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Original article

Investigation of the Biological Activities of *Colocasia esculenta* L. Schott

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Abstract

Throughout history, medicinal plants have been utilized, laying the foundation for contemporary medicine. Plant-derived compounds have been a vital source for developing medications. *Colocasia esculenta* stands out among traditional crops for its significant nutritional and medicinal potential, surpassing many other tuber crops. In this study, the antioxidant, mutagenicity, and antimutagenicity of four different extracts (hexane, acetone, methanol, and aqueous) of *C. esculenta* were investigated. Antioxidant activities of *C. esculenta* extracts were detected with the determination of total phenolic/flavonoid content (TPC/TFC), total antioxidant activity (TAC), and DPPH free radical scavenging activity. Acetone extract of C. esculenta exhibited the highest values in all TPC, TFC, TAC, and the DPPH free radical scavenging analyses. The mutagenic and antimutagenic activities of those four extracts were examined with TA98 and TA100 strains of *Salmonella typhimurium*. In higher concentrations, acetone and methanol extracts showed stronger mutagenic activity than the other extracts in both strains. The highest antimutagenic activity was observed in hexane and acetone in strain TA98.

Keywords: *Colocasia esculenta*, Antioxidant Activity, Mutagenic, Antimutagenic.

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INTRODUCTION

Plants have been crucial in supporting human health and improving quality of life for centuries, being essential ingredients in dyes, beverages, cosmetics, spices, and medicines. The belief that plants possess natural compounds capable of enhancing health and relieving ailments underpins the recent surge in herbal medicine's popularity (Chakraborty et al., 2015).

Tubers are essential sources of carbohydrates and energy, serving as a primary food in regions characterized by warm climates. They contain beneficial substances like resistant starch and mucilage, which undergo slow digestion in the lower gastrointestinal tract, gradually releasing and absorbing glucose and helping lower the risk of obesity, diabetes, and related diseases (Liu et al., 2006). Mucilage extracted from different roots and tubers has been found to possess angiotensin-converting enzyme inhibitor properties and antioxidative activities (Lee et al., 2003).

Colocasia (*Colocasia esculenta* L. Schott.), commonly referred to as arvi or taro, belongs to the Araceae family and the Colocasioidae subfamily. *C. esculenta* is a perennial starchy plant originating from Asia and the Pacific regions and is now prevalent in tropical areas around the world (Onwueme, 1999). It is a herbaceous, monocotyledonous plant that thrives in wetlands and is a significant tuber crop (Angami et al., 2015). Since ancient times, it has been used to treat a wide range of conditions such as asthma, high blood pressure, pneumonia, internal bleeding, as well as neurological and dermatological disorders (Prajapati et al., 2011).

C. esculenta tubers consist of globulins from two distinct and unrelated families, which make up 80% of the total proteins in the tubers. The total amino acid content in these tubers varies from 1.380 to 2.397 mg per 100 grams, with lysine present in relatively low amounts (Council of Scientific & Industrial Research, 1972).

Corms contain vitamin B, starch, mucilage, oil, calcium oxalate, dihydroxysterols, iron, and other compounds (Sheth et al., 2005). Tubers also contain starch, along with natural polysaccharides composed of 40% anionic compounds and 56% neutral sugars. Steamed onions have 3% sugar and 30% starch. Researchers isolated two dihydroxysterols from the tubers with β-sitosterol, cyanidin 3-glucoside, nonacosan, and stigmasterol. In *C. antiquorum* tubers which belongs to the same family as *C. esculenta*, infected by *Ceratocystis fimbriata*, an antifungal compound (9,12,13-trihydroxy-(E)-10-octadecenoic acid) was identified along with lipoxygenase and lipid hydroperoxide converting enzyme that is involved in the production of antifungal lipid peroxides (Masui et al., 1989).

The astringency in the tubers, leaves, and petioles is due to raphides, which typically decrease when the plant parts are boiled or cooked (Lim, 2015). Pressure-cooking the tubers resulted in a significant reduction or complete loss of inhibitory activity. However, for the chymotrypsin and trypsin inhibitors in taro, partial preservation of their inhibitory effects was observed. Taro tuber contains

significant levels of compounds that exhibit anti-trypsin and anti-chymotrypsin activities (Prathibha et al., 1995).

