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ORGINAL RESEARCH ARTICLE

Unveiling the antioxidant and antimicrobial properties of *Plumeria pudica* Jacq. Stem extracts, backed by qualitative and quantitative phytochemical analysis

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Abstract

The demand for herbal remedies has surged since the coronavirus outbreak due to the side effects of conventional treatments. This has led to increased research into extracting phytochemicals from plants for biomedical applications. This research aims to evaluate the phytochemical content and antioxidant and antimicrobial properties of the medicinal plant *P. pudica*. Phytochemical evaluation revealed that hydroalcoholic (HYA) and isopropanol (IPA) extracts had superior compositions, with HYA showing the highest content of phenols, flavonoids, and saponins. The HYA extract exhibited robust antioxidant activity, particularly in DPPH scavenging (70.82% inhibition) and reducing power (1.57 absorbance). Antimicrobial tests showed lower MIC zones of inhibition for IPA and HYA extracts. The findings suggest that *P. pudica* stem extracts possess significant phytochemical properties, highlighting their potential as novel therapeutic sources.

Keywords: *Plumeria pudica*, medicinal plants, phytochemicals, antioxidant potential, antimicrobial potential, LC-QTOF MS/MS analysis.

Introduction

Medicinal plants worldwide have long been acknowledged for their therapeutic properties, contributing significantly to community health through a rich array of secondary metabolites. Various plant parts, including the bark, leaves, fruits, flowers, and roots, are bioactive compounds that confer health benefits. These medicinal plants serve as reservoirs of diverse natural organic compounds (NOAs), such as polyphenols, phenolic acids, flavonoids, tannins, steroids, and carotenoids, which are esteemed for their biological efficacy, encompassing anti-hypertensive, anti-inflammatory, anticancer, anti-diabetic, and antiproliferative activities. The applications of plant-derived natural organic antioxidants (NOAs) in the cosmetics and food industries have garnered significant attention, prompting extensive efforts to identify novel sources. *P. pudica*, a member of the Apocynaceae family commonly referred to as Nag Champa or White Frangipani, is esteemed for its aesthetic appeal and potential therapeutic properties. Indigenous to tropical regions, this plant has been linked with a variety of medicinal attributes, including anti-allergic, laxative, carminative, cytotoxic, anti-microbial, and anti-inflammatory effects. In northeastern Brazil, *P. pudica* is employed in traditional medicine for its analgesic properties, albeit scientific data substantiating its pharmacological effects remains limited. Recent research has indicated that the latex of *P. pudica* exhibits both anti-inflammatory and analgesic properties [1,2]. Understanding the phytochemical composition of plants is critical, as compounds such as phenolics and flavonoids possess antioxidant characteristics that may aid in the prevention of diseases, including cancer and cardiovascular disorders. Investigations into the latex proteins derived from *P. pudica* have revealed significant influences on key elements of the inflammatory response, such as neutrophil migration and the synthesis of cytokines and inflammatory mediators. The present study conducted

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a thorough evaluation of this particular plant, aiming to ascertain the presence of phytochemicals using both qualitative and quantitative methodologies, including LC-QTOF MS/MS analysis of the stem extracts. The analysis comprised the estimation of the total content of six distinct phytochemical compounds, alongside a comprehensive assessment of the plant's antioxidant capabilities. To achieve this, two antioxidant assays were performed: the reducing power assay and the DPPH radical scavenging assay. Furthermore, the antimicrobial properties of the plant were examined, which included an antibacterial assay conducted against four bacterial species utilizing the agar well diffusion technique, in conjunction with the determination of the minimum inhibitory concentration (MIC). The cumulative findings affirm the promising applicability and inherent value of *P. pudica*, underscoring its potential for further research pursuits. Given the adverse side effects associated with synthetic antioxidants—such as tert-butyl hydroquinone, butylated hydroxytoluene, and butylated hydroxyanisole—NOAs are positioned as safer alternatives [3]. In recent years, NOAs have attracted considerable interest from researchers, scientists, and the general public alike. It is essential to recognize the significance of NOAs derived from medicinal plants, spices, fruits, vegetables, and dietary supplements, which offer broad applications and considerable health benefits.

Materials and methods

Collection of plant material

In June 2024, fresh stems of young plants of *P. pudica* were collected from various locations in the Gandhinagar district of Gujarat, situated at 23°14′ N latitude and 72°38′ E longitude. The collection took place in June, which is the beginning of the humid monsoon season, with temperatures rising from 25°C to 35°C. Most of the plants were collected from households, so they were in excellent condition due to proper maintenance. This plant is commonly grown for its ornamental appearance in homes and is readily available in local nurseries, so there is no need to obtain a license for collection. Dr. B.A. Jadeja identified the plant, and the voucher specimens (KS20A and KS20B) of the collected stems were deposited at the Department of Botany, M.D. Science College, Porbandar, Gujarat, India. The stems were thoroughly cleaned with distilled water, air-dried for ten days, and then carefully ground into a coarse powder for storage in glass bottles for further use.

Plant Extraction Process

The isolation of secondary metabolites (SMs) from plant material is influenced by several factors, including extraction time, temperature, techniques, solvents, moisture content, and particle size. Therefore, to ensure their efficient extraction, it is essential to choose an appropriate extracting solvent and method [4]. Plant extraction was conducted utilizing a Soxhlet apparatus through a hot extraction method. The powdered stems were placed in a muslin thimble within a glass chamber. The solvent was introduced at a ratio of 1:10 g/mL. The extraction procedure employed various temperatures and durations: 82.3°C for isopropyl alcohol (IPA) for 8 hours, 100°C for aqueous extraction for 15 hours, 60°C for petroleum ether for 9 hours, and 66°C for hydroalcoholic extraction for 12 hours (with a dried weight to methanol volume ratio of 6:4). The resulting supernatant was filtered using Whatman filter paper and subsequently air-dried. The dry weight of the crude extract was then determined, thereby providing pertinent data regarding the yield of the extract [5].

$$\text{Yield of plant extract} = \frac{Weight\ of\ crude\ extract\ obtained\ (gram)}{total\ weight\ of\ plant\ powder\ (gram)} \times 100$$

Qualitative phytochemical analysis

A comprehensive series of tests was conducted on four stem extracts to evaluate the presence of various primary and secondary metabolites. The assessment of primary metabolites included the analysis of proteins, amino acids, and sugars. Additionally, the presence of secondary metabolites such as alkaloids, glycosides, cardiac glycosides, saponins, terpenoids, flavonoids, and tannins was investigated. The methodologies employed for these evaluations were adapted from the protocols established by [6] and [7]. The analysis encompassed a total of 23 tests, with a "+" symbol denoting the presence of a specific compound and a "–" symbol indicating its absence. A stock solution at a concentration of 2 mg/mL was prepared for each plant extract, which served as the basis for testing various bioactive compounds.

Quantitative phytochemical analysis

Following the completion of the qualitative phytochemical analysis, we assessed the presence of various primary and secondary metabolites. The next step involves determining the overall quantity of these metabolites. This is accomplished using established standards for the tests, whereby we create a standard curve based on the relationship between concentration and absorbance values for these standards. By plotting the standard curve, we can derive the parameters from the equation $Y = mx + b$, which are essential for calculating the total content of each phytochemical identified. Specifically, a linear equation ($Y = mx + b$) is utilized to quantify the saponin content, with the concentration derived through the formula $x = (y - b) / m$. The total content is then expressed using the formula $C = x/v * m$, enabling us to accurately determine the levels of phytochemicals in each sample (for the total content estimation the concentration of 5 mg/mL was used for the stem extracts).

Total alkaloid content

This method is based on the reaction between alkaloid and bromocresol green (BCG). The stem extract was dissolved in 2 N HCl and then filtered. The pH of the phosphate buffer solution was adjusted to neutral with 0.1 N NaOH. 1 mL of this solution was transferred to a separating funnel, and then 5 mL of BCG solution along with 5 mL of phosphate buffer were added. Caffeine (500 µg/mL) was utilized as standard. The concentration series of 100,200,300,400 and 500 µg/mL was prepared for the standard. The mixture was shaken and the complex formed was extracted with chloroform by vigorous shaking. The extract was collected in a 10 mL volumetric flask and diluted to volume with chloroform. The absorbance of the complex in chloroform was measured at 470 nm. The whole experiment was conducted in three replicates. The results were described as caffeine equivalents (CE) per gram of the plant extract [8].

Total phenolic content

The Total Phenolic content in different stem extracts of *P. pudica* was evaluated using the modified Folin-Ciocalteau method. A gallic acid standard solution at 100 µg/mL was prepared, along with additional solutions at various concentrations (20, 40, 60, 80, and 100 µg/mL). For each concentration, 5 mL of 10% Folin-Ciocalteau reagent and 4 mL of 7% Na_2CO_3 were added to 5 mL of the gallic acid solution, followed by incubation at 40°C for 30 minutes, and absorbance was measured at 760 nm. Polyphenol concentrations in the plant extracts were determined using a calibration curve. The total phenolic content was calculated in milligrams of gallic acid equivalents (GAE) per gram of the sample using the formula C=x(V/m), where C is total phenolic content, x is concentration, V is volume, and m is concentration of the extract [9,10].

Total flavonoid content

The aluminum chloride assay was employed to measure the total flavonoid content in the stem extracts. A stock solution of quercetin at 100 µg/mL was prepared using methanol, followed by dilutions to 20, 40, 60, 80, and 100 µg/mL. After adding 4 mL of distilled water to each test tube, 0.3 mL of 5% $NaNO_2$ and 0.3 mL of 10% $AlCl_3$ were added after 5 minutes, followed by 2 mL of 1M NaOH after another 6 minutes. The total volume was adjusted to 10 mL with distilled water, and the absorbance was measured at 510 nm. Extracts were prepared similarly and their absorbance was recorded. The total flavonoid content was expressed as quercetin equivalents using a linear equation from a calibration curve. The formula used to calculate the total flavonoid content in mg QE/g of the extract was C = (x * V) / m, where x is the concentration, V is the extract volume, and m is the concentration of the crude extract [11].

Total protein content

The total protein content of the *P. pudica* stem was determined using the methodology outlined by [12]. The development of the blue color in the Folin reagent is linked to the reduction of phosphomolybdic-phosphotungstic components by tryptophan and tyrosine in proteins. Protein quantification was performed using Lowry's method.

Key reagents included:

- 0.1N NaOH: 0.4 g NaOH in 100 mL distilled water.
- 15% TCA: 15 g trichloroacetic acid in 100 mL water.
- Solutions A, B, C, and D were prepared for the assay.

Bovine serum albumin (BSA) was used for standard curve generation with concentrations from 200 µg/mL to 1000 µg/mL to quantify protein as quercetin equivalents. Plant extracts were prepared from five grams of stem powder, homogenized in NaOH, centrifuged, and treated with TCA to precipitate proteins. After further centrifugation, the precipitate was dissolved back into NaOH and combined with solution C, followed by solution D. Absorbance was measured at 750 nm. Protein content was calculated with the formula C = (x/V) * m, where C is total protein content (mg BSAE/g extract), x is concentration, V is extracting volume (µL), and m is the concentration of the crude extract (mg/mL).

Total saponin content

The total saponin content (TSC) of the *P. pudica* stem was determined by mixing 250 µL of stem extracts with 250 µL of 8% (w/v) vanillin and 2.5 mL of 72% (v/v) sulfuric acid. The mixture was incubated at 60°C for 10 minutes, cooled in an ice water bath for another 10 minutes, and then measured for absorbance at 560 nm, using a blank without the extract. TSC was reported as diosgenin equivalents (mg DIE/g dry weight of plant extract). A standard series was prepared and measured at 544 nm using a spectrophotometer. Various concentrations of the extracts (20, 40, 60, 80, and 100 µg/mL) were tested under the same conditions [13]. A linear equation (Y = mx + B) was used to quantify the saponin content, with x = (y - b) / m for concentration calculations. The total saponin content was expressed as mg DIE/g of plant extract using the formula C = x/v * m, allowing for accurate determination of saponin levels in each sample.

