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Exploring the Therapeutic Potential of *Colocasia esculenta* Leaves: A Study on Antioxidant, Antimicrobial and Thrombolytic Activities

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Abstract
This study explores the bioactive properties of *Colocasia esculenta* leaf extracts, focusing on their antioxidant, antimicrobial, thrombolytic, and cytotoxic activities. Several solvent extracts, including aqueous methanol, ethyl acetate, *n*-hexane, *n*-butanol, and chloroform, were analyzed for their efficacy. Among these, the aqueous methanol extract demonstrated the highest levels of phenolic and flavonoid compounds, corresponding to superior antioxidant and antimicrobial activities. Notable, significant thrombolytic activity was observed, with clot lysis percentages ranging from 27.69% to 48.78%. Hemolytic activity assays revealed varying levels of cytotoxicity among the extracts, with the aqueous methanol extract causing the least red blood cell lysis. Pearson correlation analysis revealed a positive relationship between total phenolic content, flavonoid content and inhibition potential, antimicrobial properties, and thrombolytic activities. Conversely, IC_{50} values and hemolytic activity showed negative correlations with these bioactive compounds. The findings confirm the presence of potent phenolic antioxidants in *Colocasia esculenta* leaves, supporting their potential applications in nutraceutical formulations and functional foods. Further research is recommended to elucidate the primary mechanisms of these bioactivities and validate their therapeutic potential in clinical applications.
Keywords: *Colocasia esculenta*, Antioxidant potential, Antimicrobial, Thrombolytic, Hemolytic activity.

Highlight

- The leaves of the *Colocasia esculenta* contain bioactive compounds like polyphenols, flavonoids, and alkaloids, showing therapeutic benefits.
- The leaves of *Colocasia esculenta* demonstrated efficient scavenging activity of free radicals.
- The leaf extracts demonstrated significant antibacterial activity against a variety of gram-positive and gram-negative bacteria.
- In vitro thrombolysis revealed significant clot-dissolving activity, indicating that it might be used as a natural thrombolytic drug.

1.0. Introduction

Free radicals are characterized by their unusual reactivity, instability, and short lifetimes due to the presence of unpaired electrons. Consequently, they have a strong tendency to bind with other molecules to achieve stability (Ahmad et al., 2022). During chemical reactions, molecules can transform into free radicals, which have the potential to damage cells (Kashif et al., 2024). An imbalance between excessive free radicals and insufficient antioxidant production leads to oxidative stress (Chhikara et al., 2021), which is associated with various diseases like stroke, diabetes, cancer, and myocardial infarction (Anees et al., 2023). Antioxidants play a crucial role in mitigating, delaying, and preventing oxidative damage to molecules. They achieve this by neutralizing free radicals through mechanisms such as chelation, scavenging, and other methods that inhibit lipid oxidation and act as carbonyl scavengers (Ahmad et al., 2022). These antioxidant compounds employ two primary mechanisms to neutralize free radicals: hydrogen atom transfer (HAT) and single-electron transfer (SET). Interestingly, both mechanisms yield comparable results (Munteanu & Apetrei, 2022).

Plants are significant sources of herbs and antioxidants and have a complete history of medicinal use. They have been used in folk medicines, with each plant or herb associated with specific bioactive properties (Arora & Arora, 2021; Naz et al., 2023). While synthetic drugs, such as butylated hydroxyanisole and butylated hydroxytoluene are associated with various adverse health effects. Conversely, pharmaceutical drugs derived from medicinal plants tend to produce notable and efficient outcomes with minimal or rare side effects, making them the preferred choice (Hassan et al., 2022; Kashif et al., 2024). Natural chemical compounds in plants with protective properties are referred to as phytochemicals. These compounds, responsible for the antioxidant potential of plants, primarily include phenols, flavonoids, isoflavones, anthocyanins, flavones, coumarins, catechins, and iso-catechins (Naz et al., 2023; Sun & Shahrajabian, 2023) The

evaluation of phytochemical components generally involves measuring total phenolic and total flavonoid contents, while methods such as the DPPH assay and the FRAP assay are commonly used to assess their antioxidant properties (Jafri et al., 2022).