The majority of funding from both public and private sectors for agricultural research focuses on improving the production of valuable crops intended for profitable markets or global trade. When taro is perceived as a "poor man's crop" or a "marginal crop" discourages the research needed to harness its many advantages for small-scale farming communities. Despite its diverse geographic presence, high nutrition, and significant trade as both a raw and processed product, taro and its wild relatives have attracted relatively little interest from research funding agencies. Additionally, there has been limited evaluation of its production, trade, and use, either on a small or large scale (Chivenge et al., 2015; Matthews and Ghanem, 2021).

Taro is mainly cultivated for its corm, which serves as a popular dietary essential. Its edible flowers, leaves, and stems are also utilized in making purees, sauces, soups, and stews (Ribeiro Pereira et al., 2020; Mitharwal et al., 2022). The juice extracted from the petiole of *C. esculenta* has styptic properties and effectively stops severe bleeding from arteries. It is also used to treat earaches, otorrhoea, and internal hemorrhages, and serves as a stimulant and rubefacient. Leaf juice functions as a stimulant, expectorant, astringent, and appetizer, and is used to treat otalgia. Juice from the corm is used for alopecia, while the corm itself alleviates body aches. Petiole juice mixed with salt is used to treat inflamed glands and buboes. Cooked taro acts as a nervine tonic, and a decoction of the peel is a traditional remedy for diarrhea, asthma, weight gain, piles, and portal congestion. It also serves as an antidote for insect stings. Taro is incorporated into traditional dishes like kalua and various products such as burgers, bread, flakes, chips, flour, cookies, ice cream, soups, and pakora. In West Africa, it is a staple food, offering energy and carbohydrates, particularly beneficial for diabetics and those with gastrointestinal disorders (Sudhakar et al., 2020; Patel and Singh, 2023).

Ten compounds were extracted from *C. esculenta* using ethyl acetate and butanol fractions. These compounds, which include 1-*O*-caffeoyl-D-glucoside, 1-*O*-feruloyl-D-glucoside, isoorientin, isovitexin, luteolin-7-*O*-glucoside, luteolin-7-*O*-rutinoside, orientin, rosmarinic acid, tryptophan, and vitexin, were studied for their ability to inhibit aldose reductase in rat lenses. It was found that orientin and isoorientin exhibited significant inhibitory effects on aldose reductase in rat lenses (Li et al., 2014).

C. esculenta extract was found to contain a variety of phytochemicals including alkaloids, amino acids, carbohydrates, flavonoids, glycosides, phenolic compounds, saponins, tannins, and terpenoids. These phytochemicals contributed to the extract's ability to effectively neutralize free radicals and may be linked to antioxidant activity (Sheikh and Tembhre, 2016; Sheikh and Tembhre, 2018; Akyüz, 2019).

The leaf extract of *C. esculenta* demonstrated efficacy against a wide range of bacteria, encompassing both gram-negative and gram-positive strains (Singh et al., 2011; Chakraborty et al.,

2015). Also, *C. esculenta* showed antifungal activity against selected fungal species (Ehiobu and Ogu, 2018; Elmosallamy et al., 2021). In many studies, it has been reported to have antidiabetic (Kumawat et al., 2010), antihelminthic (Kubde et al., 2010), anticancer (Brown et al., 2005; Kundu et al., 2012), and anti-inflammatory (Tuti et al., 2015) activity. Bioactive proteins found in taro extracts (TE), specifically TE-M2 and TE-M2F1, have demonstrated anti-metastatic properties in a murine model of Triple-Negative Breast Cancer (TNBC) (Kundu et al., 2021).

During ATP production for cellular energy using oxygen, reactive nitrogen species (RNS) and reactive oxygen species (ROS) are generated as by-products of cellular redox reactions. When at balanced levels, RNS and ROS support cellular functions and immune responses. However, imbalanced concentrations of these species can result in oxidative stress, potentially leading to chronic and degenerative disorders (Tungmunnithum et al., 2018). Cellular ROS concentrations are regulated by enzymatic (catalase, NADH peroxidase, etc.) and non-enzymatic (vitamin C, α-tocopherol, etc.) antioxidant systems. Polyphenols are being researched and acknowledged for their potential as natural antioxidants that promote human health by combating and preventing oxidative damage caused by free radicals (Rasouli et al., 2017).

Mutations can take the form of gene mutations, involving changes in a single base or the addition/deletion of a few bases, as well as through extensive DNA deletions/rearrangements, chromosome breaks/rearrangements, or the acquisition/loss of entire chromosomes (Mortelmans and Zeiger, 2000). Testing drug candidates for mutagenicity is a crucial regulatory step in the drug approval process because compounds with mutagenic properties pose a toxic risk to humans. Mutagenicity refers to a compound's capacity to induce mutations in DNA. The Ames test is a rapid in vitro screening method specifically developed to identify genetic damage caused by various chemicals.