Total content estimation of carbohydrates

The procedure outlines the steps to quantify carbohydrate content in a sample. It begins by hydrolysing 100 mg of glucose with 5 mL of 2.5N HCl in a water bath for 3 hours. After cooling, sodium carbonate is added until effervescence stops, and the solution is filtered and diluted to 100 mL. Working standards of glucose are prepared at specified concentrations. The sample solution is similarly diluted. Each tube receives 1 mL of phenol and 5 mL of 96% sulfuric acid, then incubated at 25-30°C for 20 minutes to produce a green compound. The absorbance is measured at 490 nm to determine glucose equivalents using a calibration curve. The total carbohydrate content is calculated, and further protein content calculations are performed using the formula C = (x/V) * m, where C is the total carbohydrate

content, x is the concentration, V is the extract volume, and m is the concentration of the crude extract [6,14].

LC-MS QTOF analysis

The components of the *P. pudica* stem were analyzed using liquid chromatography in conjunction with quadrupole time-of-flight mass spectrometry (Agilent 6545 XT Advance bio-LC/QTOF). The liquid chromatography was performed on the Agilent 1290 Infinity 2 LC system, which is part of the 6545 XT system. The Agilent Advance BIO Peptide Mapping column was the analytical column, with dimensions of 2.1 × 150 mm and a particle size of 2.7 µm (p/n 653750-902). The temperature of the column was set to 60°C, while the autosampler was maintained at 4°C. During the liquid chromatography process, 0.1% formic acid in water and 0.1% formic acid in 90% acetonitrile were used as solvents. For the mass spectrometry analysis, the gas temperature was kept at 325°C. The stem extracts were prepared at a concentration of 2 mg/mL and underwent two rounds of dilution for the analysis. In the first dilution, 100 µL of the stem extract was mixed with 900 µL of methanol. The second dilution involved taking 10 µL of the previously diluted sample and adding 990 µL of methanol. Following these dilutions, the sample was centrifuged at 4000 rpm for 10 minutes. The supernatant collected from the top of the centrifuge tubes was then used for analysis. Compound identification was carried out using the NIST library and Agilent Mass Hunter Bio Confirm B 0.9.

Antioxidant activities

Owing to the complex nature of phytochemicals, the evaluation of antioxidant activity requires at least two test systems to establish authenticity.

Reducing power assay

An increase in absorbance values may indicate the antioxidant capacity of the antioxidants or their respective extracts. Chemical compounds with antioxidant properties react with potassium ferricyanide ($K_3[Fe(CN)_6]$), resulting in the formation of potassium ferrocyanide ($K_4[Fe(CN)_6]$). This resultant compound then reacts with ferric trichloride, producing ferric ferrocyanide, which is characterized by a blue-colored complex that exhibits peak absorbance at 700 nm. To prepare the sample solution, plant extracts were used at concentrations of 200, 400, 600, 800, and 1000 µg/mL, which were combined with 1 mL of distilled water. Next, 2.5 mL of a 0.2 M pH 6.6 phosphate buffer and 2.5 mL of 1% potassium ferricyanide ($K_3[Fe(CN)_6]$) were added to the mixture. The mixtures were then incubated at 50°C for 20 minutes. After the incubation period, 2.5 mL aliquots of 10% trichloroacetic acid were added to each mixture, followed by centrifugation for 10 minutes at 1036 x g. The upper layer of each solution (2.5 mL) was then separately mixed with 2.5 mL of distilled water and 0.5 mL of 0.1% $FeCl_3$. The absorbance was measured at 700 nm using a spectrophotometer, with methanol serving as the control and ascorbic acid as the positive control [15].

DPPH assay

The antioxidant activity of the extracts was assessed using the DPPH free radical scavenging assay, with slight modifications to the previously described method by [16]. This method can be applied to both liquid and solid samples. Additionally, it is non-specific and measures the overall antioxidant capacity of the plant extract. In its oxidized state, DPPH appears as a deep violet color in methanol. When an antioxidant compound is introduced, it donates an electron to DPPH, leading to its reduction and a color change from violet to blue to yellow. DPPH solutions have an absorbance peak at 517 nm, and the scavenging of DPPH free radicals indicates the free radical scavenging capacity and antioxidant potential of plant samples. To prepare a 0.1 M DPPH solution, 0.39 mg of DPPH reagent was dissolved in methanol in a volumetric flask, resulting in a final volume of approximately 100 mL. The purple-colored DPPH solution was then stored in a freezer at -15°C for future use. Stock solutions of 2 mg/mL extracts were created by dissolving each extract in methanol and subsequently diluting to concentrations of 20, 40, 60, 80, and 100 µg/mL. To evaluate the antioxidant potential, the sample solutions were mixed with 1 mL of DPPH solution and incubated in darkness at room temperature for 30 minutes. A control solution consisting of 1 mL of methanol and 1 mL of DPPH was also prepared. After incubation, the absorbance was measured at 517 nm using a spectrophotometer, with ascorbic acid serving as the standard. The IC_{50} values of the extracts were determined from the concentration versus percentage inhibition graph, and the percentage of DPPH free radical inhibition was calculated using a specific formula.

$$\text{Inhibition (\%)} = \frac{Acontrol - Atest}{Acontrol} \times 100$$

Where Acontrol is the absorbance of the control, and the Atest is the absorbance of the reaction mixture samples (in the presence of the sample). Each test was conducted in triplicate (n=3), and the average values were calculated.

IC_{50} Value

In assessing the DPPH method, [17] employed the inhibition concentration (IC_{50}) parameter. A plot of sample discoloration against sample concentration was generated to ascertain the IC_{50} value. This value denotes the quantity of sample necessary to achieve a 50% reduction in the absorbance of DPPH.

Antimicrobial assay

The agar well diffusion technique was utilized to assess the antimicrobial properties of *P. pudica* stem extracts.

The antimicrobial efficacy was evaluated against four microorganisms: *Staphylococcus aureus* (gram-positive), *Pseudomonas aeruginosa*(gram-negative), *Salmonella enterica*(gram-negative), and *E. coli* (gram-negative), which were sourced from Pure Microbes, Pune. To prepare the inoculum, bacterial cultures were adjusted to a concentration of 10^6 cells/ml using the direct colony suspension approach. Sterilized Petri dishes were filled with Mueller-Hinton agar. Extracts from the stem were obtained using four different solvents: isopropyl alcohol, aqueous, petroleum ether, and hydro alcohol. These solvents were chosen to extract bioactive components that are polar, medium-polar, and nonpolar. The crude extracts were later dissolved in DMSO. A sterile cork borer was employed to create wells with a diameter of 6 mm in each agar plate, which were then filled with 100 µL of the plant extract solution at various concentrations. A 5% DMSO solution served as the negative control. The plates were incubated at 37°C for 18 hours. Following incubation, the diameter of the inhibition zone surrounding each well was measured using microbial callipers [18,19].

Calculation of Zone of inhibition and MIC

The agar plate dilution method was used to determine the minimum inhibitory concentration (MIC) of plant extracts. Extracts were tested at concentrations of 10, 15, 20, 25, 30, 40 and 50 mg/mL. Bacterial cultures, grown overnight in broth to a concentration of 10^8 CFU/mL, were placed onto the agar plates and incubated at 37°C for 24-48 hours. The lowest concentration of each extract that inhibited bacterial growth on Mueller-Hinton agar was recorded as the MIC, expressed in mg/mL [20,21].

Results

Extractive yield

The yield value was used to quantify the crude dry powder of the plant part employed. As depicted in Table 1, the highest yield of 77.46% was achieved for the aqueous extract, followed by a 49.81% yield for the hydroalcoholic extract, 14.58%, and 9.45% for the IPA and petroleum extracts, respectively. The primary objective of selecting various polar and non-polar solvents was to assess the impact of polarity on the overall extraction process and to isolate as many phytoconstituents as possible. The choice of solvent significantly influences all subsequent evaluations and activities related to the plant. Therefore, this study aims to assist researchers in identifying the most suitable solvent for future investigations involving this plant.

Qualitative phytochemical analysis

The extraction efficiency of four solvents—Isopropyl Alcohol (IPA), aqueous solution, petroleum ether, and hydroalcoholic solution—was evaluated based on their polarity. A comprehensive qualitative phytochemical analysis of the stem extracts yielded critical insights into the presence of various phytochemicals. In total, twenty-three tests were conducted to assess the presence of eleven distinct phytochemicals. The majority of the stem extracts exhibited significant levels of primary metabolites, including proteins, amino acids, and carbohydrates, which were consistently identified across all extracts. Additionally, alkaloids, terpenoids, phenols, flavonoids, and glycosides were present in all examined stem extracts (Table 2). Notably, saponins were absent in the IPA extract, while cardiac glycosides and steroids were not detected in the petroleum ether extracts. Furthermore, tannins were found to be absent in both the hydroalcoholic and aqueous extracts of the stem. Test results indicated the following order: IPA extract with 17/23, HYA extract with 16/23, Aqueous extract with 15/23, and Petroleum ether extract with 13/23 for the presence of various phytochemicals.

Table 1: Yield of extracts for the stem of *P. pudica*

Extract	*% Yield*	*Color of the crude*	*pH*
IPA	14.58	Brownish green	6.1
HYA	49.81	Dark green	7.2
Petroleum ether	9.45	Brown	6.6
Aqueous	77.46	Black	6.9

Quantitative phytochemical analysis

The quantitative analysis performed on six selected phytochemicals—namely alkaloids, flavonoids, carbohydrates, proteins, phenols, and saponins—in various stem extracts indicates that the plant contains significant amounts of these compounds (Table 3). The detection and overall concentration of these phytochemicals suggest a broad spectrum of antimicrobial, antioxidant, and other pharmacological activities linked to this plant. In Figure 1 a diagrammatic representation of the total content estimation of various phytochemicals is given for ease of understanding.

Total alkaloid content

The total alkaloid content in four stem extracts of *P. pudica* was assessed using the BCG method, with Caffeine serving as the standard. A calibration curve was established by plotting absorbance values from various concentrations of Caffeine. The total alkaloid content of the extracts was calculated using the regression equation derived from the calibration curve (Figure 2), resulting in the following equation:

$Y=0.0004x+0.006$

$R^2 =0.9606$

The highest total alkaloid content was found in the IPA extract of the stem, measuring 167±0.34 mg CE/g of dry-weight plant extract. This was followed by the Aqueous stem extract, which had a total alkaloid content of 145±0.53 mg CE/g of dry-weight plant extract, and the HYA extract,

Table 2: The qualitative phytochemical analysis of various stem extracts.

Test	*Result*	*PP1*	*PP2*	*PP3*	*PP4*
Primary metabolites					
Protein and amino acids					
Biuret Test	Violet color	+	-	-	+
Ninhydrin Test	Blue color	+	+	+	-
Carbohydrates					
Molisch's Test	Violet Ring on Top	+	++	+	++
Benedict's Test	Colored Precipitates	+	+	-	+
Fehling's Test	Red Precipitates	+	++	+	++
Secondary metabolites					
Alkaloids					
Mayer's Test	White Creamy Precipitates	+	+	-	-
Dragendroff's Test	Orange Precipitates	-	-	-	+
Hager's Test	Yellow precipitates	++	+	+	+
Wanger's Test	Red Brown Precipitates	+	+	+	+
Saponins					
Foam Test	White foam formation	-	+	+	+
Froth Test	White froth of 1 centimetre	-	++	-	+
Terpenoids					
Salkowski's Test	A layer of red-brown precipitates	-	+	+	+
Copper acetate Test	Green precipitates	+	++	+	-
Phenols					
Ferric chloride Test	Dark green color	+	++	+	+
Lead acetate test	White precipitates	+	+	+	-
Folin Ciocalteau test	Blue-green color	-	-	+	-
Flavonoids					
Shinoda Test	Pink crimson color	-	+	+	+
Alkaline reagent Test	Yellow color disappears	+	++	++	++
Glycosides					
Bontrager's test	Pink color	+	+	+	++
Sulfuric acid Test	Violet-green color	+	+	-	+
Cardiac Glycosides					
Keller Kiliani Test	Bluish-green ring	++	+	-	++
Steroids					
Liebermann-Burchard Test	Color change from violet to green	+	++	-	+
Tannins					
Ferric chloride test	Greenish residues	+	-	+	-
Results		17/23	16/23	13/23	15/23

PP1: IPA extract
PP2: Hydroalcoholic extract
PP3: Petroleum ether extract
PP4: Aqueous extract

with 134±0.75 mg CE/g. In contrast, the lowest total alkaloid content was observed in the petroleum ether extract of the stem, which amounted to approximately 76.5±0.52 mg CE/g of dry-weight plant extract. Alkaloid compounds are more soluble in polar solvents than in nonpolar ones. As a result, methanol extracts contain a higher Alkaloid content compared to other solvents. The high temperature of the solvent in the SEM enhances the release of secondary metabolites, particularly bound Alkaloids, phenolics, lignin, and flavonoids, from the plant material.