The world is rich in medicinal plants found in nearly every region. Pakistan, in particular, is well-known for its remarkable diversity and abundant array of medicinal plant species, which are widely used in ethnomedical practices to treat a variety of diseases. The diverse landscape of Pakistan encompasses varying climates, landforms, traditional regions, and abundant plant species (Alamgeer et al., 2018). Pakistan boasts approximately 6,000 documented species of medicinal plants distributed across its area and the Kashmir region. However, the challenge of medicinal plant waste poses significant environmental and logistical obstacles. This includes a large volume of waste, and susceptibility to spoilage (Jeswani et al., 2021). To address these issues, researchers have investigated innovative approaches and technologies to manage medicinal plant waste effectively while extracting valuable bioactive components for human health applications. Adopting sustainable practices such as reducing, reusing, and recycling plant waste can contribute to environmental preservation, enhance the sustainability of food systems, and improve food availability (Picot et al., 2021). While not all parts of the plant possess medicinal properties, every component holds the potential for therapeutic use (Iwansyah et al., 2020).

Taro, scientifically known as *Colocasia esculenta* (L.), is a tropical and perennial plant belonging to the Araceae family. It is extensively cultivated across South Asia, East Africa, Southeast America, and the Caribbean (Keshav et al., 2019). The entire plant is utilized as a food source, providing a rich supply of minerals, carbohydrates, and proteins. The leaves of *Colocasia* are characterized by their distinctive heart-shaped structure, often marked with lines, blotches, or spots in pigments ranging from light green to purple, depending upon genotype. Leaf size varies with environmental conditions and maturity. These leaves are a valuable source of essential amino acids, proteins, and peptides (Christou et al., 2023). *Colocasia* leaves possess numerous medicinal properties, including antioxidative, antidiabetic, antimicrobial, anticancer, antihypertensive, and anti-inflammatory effects. Historically, they have been used in the dietary management of various conditions, such as neurological disorders, arthritis, blood purification, and respiratory ailments (Akshatha et al., 2018; El-Mesallamy et al., 2021; Sudhakar et al., 2020).

Phenols, flavonoids, and tannins are bioactive compounds commonly found in medicinal plants and used as natural antioxidants that help to prevent various diseases. The increasing demand for plant-derived antioxidants underlines the significant potential of medicinal plants as abundant sources of these compounds for use in pharmaceutical products. This study focused on identifying the biologically active compounds and evaluating the antioxidant capabilities of solvent extracts obtained from *Colocasia esculenta* leaves. The extraction process was carried out using the ultrasonication method, employing various solvents, including *n*-hexane, chloroform, ethyl acetate, *n*-butanol, and aqueous methanol, at different concentrations.

2.0. Materials and Methods

2.1. Chemicals and Reagents

Folin-Ciocalteu (FC) reagent, trichloroacetic acid, ferric chloride, gallic acid, ascorbic acid, ammonium thiocyanate, anhydrous sodium carbonate, aluminum chloride, ferrous chloride, linoleic acid, DPPH, potassium ferricyanate, sodium nitrite, wet ice, triton X-100 and butylated hydroxytoluene were obtained from Sigma Chemicals (USA). The chemicals, including *n*-butanol, chloroform, methanol, *n*-hexane, diethyl ether, and ethanol, were purchased from the Merck Chemicals Company (Darmstadt, Germany). The components required for preparing the culture medium used in the antibiotic test were sourced from Oxoid Ltd. (Hampshire, UK).

2.2. Collection of plant material and its pretreatment

Leaves samples of *Colocasia esculenta* were collected from District Khanewal, Punjab, Pakistan. A taxonomist from the University of Agriculture, Faisalabad, Pakistan, assisted in the verification and identification of the samples. The collected leaves were chopped into smaller pieces, air-dried, and stored in plastic bags at -4 °C.

2.3. Extraction of CCEs

Colocasia esculenta leaf samples were cleaned with tap water to eliminate any impurities. The cleaned samples were shaded-dried and finely ground into a fine powder (mesh size 80) using an electric grinder. Subsequently, 10 g of the powdered sample was extracted using 100 mL of different solvents, including *n*-hexane, chloroform, ethyl acetate, *n*-butanol, and aqueous methanol (methanol: water, 80:20). The extraction process was performed using the ultrasonication method at 40 °C for 50 minutes. The recovered extract was filtered three times with filter paper (Whatman No. 1) to remove insoluble residues. The filtered extract was pooled in a new glass vial. A rotary evaporator (Ecohim Ltd. (Ekros Group of 20 companies, Saint-Petersburg) was used to evaporate excess solvents from the crude at a temperature of 45 °C. This process yielded crude concentrated extracts (CCEs), which were stored at -4 °C for further analysis (Kashif et al., 2024).

2.4. Estimation of antioxidant activity

The antioxidant activity of *Colocasia esculenta* leaf was assessed through the following assay.