The present study examined the antioxidant properties alongside the mutagenic and antimutagenic potentials inherent in four distinct extracts (hexane, acetone, methanol, and aqueous) sourced from the tubers of *C. esculenta*, thereby aiming to elucidate their biochemical activities and potential implications.

MATERIALS and METHODS

Materials

Folin-Ciocalteu's phenol reagent, potassium acetate (CH_3CO_2K), aluminium nitrate ($Al(NO_3)_3$), quercetin, DPPH (2,2-diphenyl-1-picrylhydrazyl), butylated hydroxytoluene (BHT), and 4-nitro-*o*-phenylenediamine (NPD) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Gallic acid, ammonium thiocyanate, and sodium azide (SA) were purchased from Merck (Darmstadt, Germany). Sodium carbonate (Na_2CO_3) was purchased from Pancreac (Barcelona, Spain).

Sample Collection and Extraction

Tubers of *C. esculenta* were collected from the Anamur district of Mersin in Turkiye. The collected tubers were extracted by the Soxhlet apparatus using different solvents (hexane, acetone, methanol, and aqueous) (with a 1:10 ratio) for 12 hours. Solvents were evaporated and the dry extract was stored at 4°C for further studies.

Antioxidant Activity

Determination of Total Phenolic Content (TPC)

The total amount of phenolic content of extracts was determined by Slinkard and Singleton (1977) according to the Folin-Ciocalteu reagent method with slight modifications. 4.5 mL of 0.5 N Folin-Ciocalteu reagent (30%, v/v) was added to 0.1 mL of the extract. 0.3 mL sodium carbonate (Na_2CO_3) (2%) solution was added, and the mixture was incubated for 2 hours. The same procedure was repeated in triplicate for all extracts. The absorbance value was measured at 760 nm. Gallic acid was used as positive control and TPC values were expressed in gallic acid equivalent (mg GAE/ g extract) using a standard curve generated with gallic acid.

Determination of Flavonoid Content (TFC)

Total flavonoid content was determined by Matejic et al. (2013). 0.1 mL of potassium acetate (CH_3CO_2K) was added to 0.1 mL of the extract. 0.1 mL of aluminium nitrate ($Al(NO_3)_3$) (10%, m/v) and 5.2 mL of ethanol (96%, v/v) were added to the mixture. The mixtures were incubated for 40 minutes in the dark at room temperature. The same procedure was repeated three times for all extracts. The absorbance value was measured at 415 nm. Quercetin was used as a positive control and the TFC value was expressed as quercetin equivalent (mg QE/g extract).

DPPH (2,2-diphenyl-1-picrylhydrazyl) Free Radical Scavenging Activity

The free radical scavenging activity of extracts was determined by the method described by Blois (1958) with some modifications. The prepared extract concentrations (100, 200, 400, 600 µg/mL) were mixed with 1M DPPH radical in methanol (at a ratio of 1:3) and left to incubate in the dark for 30 minutes. Butylated hydroxytoluene (BHT) was used as a standard. After incubation, absorbance values were taken at 517 nm. The results obtained were used to calculate the % DPPH free radical scavenging inhibition according to Equation 1.

$$\% \text{ Inhibition} = [(A_0 - A_1) / A_0] \times 100 \qquad \text{(Equation 1)}$$

A_0: Absorbance of control, A_1: Absorbance of sample

Determination of Total Antioxidant Activity (TAC)

The thiocyanate method was used to determine the percentage of inhibition of lipid peroxidation by the extracts (Mitsuda et al., 1966). The extracts were placed in a test tube and then phosphate buffer (pH: 7.4) and linoleic acid emulsion were added in a 1:1 ratio. The mixture was incubated at 37°C. At the sixth, twenty-fourth, and forty-eighth hours, 100 µL each extract was taken from the tubes and added to test tubes containing 4.7 mL ethanol. 100 µL Fe^{2+} and 100 µL thiocyanate (SCN^-) solution were added to the mixture. The absorbance value was measured at 500 nm by using spectrophotometry.