Total flavonoid content

The total flavonoid content in four extracts of the stem of *P. pudica* was determined using the aluminum chloride method, with quercetin serving as the standard. Absorbance values obtained at various concentrations of quercetin were utilized to create a calibration curve. The total flavonoid content of the extracts was calculated based on the regression equation derived from this calibration curve (Figure 3):

$Y = 0.0017+0.0085$

$R^2 = 0.9808$

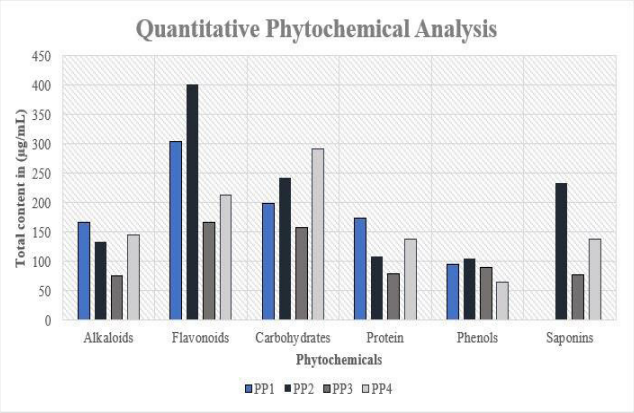

Figure 1: Total contents of various phytochemicals in the stem of *P. pudica* (PP1: IPA extract, PP2: HYA extract, PP3: Petroleum ether extract, PP4: Aqueous extract)

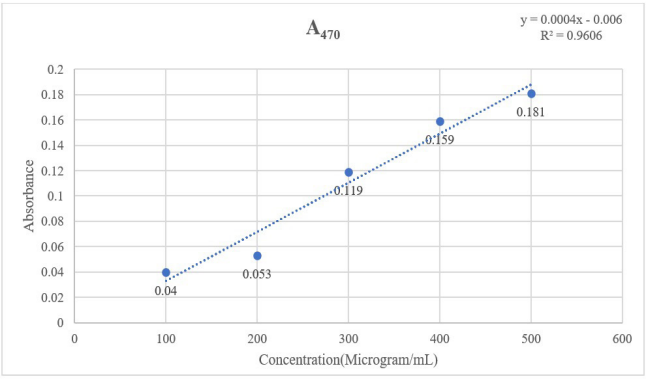

Figure 2: The standard curve for Caffeine for estimation of total alkaloid content of *P. pudica* stem.

Table 3: Total content estimation of various phytochemicals for the stem of *P. pudica*

Phytochemical	*Standard*	*Measures in.*	*PP1*	*PP2*	*PP3*	*PP4*
Alkaloids	Caffeine (500 µg/mL)	mg CE/g of plant extract.	167±0.34	134±0.75	76.5±0.52	145±0.53
Flavonoids	Quercetin (100 µg/mL)	mg QE/g of plant extract.	304.5±0.41	401.2±0.68	167.5±0.34	214±0.98
Carbohydrates	Glucose (100 µg/mL)	mg GLE/g of plant extract.	199±0.41	243±0.56	158±0.7	291.5±0.19
Protein	BSA (1000 µg/mL)	mg BSAE/g of plant extract.	173±0.70	108±0.43	80±0.92	137±0.67
Phenols	Gallic acid (100 µg/mL)	mg GAE/g of plant extract.	95±0.93	105.4±0.54	65±0.71	90.2±0.65
Saponins	Diosgenin (100 µg/mL)	mg DE/g of plant extract.	Ab.	233.4±0.45	77±0.67	138±0.71

The highest total flavonoid content (TFC) was recorded in the HYA extract derived from the stem, measuring 401.2 ± 0.68 mg QE/g of dry-weight plant extract. This was followed by the IPA stem extract, which exhibited a TFC of 304.5 ± 0.41 mg QE/g of dry-weight plant extract. The aqueous extract demonstrated a TFC of 214 ± 0.98 mg QE/g of dry-weight plant extract. Conversely, the petroleum ether extract of the stem exhibited the lowest flavonoid content, quantified at 167 ± 0.34 mg QE/g of dry-weight plant extract. Here also the polarity of the solvent plays a major role in the extraction of flavonoids as TFC was in HYA>IPA>Aqueous>Petroleum ether extracts.

Total carbohydrate content

The total carbohydrate content of the stem of *P. pudica* was determined using glucose as standard. The absorbance values obtained at various concentrations of glucose were used to create a calibration curve. The total carbohydrate content of the extracts was calculated from the regression equation of the calibration curve. The value of the equation Y= mx+B of the calibration curve is as follows (Figure 4):

Y= 0.014x-0.0805

R^2= 0.9985

The carbohydrate content of maximum value was observed in the Aqueous extract of the stem which was 291.5±0.19 mg GLUE/g dry-weight of plant extract. Followed by HYA extract of the stem with 243±0.56 mg GLUE/g dry-weight plant extract, IPA extract of the stem with 199±0.41 mg GLUE/g dry-weight plant extract, and the least amount of carbohydrates were present in Petroleum ether stem extract with 158±0. mg GLUE/g dry-weight of plant extract.

Total protein content

The total protein content in four extracts of stem of *P. pudica* was determined by Lawrey's method using BSA (Bovine Serum Albumin) as standard. The absorbance values measured across various concentrations of BSA were utilized to establish a calibration curve. The total protein content of the extracts was calculated from the regression equation of the calibration curve from which the following equations were derived (Figure 5):

Equation: Y = 0.0002+0.0176

R^2: 0.9981

The IPA extract from the stem exhibited the highest total protein content, measuring 173.1 ± 0.70 mg BSAE/g of dry-weight plant extract. This was followed by the aqueous stem extract, which contained 137 ± 0.67 mg BSAE/g of dry-weight plant extract. The HYA extract demonstrated a total protein content of approximately 108.4 ± 0.43 mg BSAE/g of dry-weight plant extract. Conversely, the petroleum ether extract exhibited the lowest total protein content, recorded at 80 ± 0.92 mg BSAE/g of dry-weight plant extract.

Total phenolic content

The total phenolic content in four stem extracts of *P. pudica* was assessed using the Folin-Ciocalteau method, with gallic acid serving as the standard. A calibration curve was established by plotting absorbance values from various

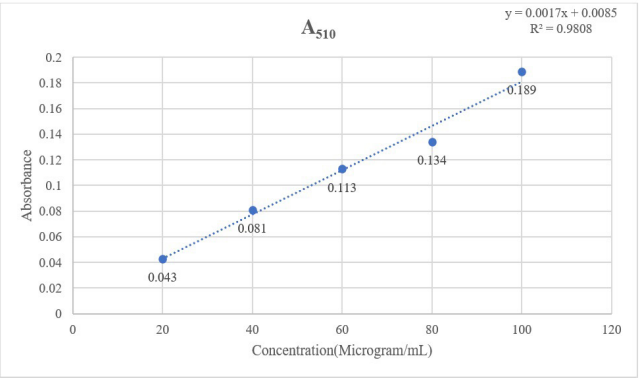

Figure 3: The standard curve for Quercetin for estimation of total Flavonoid content of *P. pudica* stem.

Figure 4: The standard curve for Glucose for estimation of total Carbohydrate content of *P. pudica* stem.

concentrations of gallic acid. The total phenolic content of the extracts was calculated using the regression equation derived from the calibration curve, resulting in the following equation (Figure 6):

Y=0.0106+0.0779

R^2 =0.9981

The highest total phenolic content was found in the HYA extract of the stem, measuring 105±0.54 mg GAE/g of dry-weight plant extract. This was followed by the IPA stem extract, which had a total phenolic content of 95±0.93 mg GAE/g of dry-weight plant extract, and the aqueous extract, with 90±0.65 mg GAE/g. In contrast, the lowest total phenolic content was observed in the petroleum ether extract of the stem, which amounted to approximately 65±0.71 mg GAE/g of dry-weight plant extract. Polyphenolic compounds are more soluble in polar solvents than in nonpolar ones. As a result, methanol extracts contain a higher polyphenolic content compared to other solvents. The high temperature of the solvent in the SEM enhances the release of secondary metabolites, particularly bound phenolics, lignin, and flavonoids, from the plant material.

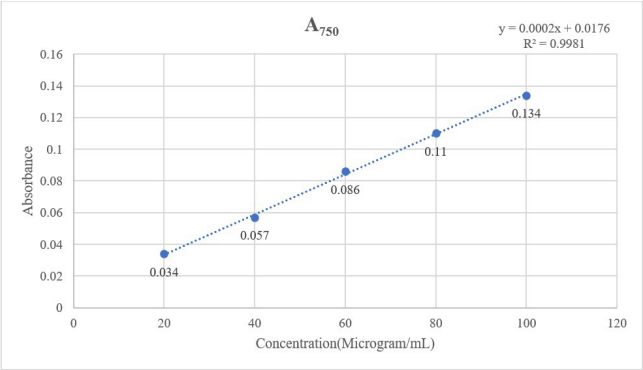

Figure 5: The standard curve for BSA for estimation of total Protein content of *P. pudica* stem.

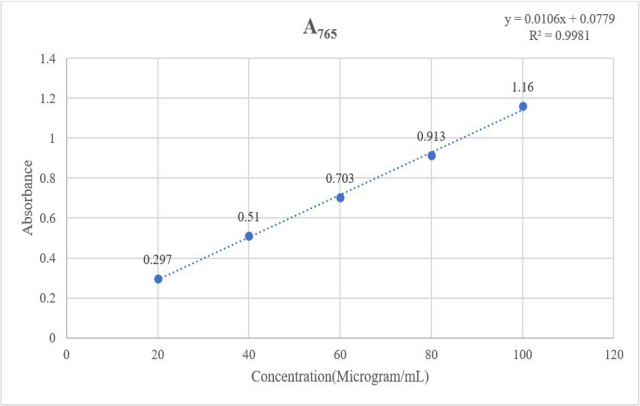

Figure 6: The standard curve for Gallic acid for estimation of total Phenolic content of *P. pudica* stem.

Total saponin content

The total saponin content in four extracts of the stem of *P. pudica* was determined by the Vanillin sulphuric acid method using diosgenin as standard. The absorbance values obtained at various concentrations of diosgenin were utilized to create a calibration curve. The total saponin content of the extracts was calculated from the regression equation of the calibration curve (Fig. 7) from which the following equations were derived:

Y = 0.0013+0.0083

R^2 = 0.9988

The highest Total saponin content was observed in the hydro alcohol extract of the stem which was 233.4±0.45 mg DIE/g dry-weight of plant extract. This was followed by Aqueous stem extract with 138±0.71 mg DIE/g dry-weight plant extract and Petroleum ether stem extract with around 77±0.67 mg DIE/g dry-weight plant extract. In the IPA extract of the stem, no saponin content was observed in the preliminary phytochemical analysis so the total saponin content measure was refrained in the IPA extract of the stem.