2.4.1 Determine the total flavonoid content in CCEs

The total flavonoid content (TFC) of *Colocasia esculenta* leaf samples was determined following the method described by Ahmad et al. (2022). To simplify, 5.0 mL of distilled water was added to CCEs at the concentration of 100 mg/mL with the addition 0.3 mL of 5% sodium nitrate. The mixture was incubated for 20 minutes. After incubation, 0.6 mL of 10% aluminum chloride and 2.0 mL of 1.0 M sodium hydroxide were sequentially added to the solution. The absorbance of the final mixture was measured at 510 nm. TFC was calculated using a catechin calibration curve and expressed as catechin equivalents (CE) per 100 g of dry matter.

2.4.2 Determine the total phenolic content in CCEs

The total phenolic content (TPC) of *Colocasia esculenta* leaf samples was determined using the method described by Ahmad et al. (2022). Briefly, 1 mg/mL CCEs were dissolved in 7.5 mL of distilled water with 0.5 mL Folin-Ciocalteu reagent and 1.5 mL of sodium carbonates. This mixture was heated in a water bath at 40 °C for 20 minutes. After incubation, the absorbance of the solution was measured at 755 nm. TPC was expressed as gallic acid equivalents (GAE) per 100 g of dry weight of the tested sample.

2.4.3 Estimation of antioxidant capacity using linoleic acid system

The antioxidant capacity of the CCEs recovered from *Colocasia esculenta* leaf samples was assessed by evaluating their ability to inhibit the oxidation of linoleic acid, as described by Kashif et al. (2024). For the assay, 5.0 mg of each green extract was mixed with 10 mL of ethanol (99.8%), 10 mL of sodium phosphate buffer (pH 7), and 0.13 mL of 0.2 M linoleic acid. Distilled water was added to the solution to achieve a final volume of 25 mL. The degree of oxidation was determined by measuring the final volume of the solution at 40 °C (Yen et al., 2000). Additionally, a mixture comprising 10 mL of 75% ethanol was mixed with 0.2 mL of 30% ammonium thiocyanate and 0.2 mL ferrous chloride (in 3.5% of 0.2 M HCl). The mixture was incubated at room temperature for 5 minutes. After that, the absorbance of the resulting solution was recorded at 500 nm using a spectrophotometer (Hitachi U-2001, Model 121-0032).

$$I(\%) = \left[\frac{Ac - A_s}{A_c}\right] \times 100 \quad \text{(1)}$$

Ac and As represent the absorbance values of the control and the sample, respectively, after 350 hours. I (%) denotes the percentage inhibition (Eq.1). A control treatment, prepared without plant extract, was also included for comparison. Positive controls used in the study were ascorbic acid ($C_6H_8O_6$) and butylated hydroxytoluene (BHT).

2.4.4 Assessment of Reducing power

The reducing power of the CCEs was determined following the method reported by Yen et al. (2000). CCEs ranging from 10 to 40 mg per dry matter were individually mixed with 5.0 mL of 0.2 M sodium phosphate buffer (pH 6.6) and 5.0 mL of 1.0% potassium ferricyanide. The mixture was heated at 50 °C for 20 minutes. After incubation, 10% of trichloroacetic acid was added, and the solution was centrifuged at 1200 rpm at 5 °C for 10 minutes using a centrifuge (CHM-17, Japan). After centrifugation, 5.0 mL of the upper layer was transferred to a separate flask and mixed with 1.0 mL of 0.1% ferric chloride and 5.0 mL of distilled water. The absorbance of the resulting mixture was measured at 700 nm. All analyses were performed in triplicate, and the data were average.

2.4.5 DPPH radical scavenging assay

The ability of the CCEs to inhibit free radicals, such as 2, 2-diphenyl-1-picrylhydrazyl (DPPH), was evaluated (Eqn. 1) using the method described by Tepe et al. (2005). A 50 µL sample of each extract, at concentrations ranging from 0.10-5.0 mg/mL, was added to 5 mL of a 0.0004% DPPH solution prepared in methanol. The mixture was incubated for 30 minutes, and its absorbance was measured at 517 nm against a blank solution as the reference.

2.5. Antimicrobial activity

2.5.1 Microorganisms tested

The *Colocasia esculenta* leaf extracts were tested individually against various pathogenic microorganisms, such as the gram-negative bacteria *Escherichia coli*, the gram-positive bacteria *Bacillus subtilis*, and fungal strains (*Aspergillus niger* and *Fusarium solani*). The microorganism strains were obtained from the Department of Bioinformatics and Biotechnology at Government College University Faisalabad. Bacterial strains were cultured on a nutrient agar medium, while fungal strains were grown on potato dextrose agar (Oxoid, UK). The incubation temperatures for bacteria and fungi were maintained at 37 °C and 30 °C, respectively.