Determination of Mutagenic and Antimutagenic Activity

The mutagenicity and antimutagenicity of the extracts were determined by using Ames/*Salmonella* assay using *Salmonella typhimurium* with the method developed by Marron and Ames (1983). Four varying concentrations (0.3, 0.6, 1.2, and 2.4 mg/mL) of extracts were utilized along with positive controls: sodium azide (SA) for TA100 and 4-nitro-*o*-phenylenediamine (NPD) for TA98. Each solvent was used as a negative control.

For each concentration, 0.1 mL of the extract, 0.1 mL of bacterial suspension from an overnight culture, and 0.5 mL of phosphate buffer were combined with 2 mL of top agar. The mixture was then vortexed and poured onto minimal glucose agar. The plates with the tested samples underwent a 48-hour incubation period at 37 °C. After this time, the revertant colonies present on each plate were counted. The assays were repeated in triplicate for each concentration group.

The mutagenicity results were evaluated by determining if the number of revertant colonies observed in the tested plates exceeded twice the count of those in the negative control. For antimutagenicity results, the inhibition rate on the plates was calculated with Equation 2; below 25% no antimutagenic activity; 25-40% moderate antimutagenic activity; 40% and above strong antimutagenic activity.

$$\text{Inhibition Rate (\%)} = \frac{A-B}{A-C} \text{ X } 100 \qquad \text{(Equation 2)}$$

A: Number of revertant colonies/plate in the positive control, B: Number of revertant colonies/plate in the presence of test compound and mutagen, C: Spontaneous colony count/plate.

RESULTS

Antioxidant Activity

Determination of Total Phenolic Content (TPC) and Total Flavonoid Content (TFC)

The acetone extract has the highest phenolic content with 355.62±3.26 mg GAE/g, while hexane extract has the lowest phenolic content with 186.15±1.15 mg GAE/g (Table 1). The acetone extract

exhibited the highest flavonoid content, determined as 295.73±1.27 mg QE/g, while the hexane extract demonstrated the lowest, with a value of 123.86±3.28 mg QE/g (Table 1).

Table 1. Total phenolic and flavonoid content of *C. esculenta* extracts.

Extract	TPC (mg GAE/g)	TFC (mg QE/g)
Hexane	186.15±1.15	123.86±3.28
Acetone	355.62±3.26	295.73±1.27
Methanol	309.93±1.67	294.63±2.56
Aqueous	270.36±2.41	187.27±1.46

Determination of Total Antioxidant Activity (TAC)

The percentage of inhibition of linoleic acid peroxidation was determined and compared with BHT. This technique relies on spectrophotometrically measuring the peroxide generated from the oxidation process within the linoleic acid emulsion. The overall antioxidant capacity of tuber extracts increases in correlation with time. The acetone extract exhibited strong antioxidant activity (82.71%) compared to BHT (77.57%), whereas the hexane extract displayed the lowest activity (16.82%) at the end of 48 h (Fig 1).

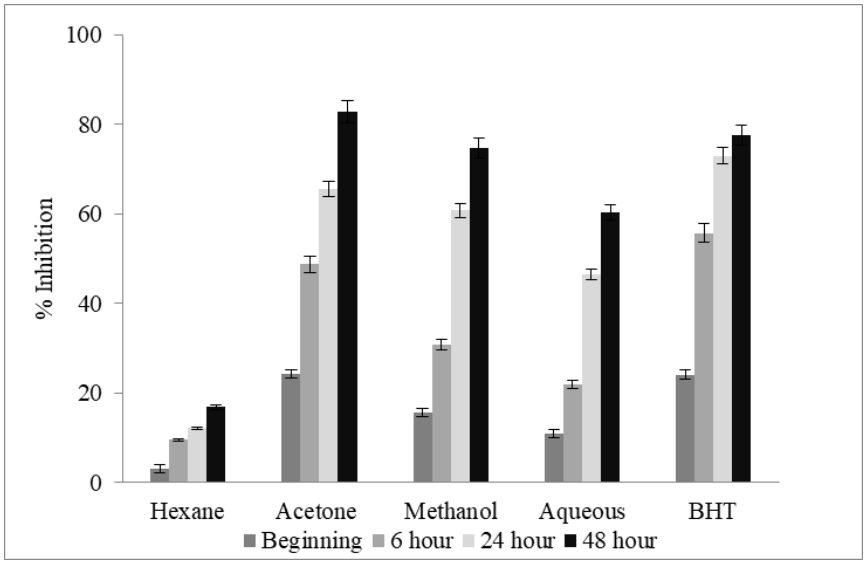

Figure 1. Total antioxidant activity of *C. esculenta* extracts.