LC QTOF MS/MS analysis

The analysis of the IPA stem extract using LC QTOF MS/MS revealed the presence of several compounds, which are detailed in the accompanying table. Notably, many of these compounds have pharmacological applications, including Thiolutin, Amsacrine, 2-O-α-D-Galactopyranuronosyl-L-rhamnose, (S)-Piperazine-2-carboxamide, Flunitrazepam, Ziprasidone, 6-Deoxyfagomine, Eremopetasitenin A2, Ilicifolinoside A, and Isocarbamide. Additionally, various glycosides and intermediates associated with numerous catabolic activities were identified. The analysis of the HYA stem extract similarly indicated the presence of several pharmacologically significant compounds, such as phosphonoacetate, bromobenzene, 2-O-α-D-Galactopyranuronosyl-L-rhamnose, (S)-Piperazine-2-carboxamide, 6-Deoxyfagomine, Todatriol glucoside, Secogalioside, Leonuriside A, and Acubin. Furthermore, various alkaloids and glycosides were detected. The HYA stem

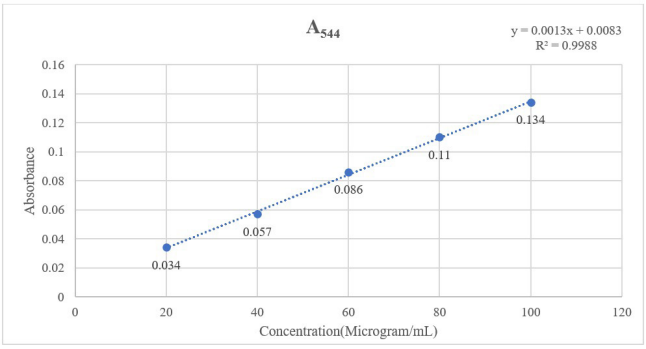

Figure 7: The standard curve for Diosgenin for estimation of total Saponin content of *P. pudica* stem.

extract contained multiple compounds with demonstrated antibiotic, anti-inflammatory, and antioxidant activities, as outlined in the table. The petroleum ether extract of the stem also revealed numerous pharmacologically relevant compounds, including quinoline, histamine, schizandrin C, 6-deoxohomodolichosterone, bruceine D, agnuside, metolozone, coniine, acubin, and isocarbamide. Many of the compounds identified in the petroleum ether extract suggest the presence of active intermediates relevant to various chemical syntheses and compound activities. Additionally, the LC-QTOF MS/MS analysis of the aqueous stem extract showed the presence of important pharmacological agents such as 1-(methylthio)-1-hexanediol, IBMX, norvaline, oxindole, and 4-quinolinol. Several primary metabolites were identified as well. Overall, the LC-QTOF MS/MS analysis of the stem extract underscores the significance of the plant as a valuable source of medicinal and pharmacologically important compounds (Table 4,5,6,7). Also, the chromatograms of the four samples are provided for ease of understanding (Fig 8,9,10,11).

Antioxidant assays

Reducing power assay

The potential of antioxidants is intricately associated with their reducing power, which serves as a crucial indicator of this capacity. Compounds exhibiting strong reducing power function as electron donors, effectively combating oxidized intermediates during lipid peroxidation processes. In the assessment, the transition of color from dark green to various shades of blue reflects the unique reducing power of each compound tested. Reducing agents facilitate the conversion of the Fe3+/ferricyanide complex to its ferrous form, with the absorbance measured at 700 nm serving as an indication of the ferrous ion concentration. An increase

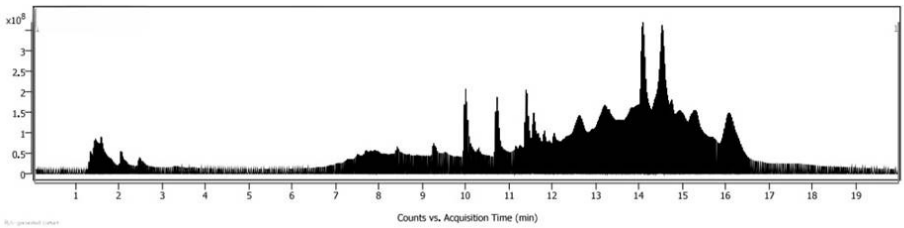

Figure 8: Sample chromatogram for IPA stem extract

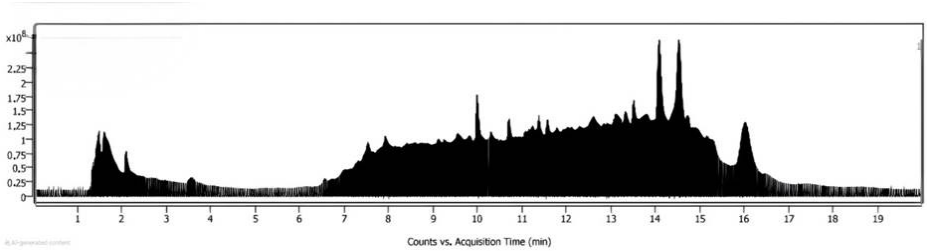

Figure 9: Sample chromatogram of stem HYA extract

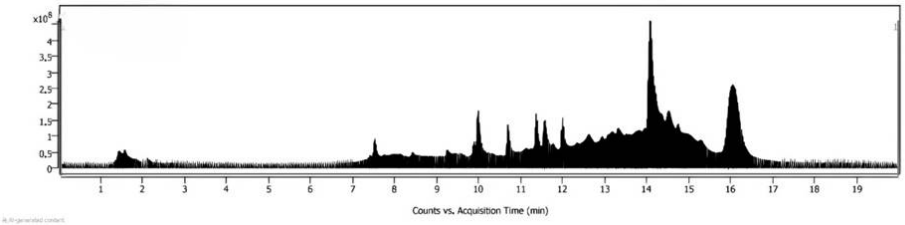

Figure 10: Sample chromatogram of stem Petroleum ether extract

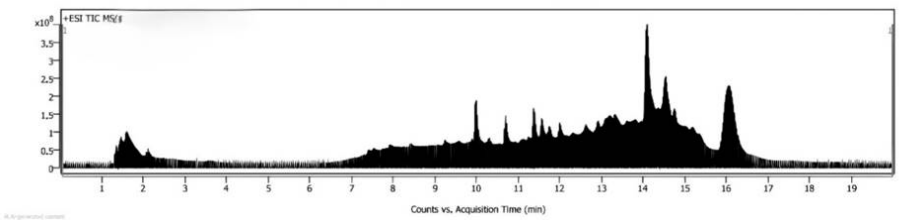

Figure 11: Sample chromatogram of stem Aqueous extract.

Table 4: LC QTOF MS/MS analysis of Stem IPA extract

Series	*Mass*	*RT*	*Base Peak*	*Molecular weight (g/mol)*	*Formula*	*Name*
1.	243.9099	0.437	180.9016	211.46	$C_6H_3Cl_3O_4$	2,3,5-Trichloro-cis, cis-muconate
2.	123.0085	0.48	64.9776	121.54	$C_3H_6ClNO_2$	3-Chloro-L-alanine
3.	149.9514	1.373	58.9604	169.64	C_2H_6Te	Dimethyl telluride
4.	248.1269	1.395	104.1072	194.22	$C_{11}H_{20}O_6$	(E)-2-Methyl-2-buten-1-ol O-beta-D-Glucopyranoside
5.	358.0924	1.49	82.9409	346.27	$C_{14}H_{19}N_2O_7P$	N1-(5-Phospho-a-D-ribosyl)-5,6-dimethyl benzimidazole
6.	393.1156	1.492	217.0723	321.37	$C_{21}H_{19}N_3O_3S$	Amsacrine
7.	86.0734	1.501	58.071	86.13	$C_5H_{10}O$	2-Methylbutanal
8.	331.0816	1.511	112.895	331.32	$C_{17}H_{15}O_7$	Malvidin
9.	210.0784	1.515	56.9677	196.22	$C_{13}H_{10}N_2O$	2-Aminoacridone
10.	180.0638	1.522	70.0665	180.16	$CH_{12}O_6$	L-Sorbose
11.	342.1165	1.534	203.0621	324.19	$C_{12}H_{22}O_{11}$	2-O-a-D-Galactopyranuronosyl-L-rhamnose
12.	312.1055	1.534	98.9801	308.27	$C_{11}H_{20}O_{10}$	6-O-(beta-D-Xylopyranosyl)-beta-D-glucopyranose
13.	194.0798	1.577	123.9478	194.34	$C_8H_{18}OS_2$	3-[(2-Mercapto-1-methyl propyl)thio]-2-butanol
14.	118.0868	1.646	58.07	117.14	$C_5H_{12}NO_2$	Trimethylammonioacetate
15.	101.1204	1.666	58.0699	101.19	$C_6H_{15}N$	Triethylamine
16.	78.014	1.667	78.9668	78.13	C_2H_6OS	Mercaptoethanol
17.	265.1587	1.688	104.1125	301.81	$C_{17}H_{19}N_3$	Acridine orange
18.	198.0166	1.713	127.9749	194.13	$C_8H_6O_6$	4,5-Dihydroxyphthalate
19.	228.0038	1.806	56.9666	172.23	$C_8H_8N_2OS_2$	Thiolutin
20.	129.9587	1.828	74.9328	129.01	$C_2H_4Cl_2O_2$	2,2-Dichloro-1,1-ethanediol
21.	156.0071	1.829	70.9608	154.24	$C_7H_8S_2$	Methyl phenyl disulfide
22.	139.9875	1.855	84.9606	138.04	$C_2H_5O_5P$	Phosphonoacetate
23.	248.1278	1.948	104.1078	240.26	$C_{12}H_{16}N_4O_2$	Histidyl proline diketopiperazine
24.	342.1175	1.956	203.0544	324.29	$C_{12}H_{22}O_{11}$	2-O-a-D-Galactopyranuronosyl-L-rhamnose
25.	200.9776	1.983	122.9867	139.16	$C_3H_7NO_5S_2$	L-Cysteine sulfonic acid
26.	129.0902	2.009	70.0658	129.14	$C_5H_{11}N_3O$	(S)-Piperazine-2-carboxamide
27.	118.0882	2.051	58.066	117.14	$C_5H_{12}NO_2$	Trimethylammonioacetate
28.	212.0482	2.079	51.4825	212.2	$C_{13}H_8O_3$	Urolithin B
29.	101.1204	2.092	58.0723	101.19	$C_6H_{15}N$	Triethylamine
30.	313.0867	2.1	136.0704	313.27	$C_{16}H_{12}FN_3O_3$	Flunitrazepam
31.	228.1476	2.112	70.0713	214.25	$C_{11}H_{20}N_2O_3$	L-isoleucyl-L-proline
32.	274.1053	2.186	85.0487	194.16	$C_{12}H_{18}O_7$	Dikegulac
33.	168.1154	2.204	60.0801	154.2	$C_{10}H_{16}O_2$	(3S)-3-Hydroxycyclocitral
34.	412.1121	2.207	233.0442	412.94	$C_{21}H_{21}ClN_4OS$	Ziprasidone
35.	131.095	2.213	370.8147	147.16	$C_6H_{13}NO_2$	6-Deoxyfagomine
36.	384.1637	2.231	275.1056	314.3	$C_{15}H_{28}O_{11}$	2,3-Butanediol apiosylglucoside
37.	396.1594	2.284	419.1408	346.4	$C_{20}H_{28}O_6S$	Eremopetasitenin A2
38.	275.1151	2.316	81.9508	263.32	$C_{15}H_{17}NO_4$	5-Amino-6-(4-hydroxy-2-butenyl)-2,2-dimethyl-4-chromanone
39.	222.1115	2.323	88.9527	222.25	$C_{10}H_{14}N_4O_2$	IBMX
40.	229.952	2.343	74.9303	202.09	$C_7H_7BrN_2S$	4-Bromophenylthiourea
41.	314.0995	2.353	86.0611	330.31	$C_{14}H_{18}O_8$	Glucovanillin
42.	420.1268	2.41	443.1162	482.5	$C_{17}H_{24}O_{12}$	Secogalioside
43.	462.173	2.447	485.1599	624.59	$C_{20}H_{30}O_{12}$	Verbasoside
44.	86.0733	2.471	58.0649	86.13	$C_5H_{10}O$	2-Methylbutanal
45.	214.0635	2.475	117.0574	240.28	$C_{13}H_{10}O_3$	4,5′-Dimethylangelicin
46.	410.1782	2.526	433.1644	346.4	$C_{21}H_{30}O_6S$	Eremopetasitenin C3
47.	229.0735	2.532	186.0515	285.33	$C_{13}H_{11}NO_3$	gamma-Fagarine
48.	264.121	2.538	193.9525	546.5	$C_{11}H_{20}O_7$	Ilicifolinoside A
49.	464.1667	2.6	320.1506	446.46	$C_{23}H_{28}O_{10}$	1-(2-Hydroxy-3,4,5,6-tetra methoxyphenyl)-3-(2,3,4,6-tetra methoxyphenyl)-2-propane-1-one
50.	185.1173	2.676	70.065	175.18	$C_8H_{15}N_3O_2$	Isocarbamid