2.5.2 Disc diffusion method

The disc diffusion method was used to evaluate the antibacterial potential of the solvent extracts (Aleem et al., 2024). Sterile discs were soaked in 100 mg/mL of each solvent extract and subsequently placed onto agar plates inoculated with

pathogenic bacterial strains. Positive controls (flumequine and amoxicillin) and negative controls (discs without any sample) were treated utilizing the same procedure to ensure consistency.

2.5.3 Micro-dilution broth assay

The microdilution method, as described by Ahmad et al. (2022), was employed to determine the minimum inhibitory concentration (MIC) of the leaf extracts. MIC is defined as the lowest concentration of the extract required to inhibit the growth of microorganisms in vitro completely. Every solvent extract from the tested samples was diluted in a 96-well plate, ranging from 5 to 100 mg/mL. Sterility controls (with leaf extract without microorganisms) and growth controls (without leaf extract) were included in this assay. For fungal and bacterial strains, 20 µL of the diluted leaf extract was added to 160 µL of sabouraud dextrose broth (SDB) and nutrient broth (NB) culture medium, respectively. Additionally, 20 µL of broth culture (5×10^5 CFU) of each microorganism was inoculated into the wells. The 96-well plate was incubated for 48 hours at 30 °C for fungal strains and 24 hours at 37 °C for bacterial strains. The formation of a white pellet at the bottom of the well indicated microbial growth. The MIC was determined as the lowest concentration at which microbial growth was completely inhibited.

2.6. Thrombolytic activity

The thrombolytic activity of selected plant samples was evaluated using the method described by Tabassum et al. (2017), with streptokinase serving as the positive control. Venous blood was collected from a healthy human volunteer to perform the clot lysis assay. 500 µL of blood was transferred into the pre-weighed Eppendorf tubes and incubated at 37 °C for 40 minutes to allow clot formation. After incubation, the serum was carefully removed from each tube using a micropipette without disturbing the clot. The tubes containing the clot were weighed again to determine the clot weight. The weight of the clot (W_3) was calculated as the difference between the weight of Eppendorf tubes (W_2) containing the clot and the weight of empty Eppendorf tubes (W_1).

$W_3 = W_2 - W_1$ _____(3)

The percentage of thrombolytic activity was evaluated using Equation 4.

Clot lysis % = $(W_3\text{-}W_4/W_3) \times 100$ _____(4)

Here

W_1 = weight of empty tube

W_2 = Weight of empty tube + clot (before lysis)

W_3 = weight of a clot (before lysis) ($W_2\text{-}W_1$)

W_4 = Weight of empty tube + clot (after lysis)

The difference in weight evaluated the percentage of clot lysis before and after clot lysis.

2.7. Hemolytic activity

The hemolytic activity of the selected leaf samples was evaluated using the method described by Zohra & Fawzia (2014). Phosphate saline buffer (PSB) was prepared and chilled in a freezer before use. Fresh human blood was collected in a falcon tube, and heparin was immediately added as an anticoagulant. The blood was washed twice with PBS to remove impurities. To wash the blood, 10 mL of PBS was added to the falcon tube containing blood and centrifuged for 10 minutes at 850 rpm. The upper serum layer was discarded, and the process was repeated twice with fresh phosphate saline buffer. The leaf extracts were prepared by dissolving 0.01 g of each extract in 1 mL of their respective solvents, such as ethyl acetate, chloroform, aqueous methanol, *n*-butanol, and *n*-hexane. Then, 20 µL of each extract was mixed with 180 µL of the blood suspension in separate Eppendorf tubes. The tubes were incubated at 37 °C for 35 minutes. After incubation, the tubes were placed in an ice bath for 5 minutes and then placed for centrifugation at 1300 rpm for 6 minutes. Then, 20 µL of the supernatant was collected and diluted with chilled phosphate saline buffer (2 - 4 µL). The tubes were again kept in an ice bath after dilution. Finally, the absorbance of the mixture was measured at 576 nm using a spectrophotometer.

2.8. Statistical Analysis

All in vitro results were reported as the mean ± SD of three parallel measurements. The data were analyzed using the student's t-test, and values of $P < 0.001$ were considered statistically significant.