The DPPH Free Radical Scavenging Activity

It was determined that the highest activity was in acetone extract (97.87%), even more than the BHT (95.77%), while the lowest was in hexane extract (33.96%) (Table 2).

Table 2. % DPPH free radical scavenging activities of *C. esculenta* extracts and BHT.

Extract	**Concentrations (µg/mL)**			
	100	**200**	**400**	**600**
Hexane	23.62	24.15	28.49	33.09
Acetone	66.19	86.37	91.85	97.87
Methanol	58.14	67.16	84.69	89.02
Aqueous	21.59	40.53	72.03	84.15
BHT	89.06	93.53	94.41	95.77

Mutagenic and Antimutagenic Activity

The mutagenic activity of four different extracts of *C. esculenta* was evaluated with Ames/*Salmonella* assay. The aqueous extract showed no mutagenic activity except against TA100 at 2.4 mg/mL. An increasing mutagenic activity was observed in both strains at concentrations of 0.6-2.4 mg/mL in acetone and methanol extracts. For hexane, TA98 showed mutagenic activity at the highest concentration while TA100 showed mutagenic activity at 1.2 and 2.4 mg/mL (Table 3).

Antimutagenicity of four different extracts of *C. esculenta* was evaluated according to the calculated inhibition rates (Table 4). While hexane and water extracts showed a decrease in antimutagenicity in both strains with increasing concentration, acetone, and methanol extracts showed an increase in antimutagenicity with increasing concentration. The lowest concentration of hexane (78.04%) and the highest concentration of acetone (77.01%) showed the highest antimutagenic activity.

Table 3. Mutagenic effects of *S. typhimurium* TA98 and TA100 strains of different concentrations of *C. esculenta*

Treatment		**Concentration (mg/mL)**	**His$^+$ Revertant Colony Count / Plate**	
			TA98	**TA100**
			Mean ± SD[a]	**Mean ± SD[a]**
Hexane		0.3	7.34±3.46	365.64±4.63
		0.6	53.00±1.63	391.28±7.33
		1.2	67.34±2.69	426.00±4.83
		2.4	126.0±2.76	447.05±6.59
Acetone		0.3	68.34±6.39	355.25±4.63
		0.6	92.67±5.93	378.48±3.29
		1.2	95.34±5.16	405.31±4.16
		2.4	102.67±4.33	467.16±2.79
Methanol		0.3	82.67±4.73	228.28±6.49
		0.6	110.67±3.66	234.09±5.49
		1.2	119.34±8.46	304.00±6.73
		2.4	128.67±7.63	371.01±4.16
Aqueous		0.3	51.34±8.39	246.23±5.73
		0.6	110.34±5.96	271.04±5.49
		1.2	134.67±7.36	298.00±4.36
		2.4	142.00±4.33	386.21±3.13
NC[b]	Hexane		53±5.36	210±4.36
	Acetone		44±6.26	176±3.73
	Methanol		54±3.73	116±4.66
	dH_2O		83±3.93	163±2.79
SC[c]			48±8.26	173±7.86
PC[d]	NPD	10^{-2}	341±5.41	
	SA	10^{-3}		717±4.76

a: Mean±standard deviation; b: negative control (100 µl/plate); c: spontaneous control; d: positive control.

Table 4. Antimutagenic effects of *S. typhimurium* TA98 and TA100 strains of different concentrations of *C. esculenta*.

Treatment		Concentration (mg/mL)	His$^+$ Revertant Colony Count / Plate			
			TA98	% Inhibition	TA100	% Inhibition
			Mean±SD[a]		Mean ±SD	
Hexane		0.3	112.34±3.43	78.04	402.12±4.69	56.25
		0.6	145.34±1.96	66.77	454.20±7.33	46.96
		1.2	205.34±6.43	46.30	531.18±4.36	33.21
		2.4	241.34±7.76	34.01	592.46±6.19	22.32
Acetone		0.3	231.67±5.53	37.31	603.24±14.3	20.35
		0.6	169.67±6.56	58.47	572.41±7.66	25.89
		1.2	151.00±7.29	64.84	497.54±6.49	39.28
		2.4	115.34±6.43	77.01	426.37±5.73	51.96
Methanol		0.3	301.07±7.69	13.42	672.08±4.49	8.03
		0.6	259.00±8.33	27.98	641.19±7.36	13.57
		1.2	243.00±2.56	33.44	565.37±5.89	27.14
		2.4	194.33±4.69	50.05	527.00±6.46	33.92
Aqueous		0.3	146.00±5.53	66.55	412.67±7.36	54.46
		0.6	168.67±4.96	58.81	495.37±6.53	39.64
		1.2	225.00±7.63	39.59	532.45±6.13	33.03
		2.4	235.67±10.29	35.94	601.09±5.39	20.71
NC[b]	Hexane		53±5.36		210±4.36	
	Acetone		44±6.26		176±3.73	
	Methanol		54±3.73		116±4.66	
	dH_2O		83±3.93		163±2.79	
SC[c]			48±8.26		157±7.86	
PC[d]	NPD	10^{-2}	341±5.41		-	
	SA	10^{-3}	-		717±4.76	