Table 5: LC QTOF MS/MS analysis of Stem HYA extract

Series	*Mass/Charge*	*Rt*	*Base Peak*	*Molecular weight*	*Chemical structure*	*Proposed compound*
1.	123.0084	0.617	64.9774	121.54	$C_3H_6ClNO_2$	3-Chloro-L-alanine
2.	149.9507	1.353	84.9585	169.64	C_2H_6Te	Dimethyl telluride
3.	319.9456	1.374	64.9713	280.98	$C_{12}H_8C_{l4}N_2$	2,2′,5,5′-Tetrachlorobenzidine
4.	174.1117	1.379	70.064	174.2	$C_6H_{14}N_4O_2$	Amino acid (Arg-)
5.	248.1263	1.399	104.1067	194.22	$C_{11}H_{20}O_6$	(E)-2-Methyl-2-buten-1-ol O-beta-D-Glucopyranoside
6.	225.9441	1.423	1521.0532	225.00	$C_6H_4C_{l2}O_5$	2,5-Dichloro-4-oxohex-2-enedioate
7.	342.117	1.455	203.0532	342.3	$C_{12}H_{22}O_{11}$	Sucrose
8.	358.0905	1.478	80.9464	354.29	$C_{15}H_{18}O_{10}$	Dihydrocaffeic acid 3-O-glucuronide
9.	194.0796	1.479	74.0221	194.19	$C_7H_{14}O_6$	3-O-Methyl-myo-inositol
10.	180.0634	1.498	71.0618	180.16	$C_6H_{12}O_6$	L-Sorbose
11.	182.0783	1.5	61.0285	182.17	$C_6H_{14}O_6$	Dulcitol
12.	115.0643	1.523	70.0661	115.13	$C_5H_9NO_2$	L-Proline
13.	250.2047	1.525	144.1029	288.42	$C_{15}H_{26}N_2O$	Retamine
14.	246.174	1.544	118.0897	285.4	$C_{15}H_{22}N_2O$	Multiflorine
15.	101.1205	1.56	58.0664	101.19	$C_6H_{15}N$	Triethylamine
16.	118.0874	1.569	58.0654	117.14	$C_5H_{12}NO_2$	Trimethylammonioacetate
17.	198.0164	1.614	127.9677	194.13	$C_8H_6O_6$	4,5-Dihydroxyphthalate
18.	165.0782	1.72	85.9813	153.16	$C_9H_{11}NO_2$	5-(3-Pyridyl)-2-hydroxytetrahydrofuran
19.	200.9764	1.775	122.9841	139.16	$C_3H_7NO_5S_2$	L-Cysteine sulfonic acid
20.	139.9874	1.875	62.982	138.04	$C_2H_5O_5P$	Phosphonoacetate
21.	86.0731	1.945	58.0648	86.14	$C_5H_{10}O$	2-Methylbutanal
22.	155.9579	1.969	79.0214	157.01	C_6H_5Br	Bromobenzene
23.	342.1173	1.991	64.9787	324.29	$C_{12}H_{22}O_{11}$	2-O-a-D-Galactopyranuronosyl-L-rhamnose
24.	129.09	2.097	70.0649	129.14	$C_5H_{11}N_3O$	(S)-Piperazine-2-carboxamide
25.	229.9516	2.141	74.9312	202.09	$C_7H_7BrN_2S$	4-Bromophenylthiourea
26.	198.0161	2.163	127.9685	194.13	$C_8H_6O_6$	4,5-Dihydroxyphthalate
27.	131.0948	2.19	55.0541	147.16	$C_6H_{13}NO_2$	6-Deoxyfagomine
28.	252.1208	2.211	84.9626	224.24	$C_{10}H_{20}O_7$	2,3-Butanediol glucoside
29.	222.1101	2.217	84.9599	208.22	$C_9H_{18}O_6$	Isopropyl beta-D-glucoside
30.	390.153	2.277	164.9105	548.6	$C_{17}H_{26}O_{10}$	Todatriol glucoside
31.	307.0562	2.325	84.9604	307.2	$C_9H_{14}N_3O_7P$	dCMP / 2′-Deoxycytidine 5′-monophosphate
32.	420.1276	2.47	317.0769	482.5	$C_{17}H_{24}O_{12}$	Secogalioside
33.	332.1108	2.475	192.0376	432.45	$C_{14}H_{20}O_9$	Leonuriside A
34.	316.1156	2.517	56.9657	300.27	$C_{14}H_{20}O_8$	Hydroxytyrosol 1-O-glucoside
35.	165.0792	2.637	103.0545	165.19	$C_9H_{11}NO_2$	Methyl N-methyl anthranilate
36.	330.0941	2.663	57.0702	302.26	$C_{14}H_{18}O_9$	3′-Glucosyl-2′,4′,6′-trihydroxy acetophenone
37.	348.0841	2.701	98.9803	340.3	$C_{17}H_{16}O_8$	alpha-(1,2-Dihydroxyethyl)-1,2,3,4-tetrahydro-7-hydroxy-9-methoxy-3,4-dioxocyclopenta[c][1]benzopyran-6-acetaldehyde
38.	346.1264	2.737	194.8724	346.33	$C_{15}H_{22}O_9$	Aucubin
39.	338.0613	2.748	153.9968	338.23	$C_9H_{15}N_4O_8$	5-Aminoimidazole-4-carboxamide-1-β-D-ribofuranosyl 5′-monophosphate
40.	367.0563	2.756	177.9773	400.36	$C_{12}H_{17}NO_{10}S$	Tetraphyllin B sulfate
41.	186.0867	2.86	174.881	174.16	$C_5H_{10}N_6O_2$	Dinitrosopentamethylenetetramine
42.	127.136	2.871	55.0548	127.21	$C_8H_{17}N$	Coniine
43.	360.1052	2.996	80.9458	376.37	$C_{15}H_{20}O_{10}$	6′-Methoxypolygoacetophenoside
44.	134.0939	3.037	74.9312	-	$C_6H_{14}O_3$	Polypropylene glycol (m w 1,200-3,000)
45.	242.0784	3.081	102.0912	186.17	$C_{11}H_{14}O_6$	Genipinic acid
46.	484.3542	3.174	102.0916	342.5	$C_{31}H_{48}O_4$	26-Methyl nigranoate
47.	440.3883	3.221	81.0696	-	$C_{27}H_{52}O_4$	MG (0:0/24:1(15Z)/0:0)
48.	460.1575	3.263	483.1512	422.46	$C_{20}H_{28}O_{12}$	Paeonolide
49.	478.1674	3.329	188.9196	344.35	$C_{20}H_{30}O_{13}$	Gentiobiosyl 2-methyl-6-oxo-2E,4E-heptadienoate
50.	432.1418	3.406	122.0684	286.27	$C_{22}H_{24}O_9$	(-)-Medicocarpin

Table 6: LC-QTOF MS/MS analysis of stem Petroleum ether extract

Series	*Mass-to-charge ratio*	*Retention time*	*Base peak*	*Molecular weight*	*Chemical structure*	*Compound*
1.	129.0581	0.45	74.9316	129.16	C_9H_7N	Quinoline
2.	120.0437	0.49	64.9777	120.11	$C_5H_4N_4$	Purine
3.	225.9445	0.516	84.9597	225.01	$C_6H_4Cl_2O_5$	2,5-Dichloro-4-oxohex-2-enedioate
4.	151.0411	1.014	58.0654	179.21	$C_3H_9N_3O_2S$	Hypotaurocyamine
5.	120.0436	1.027	64.9781	120.11	$C_5H_4N_4$	Purine
6.	149.951	1.355	84.9599	169.94	C_2H_6Te	Dimethyl telluride
7.	248.1269	1.4	104.1076	194.22	$C_{11}H_{20}O_6$	(E)-2-Methyl-2-buten-1-ol O-beta-D-Glucopyranoside
8.	83.9618	1.436	59.0687	83.45	$ClHO_3$	Chlorate
9.	194.079	1.483	85.0834	194.19	$C_7H_{14}O_6$	3-O-Methyl-myo-inositol
10.	358.089	1.485	149.9866	354.29	$C_{15}H_{18}O_{10}$	Dihydrocaffeic acid 3-O-glucuronide
11.	86.0736	1.492	58.0697	86.13	$C_5H_{10}O$	2-Methylbutanal
12.	504.1695	1.507	365.11	504.44	$C_{18}H_{32}O_{16}$	Umbelliferose
13.	135.0546	1.514	119.0409	135.13	$C_5H_5N_5$	Adenine
14.	347.0723	1.529	203.0648	282.19	$C_{18}H_{15}ClFNO_3$	Flumipropyn
15.	254.1	1.535	81.9965	244.23	$C_9H_{18}O_8$	2-(beta-D-Glucosyl)-sn-glycerol
16.	162.0529	1.539	56.9715	164.14	$C_6H_{10}O_5$	L-Fucono-1,5-lactone
17.	180.0636	1.57	68.9651	180.16	$C_6H_{12}O_6$	L-Sorbose
18.	101.1206	1.65	58.0676	101.19	$C_6H_{15}N$	Triethylamine
19.	198.0167	1.709	127.9744	194.13	$C_8H_6O_6$	4,5-Dihydroxyphthalate
20.	202.0247	1.767	103.0079	186.12	$C_4H_{11}O_7P$	D-Erythritol 4-phosphate
21.	342.1159	1.838	70.0638	342.30	$C_{12}H_{22}O_{11}$	Cellobiose
22.	196.0239	1.853	61.0108	194.14	$C_6H_4N_4O_4$	Xanthine-8-carboxylate
23.	180.0632	2	59.0484	180.16	$C_6H_{12}O_6$	b-D-Galactopyranose
24.	111.08	2.104	51.027	111.15	$C_5H_9N_3$	Histamine
25.	131.0949	2.23	55.0499	147.16	$C_6H_{13}NO_2$	6-Deoxyfagomine
26.	237.9363	2.243	51.474	296.12	$C_8H_5Cl_3O_2$	Chlorfenac
27.	384.1579	2.262	407.1464	416.44	$C_{22}H_{24}O_6$	Schizandrin C
28.	198.0167	2.267	127.961	194.13	$C_8H_6O_6$	4,5-Dihydroxyphthalate
29.	229.9519	2.267	74.9273	216.06	$C_7H_7BrN_2S$	4-Bromophenylthiourea
30.	222.1104	2.34	84.9529	194.22	$C_9H_{18}O_6$	Isopropyl beta-D-glucoside
31.	164.0698	2.451	74.9265	178.35	$C_7H_{16}S_2$	Butyl isopropyl disulfide
32.	412.1344	2.458	205.0342	375.33	$C_{15}H_{20}N_6O_8$	N6-Carbamoyl-L-threonyladenosine
33.	146.0845	2.518	77.0352	145.18	$C_9H_{10}N_2$	Myosmine
34.	260.0896	2.576	233.1354	242.24	$C_{11}H_{16}O_7$	3-Furanmethanol glucoside
35.	307.9618	2.612	74.9298	308.60	$C_8H_{11}Cl_3O_6$	Chloralose
36.	155.957	2.653	56.9406	157.01	C_6H_5Br	Bromobenzene
37.	165.0786	2.661	103.0543	165.19	$C_9H_{11}NO_2$	Methyl N-methyl anthranilate
38.	185.1173	2.688	70.0648	179.22	$C_8H_{15}N_3O_2$	Isocarbamid
39.	346.1274	2.699	206.0529	344.36	$C_{15}H_{22}O_9$	Aucubin
40.	229.9518	2.721	74.9307	216.06	$C_7H_7BrN_2S$	4-Bromophenylthiourea
41.	127.1364	2.731	55.0542	127.21	$C_8H_{17}N$	Coniine
42.	365.06	3.026	86.9051	365.41	$C_{16}H_{16}ClN_3O_3S$	Metolazone
43.	462.3697	3.224	102.0912	402	$C_{29}H_{50}O_4$	6-Deoxohomodolichosterone
44.	466.1462	3.234	305.099	492.46	$C_{22}H_{26}O_{11}$	Agnuside
45.	134.0947	3.243	74.9314	-	$C_6H_{14}O_3$	Polypropylene glycol (m w 1,200-3,000)
46.	410.1561	3.374	80.9449	519.50	$C_{20}H_{26}O_9$	Bruceine D
47.	222.1101	3.433	196.9102	-	$C_9H_{18}O_6$	Isopropyl beta-D-glucoside
48.	121.0887	3.546	77.0378	121.18	$C_8H_{11}N$	Phenylethylamine
49.	208.0746	3.619	128.0636	208.22	$C_{11}H_{12}O_4$	Benzyl succinate
50.	192.1011	3.725	96.9281	194.39	$C_9H_{20}S_2$	1,9-Nonanedithiol