3.0. Results and Discussion

The present study focused on the activities of the bioactive compounds in the leaves of *Colocasia esculenta*, a plant known for its rich phenolic compounds with remarkable therapeutic properties. The growing interest in natural plants for their health benefits continues to rise. The extract of the leaves of *Colocasia esculenta* demonstrated significant antioxidants and thrombolytic, hemolytic, and antimicrobial activities.

3.1. Yield of extracts

Table 1 shows the (g/100g) yield extracts of *Colocasia esculenta* leaf with different solvents and concentrations. The leaf extract yield has antioxidative constituents ranging from 3.39-4.48 g/100 g per dry substance. Aqueous methanol extract recovered the maximum yield however, the least was obtained from chloroform extract. The result revealed that the extract yield varied significantly ($p < 0.05$) depending on the components and solvent type, with aqueous methanol yielding the highest amount of *Colocasia esculenta* leaf extracts, demonstrating that solvents are more effective at restoring antioxidant constituents (Kasote et al., 2011).

3.2. Total phenolic and flavonoid content

Wojdyło et al. (2007) found that the food industry is increasingly focusing on plants with anti-carcinogenic properties and the ability to prevent lipid oxidation. Natural antioxidants, such as phenolic compounds, are commonly derived from plants (Awika et al., 2003). Numerous studies have emphasized that the antioxidant activity of fruits and vegetables is primarily attributed to their total flavonoid and phenolic content (Katalinic et al., 2006).

TPC and TFC recovered from *Colocasia esculenta* leaf extracts using different solvents are presented in Table (1). The phenolic and flavonoid compounds isolated from the extracts ranged from 231–438 mg GAE/100 g and 185–311 mg CE/100 g, respectively. Aqueous methanol extract exhibited the highest TPC and TFC values, while the chloroform extract showed the lowest. The phenolic compound content extracted from the plant varied significantly ($p < 0.05$) depending on the solvent used. Aqueous methanol is often preferred for antioxidant extraction from leaf samples due to its higher extraction efficiency and lower toxicity (Kasote et al., 2011). The solvent extraction efficiency for total phenolic and flavonoid contents followed the order: aqueous methanol > ethyl acetate > *n*-hexane > *n*-butanol > chloroform.

Jeffery et al. (2003) reported that the amounts of TFC and TPC in the plant leaves are influenced by plant maturity at the time of harvest, habitat conditions, and soil composition. The amount of total phenolic and flavonoid found in this study is comparable with those previously documented for *Colocasia esculenta* leaves. When compared to other studies, the current research demonstrated higher levels of both total phenolics and flavonoids in the examined *Colocasia esculenta* leaf samples (Christou et al., 2023).

Table 1: Yield (%), total phenolic content and total flavonoid content of *Colocasia esculenta* leaf.

Extracting solvent	Yield (%) (g/100g of dry matter)	TPC GAE (mg/100g)	TFC CE (mg/100g)
n-hexane	3.69 ± 0.05	311 ± 0.71	259 ± 0.47
Aqueous methanol	4.48 ± 0.07	438 ± 0.86	311 ± 0.63
Ethyl acetate	4.17 ± 0.05	342 ± 0.39	277 ± 0.52
Chloroform	3.29 ± 0.08	231 ± 0.69	185 ± 0.48
n-butanol	3.51 ± 0.04	278 ± 0.57	219 ± 0.84

Values are averaged (mean ± SD) of three replicates that are examined independently.

3.3. DPPH radical scavenging assay

The natural free radical DPPH exhibited a dark violet color with an absorption peak in the range of 515-528 nm. Upon accepting a proton from hydrogen donors, typically phenolic compounds, it undergoes a color change from violet to yellow. It is widely recognized that higher levels of phenolic components or greater hydroxylation enhance the scavenging ability of DPPH radicals, thereby improving the antioxidant properties of leaf extracts (Sánchez-Moreno et al., 1999; Younas et al., 2024).

Colocasia esculenta leaf extracts exhibited excellent radical scavenging activity, with IC_{50} values ranging from 10.61 to 17.76 µg/mL, as shown in Table (2). The lowest IC_{50} value indicates the highest free radical scavenging activity, which was observed in the aqueous methanol extract. Statistical analysis confirmed that the radical scavenging potential of the aqueous methanol extract was significantly higher than that of other solvents ($p < 0.05$). However, the scavenging efficacy of the studied extracts was lower compared to the synthetic antioxidant butylated hydroxytoluene (BHT). The DPPH radical scavenging activity of the extracts is strongly associated with their phenolic content (Siddhuraju et al., 2002).