a: Mean±standard deviation; b: negative control (100 µl/plate); c: spontaneous control; d: positive control.

DISCUSSION

This study provides a comprehensive evaluation of the antioxidant, mutagenic, and antimutagenic activities of different *C. esculenta* extracts, highlighting their potential health implications. Our results underscore the significance of solvent type and concentration in determining the bioactive properties of these extracts.

Among the different solvents tested, the acetone extract had the highest phenolic and flavonoid content, while the hexane extract had the lowest. These results highlight the superior efficiency of acetone in extracting phenolic and flavonoid compounds from *C. esculenta*.

Nur-Hadirah et al. (2021) also investigated TPC and antioxidant activities using various solvents (ethanol, methanol, and ethyl acetate) for taro petioles. Their findings indicated that methanol extracts had the highest TPC, but ethanol extracts demonstrated the greatest DPPH scavenging activity with an IC_{50} value of 308 µg/mL. This contrasts with our results, where acetone extracts outperformed both BHT and other solvents in antioxidant assays. This inconsistency could be due to differences in the plant parts used and the specific phenolic compounds present. Kasote et al. (2011) evaluated the TPC and antioxidant activity of methanol extracts of *C. esculenta* corms, finding a significantly lower TPC (0.0137 ± 1.33 mg GAE/g) compared to our study. Their methanol extract displayed moderate DPPH radical scavenging activity, which was less potent than the acetone extract in our study. This variance may be attributed to differences in extraction protocols, plant materials, and geographic variations in plant chemistry.

Das et al. (2023) examined the phenolic content of taro powder enriched with natural colorants, reporting a TPC of 46.78±0.19 mg GAE/100g for crude *C. esculenta*. This value is considerably lower than our findings for acetone extracts. The addition of colorants, such as *Syzygium cumini* and *Rosa indica*, increased the TPC, suggesting that these additives can enhance the antioxidant properties of taro products. However, our study's focus on solvent extraction alone highlights the high baseline antioxidant potential of *C. esculenta*. In another study, Kim et al. (2019) compared the TPC and TFC of steamed and unsteamed Fijian taro corms using various solvents. Their highest TPC values were obtained with methanol extracts of steamed corms (42.77 ± 3.39 mg GAE/g), and the highest TFC with methanol extracts (12.68 ± 4.85 mg CE/g). These values are significantly lower than the TPC and TFC obtained from our methanol extracts, reinforcing the superior extraction efficiency of methanol for these compounds.

Karigidi et al. (2019) evaluated the effects of different cooking methods on the phenolic and flavonoid contents and antioxidant capacities of *C. esculenta*. They found that roasted *C. esculenta* had the highest TPC (45.65±0.50 mg GAE/100g) and TFC (26.45±0.39 mg QUE/100g), while raw *C. esculenta* exhibited the highest total antioxidant capacity (TAC) (52.19±0.40 mg AsAE/100g). These values, although lower than our acetone extract findings, underscore the impact of cooking methods on the antioxidant properties of taro, suggesting that thermal processing can significantly alter its bioactive compound profile. The findings of this study highlight significant variances in the DPPH free radical scavenging activity of different *C. esculenta* extracts, providing insights into the impact of extraction solvents on antioxidant properties. Our study found that the acetone extract exhibited the highest DPPH free radical scavenging activity at 97.87%, surpassing the activity of BHT (95.77%). This demonstrates the exceptional efficiency of acetone as an extraction solvent for phenolic compounds with potent antioxidant properties. In contrast, the hexane extract showed the lowest activity at 33.96%, emphasizing the influence of solvent choice on antioxidant extraction efficiency. Comparing these results with the

literature, Akshatha et al. (2018) performed a DPPH analysis using methanol extracts of *C. esculenta* corms and leaves obtained through *in vitro* micropropagation. They reported IC_{50} values of 36.8 µg/mL and 23.3 µg/mL for greenhouse-stored plant leaves and corms, respectively, and 21.2 µg/mL and 21.4 µg/mL for micro-propagated leaves and corms. However, they confirm the high efficacy of methanol extracts, which aligns with the high activity observed in our acetone extract.