Table 7: LC-QTOF MS/MS analysis of stem Aqueous extract

Series	*Mass-to-charge ratio*	*Retention time*	*Base peak*	*Molecular weight*	*Chemical structure*	*Compound*
1.	117.0785	0.092	493.4851	117.15	$C_5H_{11}NO_2$	Valine
2.	120.0438	0.133	67.0278	120.11	$C_5H_4N_4$	Purine
3.	155.0345	0.193	83.0568	155.10	$C_3H_{10}NO_4P$	D-1-Aminopropan-2-ol O-phosphate
4.	118.0872	0.464	76.9276	117.15	$C_5H_{12}NO_2$	Trimethylammonioacetate
5.	238.143	0.478	111.8891	238.29	$C_{11}H_{18}N_4O_2$	Pirimicarb
6.	177.1153	0.643	91.0558	-	$C_{11}H_{15}NO$	Valerianine
7.	248.1275	1.426	104.1071	252.28	$C_{12}H_{16}N_4O_2$	Histidyl proline diketopiperazine
8.	219.9375	1.448	962.4854	220.00	C_6H_5IO	2-Iodophenol
9.	342.1224	1.53	80.9513	-	$C_{18}H_{18}N_2O_5$	Dictyoquinazol C
10.	194.0789	1.56	123.9465	194.19	$C_7H_{14}O_6$	3-O-Methyl-myo-inositol
11.	202.0238	1.656	103.0087	200.12	$C_4H_{11}O_7P$	D-Erythritol 4-phosphate
12.	238.1423	1.851	154.8993	238.29	$C_{11}H_{18}N_4O_2$	Pirimicarb
13.	200.9773	1.916	61.0112	153.17	$C_3H_7NO_5S_2$	L-Cysteinesulfonic acid
14.	164.0689	2.289	108.955	150.29	$C_7H_{16}S_2$	1-(Methylthio)-1-hexanethiol
15.	133.0523	2.364	74.93	133.15	C_8H_7NO	Oxindole
16.	111.9487	2.453	71.9294	112.95	$C_2H_2Cl_2O$	Chloroacetyl chloride
17.	214.0624	2.54	143.0717	244.28	$C_{13}H_{10}O_3$	4,5′-Dimethylangelicin
18.	301.1026	2.662	144.9297	301.30	$C_{16}H_{13}F_2N_3O$	Flutriafol
19.	237.0642	2.731	116.0484	221.21	$C_{11}H_{11}NO_5$	N-Benzoyl aspartic acid
20.	208.0946	2.739	84.962	194.19	$C_8H_{16}O_6$	Ethyl beta-D-glucopyranoside
21.	294.1061	2.772	64.9786	215.19	$C_{10}H_{18}N_2O_8$	N-Glycosyl-L-asparagine
22.	252.0592	2.777	165.0563	257.38	$C_8H_{16}N_2O_3S_2$	S-(4-Methylthiobutylthiohydroximoyl)-L-cysteine
23.	127.1361	2.822	55.0549	127.22	$C_8H_{17}N$	Coniine
24.	246.0516	2.858	58.96	747.93	$C_6H_{15}O_8P$	Phosphatidyl glycerol
25.	117.0793	2.862	52.9996	11.715	$C_5H_{11}NO_2$	Valine
26.	99.0683	2.921	58.9569	99.13	C_5H_9NO	(2R)-2-Hydroxy-2-methyl-butane nitrile
27.	120.0436	2.928	67.0297	120.11	$C_5H_4N_4$	Purine
28.	222.1112	2.996	64.9754	222.24	$C_{10}H_{14}N_4O_2$	IBMX
29.	213.0789	3.051	154.0661	213.22	$C_{13}H_{11}NO_2$	Salicylanilide
30.	462.3697	3.138	102.0915	396.57	$C_{29}H_{50}O_4$	6-Deoxohomodolichosterone
31.	440.3884	3.144	81.0686	603.92	$C_{27}H_{52}O_4$	MG (0:0/24:1(15Z)/0:0)
32.	406.0906	3.273	142.9396	274.31	$C_{19}H_{18}O_{10}$	Lancerin
33.	204.1368	3.402	180.9052	190.23	$C_{10}H_{20}O_4$	(2xi,6xi)-7-Methyl-3-methylene-1,2,6,7-octane petrol
34.	155.0352	3.447	67.0154	141.06	$C_3H_{10}NO_4P$	N-Methyl ethanolamine phosphate
35.	178.0838	3.528	74.9305	164.16	$C_7H_{14}O_5$	D-Thevetose
36.	418.3454	3.587	102.0929	402.65	$C_{27}H_{46}O_3$	7alpha,25-dihydroxy cholesterol
37.	252.1212	3.68	76.9285	194.19	$C_{10}H_{20}O_7$	2,3-Butanediol glucoside
38.	274.1043	3.704	229.102	194.18	$C_{12}H_{18}O_7$	Dikegulac
39.	238.1423	3.721	216.092	238.29	$C_{11}H_{18}N_4O_2$	Pirimicarb
40.	280.0942	3.754	64.9756	280.45	$C_{14}H_{16}O_6$	Gravolenic acid
41.	226.1685	3.833	80.9645	226.31	$C_{12}H_{22}N_2O_2$	1,8-Diazacyclotetradecane-2,9-dione
42.	326.0748	3.855	62.9817	-	$C_{13}H_{14}N_2O_8$	Miraxanthin-II
43.	298.0973	3.938	229.0142	298.76	$C_{15}H_{19}CO_4$	8-Deoxy-11-hydroxy-13-chlorogrosheimin
44.	117.0789	3.98	76.928	117.15	$C_5H_{11}NO_2$	Norvaline
45.	133.0523	4.016	58.9575	133.15	C_8H_7NO	Oxindole
46.	342.0699	4.07	62.9813	342.84	$C_{17}H_{15}ClN_4S$	Etizolam
47.	111.9482	4.317	71.9282	112.95	$C_2H_2Cl_2O$	Chloroacetyl chloride
48.	160.11	4.364	52.9472	160.21	$C_8H_{16}O_3$	7-Hydroxyoctanoic acid
49.	320.0884	4.498	80.9492	-	$C_{16}H_{16}O_7$	DHK-OH
50.	145.05	4.53	58.065	145.16	C_9H_7NO	4-Quinolinol

in absorbance within the reaction mixture signifies a greater reducing power of the extracts. As illustrated in Figure 12, at the highest concentration of 1000 µg/mL, ascorbic acid demonstrated an absorbance of 1.486, while the HYA extract exhibited the highest absorbance at 1.567, followed by the IPA extract at 1.35, the aqueous extract at 1.133, and the petroleum ether extract, which showed the lowest absorbance at 0.7. These findings suggest that the HYA and IPA extracts possess excellent antioxidant properties.

DPPH Assay

The DPPH stable free-radical technique provides a reliable and sensitive method for evaluating the antioxidant activity of specific compounds or plant extracts. This methodology is characterized by its rapid and straightforward nature, with its efficacy influenced by both the type of plant and the extraction procedure employed. The detection of DPPH radical scavenging efficiency is conducted at a wavelength of 517 nm. For DPPH to transition into a stable diamagnetic molecule, it must accept either an electron or a hydrogen radical. A notable reduction in the absorbance of DPPH radicals results in a color change from purple to yellow or greenish, which indicates the presence of antioxidants in the solution that mitigate the effects of free radicals [22]. The degree of inhibition serves as an indicator for assessing the antioxidant activity of the extract and its ability to neutralize free radicals. At the highest concentration of 100 µg/mL, the percentage of inhibition exhibited by standard ascorbic acid was 68.89%, while the percentage of inhibition measured for HYA extract was 70.82%, IPA extract at 64.45% aqueous extract at 50.81%, and petroleum ether extract at 34.67% (Fig. 13). The IC_{50} value is defined as the concentration of the sample required to inhibit 50% of free radicals, with a lower IC_{50} value indicating a higher antioxidant potential [23]. In the context of this study, the IC_{50} value for standard ascorbic acid was approximately 35.19 µg/mL, whereas the HYA extract exhibited the lowest IC_{50} value at 33.7 µg/mL (Table 8). A study conducted showed that the ethanolic extract of *Plumeria alba* exhibited an inhibition percentage of 51.50% and *Plumeria rubra* of 41.30%, both lower than the hydroalcoholic extract of *P. pudica* stem, which showed 70.82%. This demonstrates the potential of these plants.

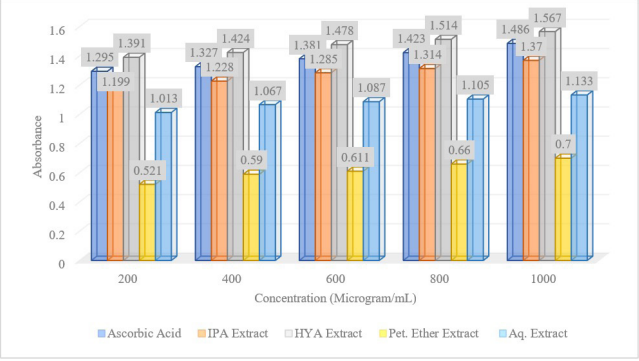

Figure 12: Comparison of the absorbance values at 700nm of the stem extracts for the reducing power assay.

Antibacterial assay

The results of the antimicrobial assay indicated that the stem extract of IPA exhibited the most significant antibacterial activity against all selected bacterial strains, with a common minimum inhibitory concentration (MIC) of 20 mg/mL required for effective inhibition (Fig. 14). The zones of inhibition (ZOI) varied, measuring 14 mm against *Pseudomonas aeruginosa*, 11 mm against *Escherichia coli*, 8 mm against *Staphylococcus aureus*, and 5 mm against *Salmonella enterica*. Additionally, the HYA stem extract demonstrated commendable activity, with MIC values of 25 mg/mL against *Staphylococcus aureus* and 30 mg/mL against other bacterial species (Fig. 15). The corresponding ZOIs were 13 mm for *Staphylococcus aureus*, 10 mm for *Pseudomonas aeruginosa*, and 7 mm as well as 8 mm for *Salmonella enterica* and *Escherichia coli*, respectively, at the 30 mg/mL MIC. Furthermore, the aqueous extract of the stem displayed notable antimicrobial effects, achieving an MIC of 25 mg/mL against both *Staphylococcus aureus* and *Pseudomonas aeruginosa*, and an MIC of 30 mg/mL against *Escherichia coli* and *Salmonella enterica* (Fig. 16). The most substantial zone of inhibition was recorded against *Salmonella enterica* at 21 mm, followed by 15 mm against *Staphylococcus aureus* and 14 mm against *Pseudomonas*

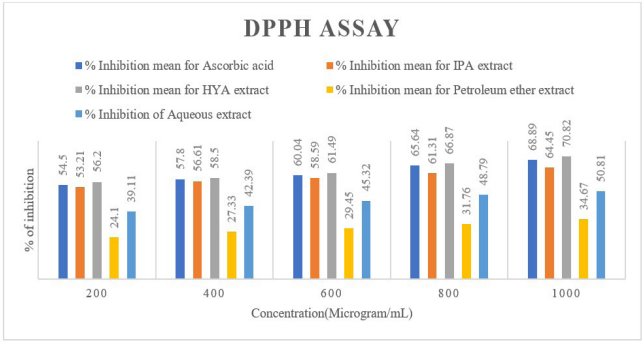

Figure 13: Comparison of % of inhibition of standard with the stem extracts of *P. pudica* (Concentration in µg/mL) for DPPH assay.