3.4. Antioxidant activity in linoleic acid system

Linoleic acid (unsaturated fatty acid) undergoes oxidization to produce peroxides. The produced peroxides further oxidize Fe^{2+} to Fe^{3+}, which then reacts with SCN^- to form a complex (Younas et al., 2025). The concentration of this compound is determined by measuring its absorbance at 500 nm. Higher absorbance values indicate lower antioxidant activity, as they reflect a greater concentration of peroxides (Ahmad et al., 2022).

The antioxidant potential of *Colocasia esculenta* leaf extracts in preventing lipid peroxidation is presented in Table (2). The extracts exhibited inhibition percentages ranging from 48.53% to 67.21%. Among the tested solvents, the aqueous methanol extract demonstrated a significantly higher concentration of phenolic compounds, resulting in superior

protection against peroxidation compared to other extracts ($p < 0.05$). However, when compared to chemical antioxidants such as BHT, all studied leaf extract samples showed relatively lower effectiveness in preventing linoleic acid oxidation. The inhibition efficacy of *Colocasia esculenta* leaf extracts followed this order: aqueous methanol > ethyl acetate > *n*-hexane > *n*-butanol > chloroform. Notably, the results of this study indicate a higher lipid peroxidation inhibition capacity compared to a previous study reported by Christou et al. (2023).

Table 2: DPPH radical scavenging activity and (%) inhibition of *Colocasia esculenta* leaf extracts.

Extracting solvent	IC_{50} value (µg/mL)	% Inhibition
n-hexane	13.88 ± 0.07	56.38 ± 0.61
Aqueous methanol	10.61 ± 0.08	67.21 ± 0.76
Ethyl acetate	12.57 ± 0.04	61.87 ± 0.49
Chloroform	17.76 ± 0.07	48.53 ± 0.89
n-butanol	15.93 ± 0.03	52.28 ± 0.67

Values are averaged (mean ± SD) of three replicates that are examined independently.

3.5. Reducing power of the extract

The antioxidant activity of the solvent extracts of the tested leaf was assessed by measuring their capacity to exhibit reducing power. In this assay, yellow-colored ferric ions are reduced to bluish-green ferrous ions. The reducing potential of the plant extracts is directly related to the intensity of this color change, which occurs due to the presence of antioxidant compounds. A more substantial color change corresponds to higher absorbance, indicating a more significant antioxidant potential (Zou et al., 2004). It is described by Ahmad et al. (2022) that the reducing potential of bioactive compounds is closely linked to its antioxidant capacity, establishing a strong relationship between the two.

In the current study, all the examined extracts showed a consistent increase in reducing potential as the extract concentration increased, as illustrated in Fig. (1). Leaf extract concentrations ranging from 10 to 40 mg/mL were evaluated for their reducing power. The absorbance values obtained ranged from 0.217 to 0.494. Among the *Colocasia esculenta* leaf extracts, the highest absorbance (0.494) was recorded for the aqueous methanol extract, while the lowest absorbance (0.217) was observed for the chloroform extract. The order of reducing potential across the different extracts was as follows: aqueous methanol > ethyl acetate > *n*-hexane > *n*-butanol > chloroform. Notably, the differences in reducing potential among the various extracts were statistically significant ($p < 0.05$).

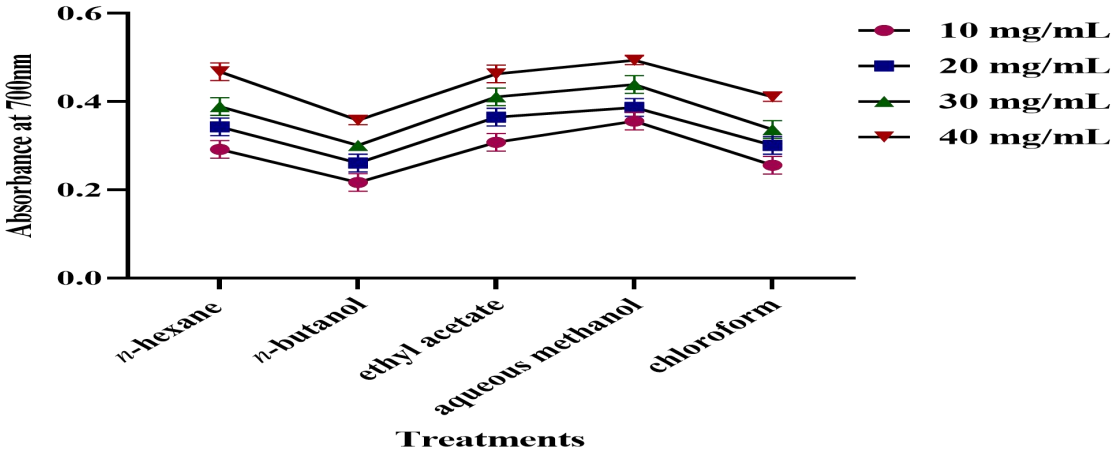

Figure 1: Reducing power of *Colocasia esculenta* leaf extract.