Chakraborty et al. (2015) found that methanol extracts of taro leaves exhibited higher antioxidant activity (81.77%) compared to tuber extracts (78.73%). These values are comparable to the high activities observed in our acetone extract, supporting the notion that the type of extract and plant part used significantly affect antioxidant activity. Keshav et al. (2019) analyzed the DPPH scavenging activity of ethanol, methanol, and chloroform extracts of fresh taro leaves. They reported activities of 84% for ascorbic acid, 78.92% for ethanol, 76.46% for methanol, and 72.46% for chloroform.

Kapcum et al. (2019) examined the impact of various cooking methods on the antioxidant activity of taro corms, reporting high DPPH values for raw taro (12826.98±53.04 mg Trolox/100 g). However, all cooking methods led to a reduction in antioxidant activity, with boiled taro exhibiting the lowest activity (1938.70±42.00 mg Trolox/100 g) and baked taro retaining relatively high activity (2122.66±91.77 mg Trolox/100 g). This underscores the significant influence of preparation methods on antioxidant properties, which might account for some variability in results across studies.

The evaluation of the mutagenic and antimutagenic activities of *C. esculenta* extracts in this study reveals significant insights into their potential health implications. Using the Ames/*Salmonella* assay, our study investigated four different extracts (aqueous, acetone, methanol, and hexane) for their mutagenic properties. The results indicate distinct variations among these extracts in terms of both mutagenicity and antimutagenicity. In our study, the aqueous extract of *C. esculenta* showed no mutagenic activity except against the TA100 strain at the highest concentration of 2400 µg/ml. This contrasts with the findings of Thepouyporn et al. (2006), where neither water nor ethanol extracts exhibited mutagenicity at 10 mg/plate for TA98 or TA100 strains, with or without S9 activation. This inconsistency could be attributed to differences in concentration and the specific conditions under which the assays were performed.

The acetone and methanol extracts in our study displayed increasing mutagenic activity in both TA98 and TA100 strains at concentrations ranging from 600 to 2400 µg/mL. These results align with the general observation in the literature that certain solvents can enhance the extraction of mutagenic compounds from plant materials. For instance, Nogodula et al. (2012) found that the aqueous-ethanolic extract of taro leaves demonstrated mutagenicity when tested with the *Salmonella typhimurium* TA98 strain, supporting our findings that certain solvent extracts can indeed exhibit mutagenic properties.

The hexane extract in our study showed mutagenic activity for the TA98 strain only at the highest concentration, while the TA100 strain exhibited mutagenicity at both 1200 and 2400 µg/mL. This is somewhat in contrast to Botting et al. (1999), who reported that methanol, ethyl acetate, and heptane extracts of various plants, including taro, did not result in a significant reduction in mutagenicity, suggesting that the mutagenic potential of hexane extracts might vary significantly with concentration and the specific strain tested.

Regarding antimutagenicity, our study found that the hexane and aqueous extracts showed a decrease in antimutagenicity in both strains with increasing concentration. Conversely, the acetone and methanol extracts exhibited an increase in antimutagenicity with increasing concentration. Notably, the lowest concentration of hexane (78.04%) and the highest concentration of acetone (77.01%) displayed the highest antimutagenic activity. This pattern is supported by Thepouyporn et al. (2006), who found that the ethanol extract at lower concentrations (0.1-0.5 mg/plate) was antimutagenic against various carcinogens in both strains, while higher concentrations showed cytotoxic effects. The observed cytotoxicity at higher concentrations might explain the decrease in antimutagenicity for some extracts in our study. Botting et al. (1999) did not observe a significant antimutagenic effect with methanol extracts of taro leaves, potentially due to increased bacterial growth from additional nutrients in the medium. This highlights the complexity of interactions in the assay environment that can influence the observed outcomes. Our findings suggest that solvent type and concentration are crucial factors in determining the antimutagenic efficacy of *C. esculenta* extracts.

Our study demonstrates that while certain extracts of *C. esculenta* exhibit promising antimutagenic properties, others may present mutagenic risks at higher concentrations. These observations, consistent with existing literature, emphasize the need for meticulous assessment of these extracts in therapeutic applications. Future research should aim to isolate specific bioactive compounds to further elucidate their mechanisms and optimize their beneficial use in health-related interventions.