Table 8: IC_{50} values of DPPH Assay for various stem extracts of *P. pudica*

Sample	*IC_{50} (µg/mL)*
Ascorbic acid	35.1996
Isopropyl alcohol	36.91
Hydro-alcohol	33.7
Petroleum ether	176.47
Aqueous	99.05

aeruginosa. The lowest ZOI, measuring 12 mm, was observed for *Escherichia coli* when subjected to the aqueous stem extract. Conversely, the petroleum ether extract of the stem demonstrated the least antibacterial activity, with a MIC of 30 mg/mL, failing to exhibit any inhibition against *Salmonella enterica* even at a concentration of 50 mg/mL (Fig. 17). The most considerable ZOI recorded with this extract was 8 mm against *Staphylococcus aureus*, followed by a ZOI of 5 mm against *Escherichia coli* and a slightly larger inhibition of 10 mm against *Pseudomonas aeruginosa* (Table 9).

Correlations

The correlations among the results of the Total contents of alkaloid, flavonoid, protein, carbohydrates, saponin and phenol, DPPH, and Reducing Power assays for various extracts of *P. pudica* stem are illustrated in Fig. 18. The correlation coefficients (r) for the different content estimations and antioxidant studies ranged from 0.3 to 0.9975, indicating a strong correlation across these assays. Notably, the highest correlation was observed between the Total Phenolic Content and the reducing power assay (r = 0.9975), while the DPPH assay also showed a strong correlation with Total Phenolic Content (r = 0.97). A strong correlation coefficient was recorded between the Reducing Power assay and Total Flavonoid Content (r = 0.97) and DPPH assay and TFC(r=0.97). The significant correlation between Total Phenolic Content and Total Flavonoid Content across various stem extracts suggests that polyphenolic compounds and flavonoids constitute the primary phytochemicals responsible for the plant's overall antioxidant potential.

Table 9: The antibacterial activity of various stem extracts of *P. pudica*.

Extract	*Organism*	*MIC*	*ZOI*
Isopropyl alcohol stem extract	*E. coli*	20 mg/mL	11 mm
	S. aureus	20 mg/mL	8 mm
	S. enterica	20 mg/mL	5 mm
	P. aeruginosa	20 mg/mL	14 mm
Hydro-alcohol stem extract	*E. coli*	30 mg/mL	8 mm
	S. aureus	25 mg/mL	13 mm
	S. enterica	25 mg/mL	7 mm
	P. aeruginosa	25 mg/mL	10 mm
Petroleum ether stem extract	*E. coli*	30 mg/mL	5 mm
	S. aureus	30 mg/mL	8 mm
	S. enterica	50 mg/mL	-
	P. aeruginosa	30 mg/mL	12 mm
Aqueous stem extract	*E. coli*	30 mg/mL	13 mm
	S. aureus	25 mg/mL	15 mm
	S. enterica	30 mg/mL	21 mm
	P. aeruginosa	25 mg/mL	14 mm

Discussion

Solvent extraction is the most prevalent method employed to isolate antioxidant compounds from plant materials. The yield of extracts and the antioxidant activity of these plant materials is significantly influenced by the choice of solvent. This phenomenon arises from various antioxidant compounds' diverse chemical properties and polarities, which determine their solubility in specific solvents. Polar solvents are frequently utilized to extract polyphenols from

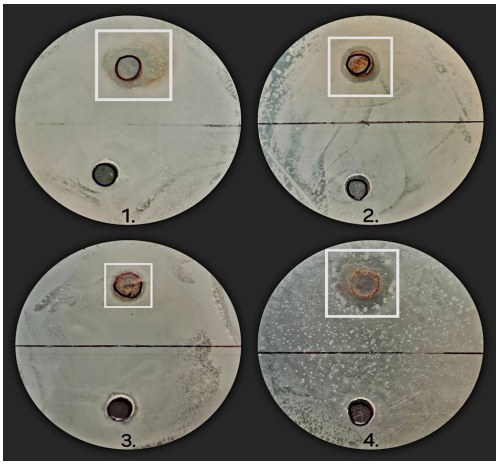

Figure 14: Zone of inhibition caused by IPA stem extract against *Escherichia coli (1), Staphylococcus aureus (2), Salmonella enterica (3), and Pseudomonas aeruginosa (4).*

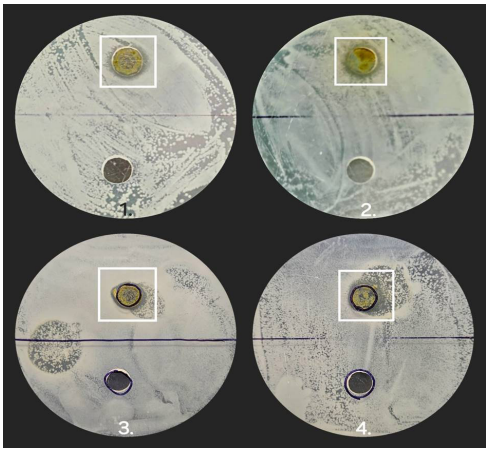

Figure 15: Zone of inhibition caused by HYA stem extract against *Escherichia coli (1), Staphylococcus aureus (2), Salmonella enterica (3), and Pseudomonas aeruginosa (4).*

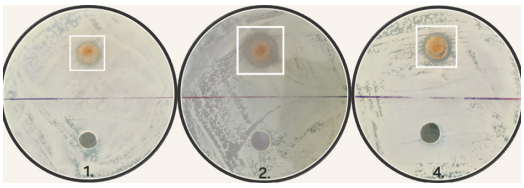

Figure 16: Zone of inhibition caused by Petroleum ether stem extract against *Escherichia coli (1), Staphylococcus aureus (2), and Pseudomonas aeruginosa (4).*

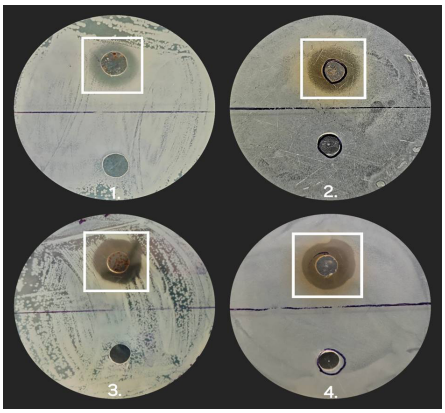

Figure 17: Zone of inhibition caused by Aqueous stem extract against *Escherichia coli (1), Staphylococcus aureus (2), Salmonella enterica (3), and Pseudomonas aeruginosa (4).*

plant matrices [24]. This study assesses the impact of solvent polarity on extraction efficiency, indicating that polar solvents such as HYA and IPA produced superior results, in contrast to non-polar solvents like petroleum ether, which resulted in the lowest yields. Notably, the HYA extract of *P. pudica* exhibited the highest total flavonoid content at 401.2 ± 0.68 mg QE/g dry weight of the plant extract. In contrast, the petroleum ether extract demonstrated the lowest total flavonoid content at 167.5 ± 0.34 mg QE/g dry weight of the plant extract. In comparison, the ethanolic extract of *P. alba* yielded 31.26 mg QE/g dry weight of the plant extract [25]. In contrast, the ethanolic extract of *P. rubra* yielded 117.83 mg QE/g dry weight of the plant extract [26], which aligns closely with the total flavonoid content of *P. pudica*. The greatest alkaloid content was observed in the IPA extract

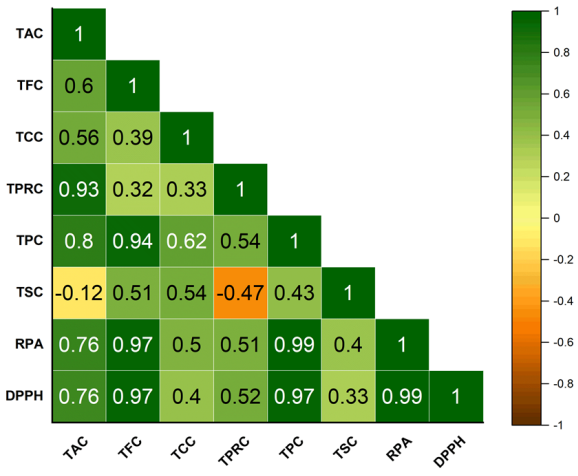

Figure 18: Correlation among various assays viz. TAC, TFC, TCC, TPRC, TPC, TSC, RPA and DPPH. (Here TAC=Total alkaloid content, TFC=Total flavonoid content, TFC= Total flavonoid content, TCC=Total carbohydrate content, TPRC= Total protein content, TPC= Total phenolic content, TSC=Total saponin content, RPA= Reducing power assay).

of the stem, with a value of 167 ± 0.34 mg CE/g dry weight of the plant extract, while the petroleum ether extract had the lowest total alkaloid content at 76.5 ± 0.52 mg CE/g dry weight of the plant extract. The highest protein content was recorded in the IPA extract, amounting to 173 ± 0.7 mg BSAE/g dry weight of the plant extract, in contrast to the petroleum ether extract, which had the lowest protein content at 80 ± 0.92 mg BSAE/g dry weight of the plant extract. Furthermore, the HYA extract displayed the highest total phenolic content at 105.4 ± 0.54 mg GAE/g dry weight of the plant extract, whereas the petroleum ether extract had a total phenolic content of 65 ± 0.71 mg GAE/g dry weight of the plant extract. Reportedly, *P. alba* contained 48.43 mg GAE/g dry weight of the plant extract, which is notably lower than that of *P. pudica*. The aqueous extract presented the highest carbohydrate content of 291.5 ± 0.19 mg GLUE/g dry weight of the plant extract, while the petroleum ether extract recorded the lowest carbohydrate content at 158 ± 07 mg GLUE/g dry weight of the plant extract. The hydroalcoholic extract exhibited the highest saponin content at 233.4 ± 0.45 mg DIE/g dry weight of the plant extract, whereas the petroleum ether extract yielded the lowest saponin content at 77 ± 0.67 mg DIE/g dry weight of the plant extract, with IPA extract lacking detectable saponins. These findings substantiate the hypothesis that solvent polarity has a profound effect on the extraction of phytoconstituents. The qualitative and quantitative analyses of the phytoconstituents thus corroborate the hypothesis concerning the choice between polar and non-polar solvents about extraction potential, which will ultimately influence subsequent biochemical studies.

