pharmacy and Pharmacology, 49*(1), 105-107. https://doi.org/10.1111/j.2042-7158.1997.tb06761.x

Syarifah, A. L., Retnowati, R., & Soebiantoro (2019). Characterization of Secondary Metabolites Profile of Flavonoid from Salam Leaves (*Eugenia polyantha*) Using TLC and UV Spectrophotometry. *Pharmaceutical Sciences and Research, 6*(3), 4. https://doi.org/10.7454/psr.v6i3.4219

Whitelaw, A. (1826). *Materia Indica*. (Vol. 2). London, UK: A. & R. Spottiswoode.

Zimila, H. E., Mandlate, J. S., Artur, E. M., Muiambo, H. F., & Uamusse, A. A. (2021). Vortex-assisted Solid-Liquid Extraction for Rapid Screening of Oil Content in *Jatropha* Seed: An Alternative to the Modified Soxhlet Method. *South African Journal of Chemistry, 75*, 1-5.