3.6. Antimicrobial activity

The *Colocasia esculenta* leaf extracts have demonstrated significant antibacterial and antifungal activity against pathogenic microorganisms, as determined by minimum inhibitory concentration (MIC) and the disc diffusion method. The leaf extracts exhibited notable zone of inhibition (ZOI) against *Bacillus subtilis*, ranging from 6 to 12 mm, indicating their potent antimicrobial properties. The leaf extracts also displayed the lowest MIC values against *Escherichia coli*, ranging from 206 to 279 µg/mL, indicating strong antimicrobial efficacy when compared to *Bacillus subtilis* (Table 3). Additionally, the leaf extracts showed substantial inhibition zones against *Fusarium solani*, ranging from 2 to 7 mm, highlighting their potent antifungal properties. The leaf extracts exhibited the lowest MIC values ranging from 165 to 238

µg/mL against *Aspergillus niger*, indicating more potent antifungal efficacy compared to *Fusarium solani* (Table 4). Amoxicillin and Terbinafine were used as standard references for bacterial and fungal strains, respectively.

Previous research, as indicated by Yesil-Celiktas et al. (2007) has highlighted that variations in the chemical composition of extracts can lead to differences in their biological effects. In the current study, the extraction of *Colocasia esculenta* leaves using various solvents yielded extracts with distinct chemical profiles and varying levels of antimicrobial and antioxidant potential. These differences in biological activity can be attributed to the diverse range of bioactive compounds present in the leaf extracts, with each solvent being capable of extracting specific antibacterial and antioxidant substances.

Table 3: Antibacterial activity of *Colocasia esculenta* leaf extracts.

Extracting solvent	Antibacterial activity			
	Bacillus subtilis		***Escherichia coli***	
	ZOI (mm)	**MIC (µg/mL)**	**ZOI (mm)**	**MIC (µg/mL)**
n-hexane	8	261	10	243
Aqueous methanol	12	214	14	206
Ethyl acetate	11	249	11	228
Chloroform	6	303	7	279
n-butanol	7	287	8	256
Positive control (Amoxicillin)	-	-	-	-

Values are averaged (mean ± SD) of three replicated that are examined independently.

Table 4: Antifungal activity of *Colocasia esculenta* leaf extracts.

Extracting solvent	Antifungal activity			
	Fusarium solani		***Aspergillus niger***	
	ZOI (mm)	**MIC (µg/mL)**	**ZOI (mm)**	**MIC (µg/mL)**
n-hexane	4	218	7	207
Aqueous methanol	7	173	10	165
Ethyl acetate	6	199	8	186
Chloroform	2	249	3	238
n-butanol	4	227	5	221
Positive control (Terbinafine)	21	142	24	133

Values are averaged (mean ± SD) of three replicated that are examined independently.

3.7. Thrombolytic activity

The thrombolytic activity of *Colocasia esculenta* leaf extracts were evaluated against human red blood cells. The rate of thrombolysis for the various extracts is presented in Table 5. The results indicate that all extracts exhibited significant thrombolytic activity. The percentage of clot lysis ranged from 27.69% to 48.78%, with the aqueous methanol extract showing the highest clot lysis, while the lowest was observed with the chloroform extract. The positive control, streptokinase, demonstrated a clot lysis of 72.13%.

3.8. Hemolytic activity

Cytotoxicity was assessed by examining the hemolytic activity of various solvent extracts against human red blood cells (RBCs). Triton X-100 was used as the positive control, inducing approximately 97.82% RBC lysis, while phosphate-buffered saline (PBS) did not cause any RBC lysis. A comparison of the results between the leaf extracts and controls revealed varying levels of RBC lysis. Specifically, the aqueous methanol extract exhibited 6.13%, ethyl acetate 7.47%, chloroform 14.35%, *n*-hexane 9.28%, and *n*-butanol showed 11.53% RBC lysis (Table 5). The stability of the erythrocyte membrane offers a more reliable indicator of the cytotoxicity of different chemical constituents studied in vitro. Hemolysis, the breakdown of red blood cells, is a phenomenon associated with infectious diseases caused by microbial activity (Sharma & Sharma, 2001).

Table 5: Thrombolytic and hemolytic activity of *Colocasia esculenta* leaf extract.