Free radicals contribute to serious diseases by causing oxidative stress, leading to cell damage and conditions like diabetes, atherosclerosis, and cancer. Antioxidant compounds such as phenolic and flavonoid substances in plant extracts play a crucial role in mitigating these effects. Phytochemicals from fruits, vegetables, and herbs can significantly reduce the risk of chronic diseases. This has led to increased interest in discovering new natural antioxidants and therapeutic agents. Historically, ancient Egyptians used various plant parts for medicinal purposes in forms like pastes and ointments [27]. The Antioxidant potential was assessed using the DPPH assay and the reducing power assay. At 100 µg/mL, ascorbic acid exhibited 68.89% inhibition, while HYA extract showed the highest inhibition at 70 %. Comparable studies by [28], and [29] show varied inhibition percentages for other Plumeria species, indicating that *P. pudica* exhibits notable antioxidant potential. The IC_{50} value for ascorbic acid was 35.1996 µg/ml, with the HYA extract showing the lowest IC_{50} value (33.7 µg/mL), indicating superior antioxidant activity. [26] found higher IC_{50} values in *P. rubra* and *P. alba*, suggesting *P. pudica* has better antioxidant properties. The reducing power assay corroborated these findings, with the HYA extract showing the highest absorbance (1.57) at 1000 µg/mL, compared to

ascorbic acid (1.48) and petroleum ether extract (0.7). With these assays, we can conclude that there is a correlation between the presence of phenolics and flavonoids and their attribution to the antioxidant potential of the extract as the HYA stem extract had the highest TPC (105.4±0.54 mg GAE/g dry-weight of plant extract) and the highest TFC (401.2 QE/g of dry-weight plant extract), thus it showed best results in both reducing power assay and DPPH assay. The antibacterial assay showcased that the IPA and HYA stem extracts showed excellent antimicrobial activity at low MIC. Also, the Aqueous and Petroleum ether extracts showed competent activity against the selected bacterial strains as well. The LC-QTOF MS/MS analysis unveiled the presence of many pharmacologically important compounds to be present in the extracts. Various secondary metabolites, various antibiotics, and various intermediates of metabolic processes were detected proving the plants' potency for further clinical and pharma research. Further comprehensive studies are necessary to explore its anti-cancer, anti-inflammatory, and anti-nociceptive properties and its broader therapeutic potential.

Environmental considerations and commercial aspects

This plant can be propagated without seeds through grafting, and grafts are readily available at nurseries at an affordable price. The growth requirements for this plant are minimal; it needs adequate water and only a small amount of manure and pesticides. Once planted, the plant demonstrates substantial growth in a relatively short period, so it has been chosen for cultivation. Many citizens in Gandhinagar have planted this species for ornamental purposes. Studies like this highlight the importance of the plant, as its easy propagation and maintenance make it valuable in many respects. However, despite this plant's rich constituents and activities unveiled in this paper, its real potential for providing benefits to households is rarely realized. In other words, although this plant is recognized for its pharmacological, bio-medicinal, and therapeutic significance, many pharmaceutical and Ayurvedic medicine companies are still not fully utilizing it. The commercial scalability, research, and development of herbal products and various drugs, as well as the isolation of drug agents from plants, are progressing slowly. Therefore, the success of studies like this will only lead to real-world applications if the plants are used in a more resourceful and appropriate manner.

Conclusion

This comprehensive study highlights the significant pharmacological potential of *P. pudica* stem extracts, emphasizing their therapeutic value through both qualitative and quantitative analyses. The hydroalcoholic extract (HYA) proved to be the most effective solvent, demonstrating a superior phytochemical profile with high concentrations of phenols, flavonoids, and saponins. It also exhibited the strongest antioxidant activity, as shown by a DPPH scavenging activity of 70.82% inhibition and impressive reducing power. Furthermore, antimicrobial assays revealed significant activity of both HYA and isopropyl alcohol (IPA) extracts against bacterial strains, including *E. coli, Salmonella enterica, Staphylococcus aureus,* and *Pseudomonas aeruginosa*. These results support their potential therapeutic applications. The LC-QTOF MS/MS analysis uncovered a diverse range of bioactive compounds, such as alkaloids, glycosides, and flavonoids, which are known for their antioxidant, anti-inflammatory, and antimicrobial properties. These findings reinforce the idea that this plant can serve as a source of natural compounds, providing alternatives to synthetic drugs, particularly in light of the adverse effects associated with synthetic antioxidants and antibiotics. However, certain limitations must be addressed, including the need for in vivo studies to confirm the efficacy and safety of these bioactive compounds. Additionally, exploring the synergistic effects of these compounds could reveal broader therapeutic applications. Future research should prioritize isolating and characterizing individual compounds, conducting molecular docking studies, and performing clinical trials to validate the therapeutic potential of *P. pudica* compounds. This would pave the way for their potential use in the pharmaceutical, nutraceutical, and cosmeceutical industries. By connecting traditional knowledge with modern scientific methodologies, this research significantly contributes to the field of plant-based therapeutics, encouraging further exploration of this valuable medicinal plant.

Data availability

All the data that has been generated during this research are included in the manuscript.

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Research compliance statement

All field studies and experimental research presented in this article, including the collection of plant material and laboratory tests, adhere to ethical standards. These activities comply with the guidelines, legislation, and standards set by the institution (M.D. Science College), as well as national and international regulations.

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Conflict of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Authorship contribution statement

The conceptualization of the study was carried out by KS and BAJ. Data curation and the performance of experiments were handled solely by KS. Formal analysis and validation were conducted by BAJ, who also oversaw the project administration. Methodology development was a collaborative effort involving KS, BAJ, and KNO. The original draft of the manuscript was written by KS and KNO. All data were generated in-house, and the authors collectively agree to be accountable for all aspects of the work, ensuring its integrity and accuracy.

Declaration of generative AI in scientific writing

This manuscript has benefited from the use of generative AI tools, including ChatGPT by OpenAI, for language refinement and improvement. The authors confirm that all intellectual content, scientific accuracy, and interpretations presented in this manuscript are their own and have not been influenced by the AI tool.

References

Aiyengar OA and Okoh AI (2010). Preliminary phytochemical screening and in vitro antioxidant activities of the aqueous extract of *Helichrysum longifolium* DC. *BMC Complementary and Alternative Medicine* 10(1): 1–8. DOI: 10.1186/1472-6882-10-21.

Arya V, Thakur N and Kashyap CP (2012). Preliminary phytochemical analysis of the extracts of *Psidium* leaves. *Journal of Pharmacognosy and Phytochemistry* 1: 01–05.

Attasih M, Pambudi DB and Saad M (2024). Determination of total phenolic, flavonoid contents, and antioxidant activity evaluation of ethanolic extract from *Plumeria alba*. *Journal of Nutraceuticals and Herbal Medicine*: 14–27.

Chamakuri SR, Suttee A and Mondal P (2017). An eye-catching and comprehensive review of *Plumeria pudica* Jacq. (Bridal Bouquet). *Plant Archives* 20(2): 2076–2079.

Clinical and Laboratory Standards Institute (CLSI) (2016). Standards for antimicrobial susceptibility testing, CLSI supplement M100S. Wayne, PA: *Clinical and Laboratory Standards Institute*.

Hiai S, Oura H and Nakajima T (1976). Color reaction of some sapogenins and saponins with vanillin and sulfuric acid. *Planta Medica* 29(2): 116–122.

Hsu CL, Chen W, Weng YM and Tseng CY (2003). Chemical composition, physical properties, and antioxidant activities of yam flours as affected by different drying methods. *Food Chemistry* 83: 85–92. https://doi.org/10.1016/S0308-8146(03)00053-0

Jadid N, Hidayati D, Hartanti SR, Arraniry BA, Rachman RY and Wikanta W (2017). Antioxidant activities of different solvent extracts of *Piper retrofractum* Vahl. using DPPH assay. In: *AIP Conference Proceedings* 1854(1). AIP Publishing. https://doi.org/10.1063/1.4985410

Jadid N, Hidayati D, Hartanti SR, Arraniry BA, Rachman RY and Wikanta W (2017). Antioxidant activities of different solvent extracts of *Piper retrofractum* Vahl. using DPPH assay. In: *AIP Conference Proceedings* 1854(1). AIP Publishing. https://doi.org/10.1063/1.4985410

Karabegović IT, Stojičević SS, Veličković DT, Todorović ZB, Nikolić NČ and Lazić ML (2014). The effect of different extraction techniques on the composition and antioxidant activity of cherry laurel (*Prunus laurocerasus*) leaf and fruit extracts. *Industrial Crops and Products* 54: 142–148.

Kupe M, Karatas N, Unal MS, Ercisli S, Baron M and Sochor J (2021). Phenolic composition and antioxidant activity of peel, pulp, and seed extracts of different clones of the Turkish grape cultivar 'Karaerik'. *Plants* 10: 2154.

Mokrani A, Krisa S, Cluzet S, Da Costa G, Temsamani H, Renouf E and Richard T (2020). Phenolic contents and bioactive potential of peach fruit extracts. *Food Chemistry* 202: 212–220. https://doi.org/10.1016/j.foodchem.2015.12.026

Muhtadi M and Wiyono AAF (2021). Testing antioxidant activity of *Plumeria alba* and *Plumeria rubra* ethanolic extracts using DPPH and FRAP methods and determining their total flavonoid and phenolic levels. *Journal of Nutraceuticals and Herbal Medicine* 3: 38–50.

Nithianantham K, Shyamala M, Chen Y, Latha LY, Jothy SL and Sasidharan S (2011). Hepatoprotective potential of *Clitoria ternatea* leaf extract against paracetamol-induced damage in mice. *Molecules* 16(12): 10134–10145. https://doi.org/10.3390/molecules161210134

Osman MA, Mahmoud GI and Shoman SS (2020). Correlation between total phenols content, antioxidant power, and cytotoxicity. *Biointerface Research in Applied Chemistry* 11: 10640–10653.

Patel P, Patel N, Patel D, Desai S and Meshram D (2014). Phytochemical analysis and antifungal activity of *Moringa oleifera*. *International Journal of Pharmacy and Pharmaceutical Sciences* 6(5): 144–147.

Sa-Eed A, Donkor ES, Arhin RE, Tetteh-Quarcoo PB, Attah SK, Kabotso DE and Dayie NT (2023). In vitro antimicrobial activity of crude propolis extracts and fractions. *FEMS Microbes* 4: xtad010.

Salamah N and Ningsih DS (2017). Total alkaloid content in various fractions of *Tabernaemonata sphaerocarpa* Bl. (Jembirit) leaves. In: *IOP Conference Series: Materials Science and Engineering* 259(1). IOP Publishing.

Samanta A, Das G and Das SK (2011). Roles of flavonoids in plants. *Carbon* 100: 12–35.

Sannigrahi S, Mazumder UK, Pal DK, Parida S and Jain S (2010). Antioxidant potential of crude extract and different fractions of *Enhydra fluctuans* Lour.

Santana LDAB, Aragão DP, Araújo TDSL, de Sousa NA, de Souza LKM, Oliveira LES and de Oliveira JS (2018). Antidiarrheal effects of water-soluble proteins from *Plumeria pudica* latex in mice. *Biomedicine & Pharmacotherapy* 97: 1147–1154.

Shraim AM, Ahmed TA, Rahman MM and Hijji YM (2021). Determination of total flavonoid content by aluminum chloride assay: A critical evaluation. *LWT* 150: 111932.

Singleton VL and Lamuela-Raventos R (1999). Analysis of total phenols and other oxidation substrates and antioxidants through a Folin-Ciocalteu reagent. *Methods in Enzymology* 299: 152.

Stankovic MS (2011). Total phenolic content, flavonoid

concentration, and antioxidant activity of *Marrubium peregrinum* L. extract. *Kragujevac Journal of Science* 33: 63–72.

Sultana B, Anwar F and Ashraf M (2009). Effect of extraction solvent/technique on the antioxidant activity of selected medicinal plant extracts. *Molecules* 14(6): 2167–2180.

Tamboli FA, More HN, Bhandugare SS, Patil AS, Jadhav NR and Killedar SG (2020). Estimation of total carbohydrate content by phenol sulphuric acid method from *Eichhornia crassipes* (Mart.) Solms.

Wald M, Schwarz K, Rehbein H, Bußmann B and Beermann C (2016). Detection of antibacterial activity of an enzymatic hydrolysate generated by processing rainbow trout by-products with trout pepsin. *Food Chemistry* 205: 221–228.

Wiegand I, Hilpert K and Hancock RE (2008). Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. *Nature Protocols* 3: 163–175.

Wrasiati LP, Wirawan IGP, Bagiada NA and Astawa INM (2011). Antioxidant capacity of frangipani (*Plumeria alba*) powder extract. *Indonesia Journal of Biomedical Science* 5.