Extracting solvent	Clot lysis (%)	RBC lysis (%)
n-hexane	39.81 ± 1.77	9.28 ± 0.06
Aqueous methanol	48.78 ± 1.18	6.13 ± 0.06
Ethyl acetate	42.57 ± 0.94	7.47 ± 0.04
Chloroform	27.69 ± 1.27	14.35 ± 0.09
n-butanol	33.43 ± 1.13	11.53 ± 0.07
Positive control (streptokinase)	72.13 ± 1.53	-
Positive control (Triton X-100)	-	97.82 ± 1.07

Values are averaged (mean ± SD) of three replicates that are examined independently.

3.9. Correlation study among TF, TP, and biological potential

The Pearson correlation method was used to analyze the relationships at a significant level of P = 0.001 (Tables 6 and 7), revealing a positive association between total phenolic (TP) and total flavonoid (TF) content with inhibition potential, antimicrobial properties and thrombolytic activities (P = 0.704, 0.676-0.722, and 0.639, respectively) as well as (P = 0.984, 0.977-0.990, and 1.000). Conversely, the IC_{50} value and hemolytic activity were found to be negatively correlated with total phenolic content (TPC) and total flavonoid content (TFC) values, with P = -0.998 and -0.993 for IC_{50} and P = -0.655 and -0.622 for hemolytic activity, respectively.

The *Colocasia esculenta* leaf from Khanewal, Pakistan, was evaluated for its nutritional value and potential bioactive compounds due to the significant interest in its medicinal properties. This study is the first to confirm the presence of phenolic antioxidant compounds in these particular sections. By demonstrating the significant biological activities of these phenolic antioxidants, which were previously underexplored, the research significantly enhances scientific understanding. The findings suggest that *Colocasia esculenta* leaf could be utilized in the development of various nutraceutical formulations and functional foods that offer numerous health benefits.

Table 6: Correlation between TP and biological activity of *Colocasia esculenta* leaf.

	TPC	IC_{50} value	Inhibition Potential	Antibacterial activity		Antifungal activity		Thrombolytic activity	Hemolytic activity
				B. subtilis	*E. coli*	*F. solani*	*A. niger*		
TPC	1								
IC_{50} value	-0.655**	1							
Inhibition Potential	0.704**	-0.991**	1						
B. subtilis	0.676**	-0.991**	0.989**	1					
E. coli	0.686**	-0.995**	0.990**	0.988**	1				
F. solani	0.722**	-0.985**	0.992**	0.985**	0.995**	1			
A. niger	0.707**	-0.992**	0.999**	0.989**	0.994**	0.995**	1		
Thrombolytic activity	0.639*	-0.999**	0.985**	0.990**	0.993**	0.980**	0.985**	1	
Hemolytic activity	-0.622*	0.992**	-0.975**	-	-	-	-	-	1

** Correlation is prominent at the 0.01 level.

Table 7: Correlation between TF and biological activity of *Colocasia esculenta* leaf.

	TFC	IC_{50} value	Inhibition Potential	Antibacterial activity		Antifungal activity		Thrombolytic activity	Hemolytic activity
				B. subtilis	*E. coli*	*F. solani*	*A. niger*		
TFC	1								
IC_{50} value	-0.998**	1							
Inhibition Potential	0.984**	-0.991**	1						
B. subtilis	0.987**	-0.991**	0.989**	1					
E. coli	0.990**	-0.995**	0.990**	0.988**	1				
F. solani	0.977**	-0.985**	0.992**	0.985**	0.995**	1			
A. niger	0.984**	-0.992**	0.999**	0.989**	0.994**	0.995**	1		
Thrombolytic activity	1.000**	-0.999**	0.985**	0.990**	0.993**	0.980**	0.985**	1	
Hemolytic activity	-0.993**	0.992**	-0.975**	-	-	-	-	-	1

** Correlation is prominent the 0.01 level.

4.0. Conclusion

This study provides a comprehensive evaluation of the *Colocasia esculenta* leaf extracts, highlighting their significant antioxidant, antimicrobial, thrombolytic, and cytotoxic properties. The aqueous methanol extract consistently exhibited the highest efficacy across various assays, owing to its superior extraction of phenolic and flavonoid compounds. The positive correlation between total phenolic and flavonoid content, inhibition potential, and antimicrobial and thrombolytic properties underscores the therapeutic potential of these bioactive compounds. The results significantly contribute to the scientific understanding of *Colocasia esculenta* leaves, paving the way for their integration into health-promoting products.

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