Conclusion

In developing countries, tubers are often among the most affordable sources of dietary energy, providing carbohydrates. Taro, a tropical tuberous plant, is primarily cultivated for its subterranean corms and is widely eaten in tropical regions. In terms of nutrition, taro provides a wider array of nutrients and vitamins compared to other tubers. Despite being a reservoir of numerous nutrients and bioactive compounds, *Colocasia esculenta* (L.) leaves remain underutilized. Due to their high protein content with a well-rounded amino acid profile, along with a variety of micronutrients and bioactive compounds, taro leaves are considered advantageous compared to other leafy greens for potential use in nutraceuticals and functional foods. Acetone and methanol extracts showed strong DPPH radical scavenging activity along with high TPC and TFC content. These results can be evaluated as a potential antioxidant in future *in vitro* and *in vivo* studies. The results suggest that while the hexane and water

extracts of *C. esculenta* exhibit a decrease in antimutagenic activity with increasing concentration, the acetone and methanol extracts demonstrate a promising increase in antimutagenic activity at higher concentrations, with the hexane extract at its lowest concentration and the acetone extract at its highest concentration showing the most significant antimutagenic effects. These findings highlight the potential of specific extracts of *C. esculenta* in alleviating mutagenic effects, highlighting the need for further investigation into their mechanisms and applications.

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Conflicts of interest

There is no conflict of interest between the authors of the article.

REFERENCES

Akshatha, M. D., Kavadikeri, S., & Rao, N. N. (2018). In vitro micropropagation and antioxidant assay in *Colocasia esculenta*. *Plant Tissue Cult. Biotechnol*, *28*, 183-190. https://doi.org/10.3329/ptcb.v28i2.39677

Akyüz, M. (2019). Determination of Antioxidant Activity of Ethanol Extract of Gölevez [(*Colocasia esculenta* (L.)] Tubers. *Kahramanmaraş Sütçü İmam Üniversitesi Tarım ve Doğa Dergisi*, *22*, 388-394.

Angami, T., Jha, A. K., Buragohain, J., Deka, B. C., Verma, V. K., & Nath, A. (2015). Evaluation of taro (*Colocasia esculenta* L.) cultivars for growth, yield, and quality attributes. *Journal of Horticultural Sciences*, 183-189.

Blois, M. S. (1958). Antioxidant determinations by the use of a stable free radical. *Nature*, 181 (4617), 1199-1200. https://doi.org/10.1038/1811199a0

Botting, K. J., Young, M. M., Pearson, A. E., Harris, P. J., & Ferguson, L. R. (1999). Antimutagens in food plants eaten by Polynesians: micronutrients, phytochemicals and protection against bacterial mutagenicity of the heterocyclic amine 2-amino-3-methylimidazo [4, 5-f] quinoline. *Food and Chemical Toxicology*, *37*(2-3), 95-103.

Brown, A. C., Reitzenstein, J. E., Liu, J., & Jadus, M. R. (2005). The anti-cancer effects of poi (*Colocasia esculenta*) on colonic adenocarcinoma cells in vitro. *Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives*, *19*(9), 767-771. https://doi.org/10.1002/ptr.1712

Chakraborty, P., Deb, P., Chakraborty, S., Chatterjee, B., & Abraham, J. (2015). Cytotoxicity and antimicrobial activity of *Colocasia esculenta*. *Journal of Chemical and Pharmaceutical Research*, 7(12), 627-635. https://doi.org/10.5555/20163058035

Chivenge, P., Mabhaudhi, T., Modi, A. T., & Mafongoya, P. (2015). The potential role of neglected and underutilized crop species as future crops under water-scarce conditions in Sub-Saharan

Africa. *International Journal of Environmental Research and Public Health*, *12*(6), 5685-5711. https://doi.org/10.3390/ijerph120605685

Council of Scientific & Industrial Research (India). (1972). *The Wealth of India: A Dictionary of Indian Raw Materials and Industrial Products* (Vol. 9).

Das, S., Kandali, R., & Baishya, S. (2023). Development and Nutritional Analysis of Taro Powder [*Colocasia esculenta* (L.) Schott.] Enriched with Natural Colorants. *International Journal of Environment and Climate Change*, *13*(9), 2883-2895. https://doi.org/10.9734/IJECC/2023/v13i92523

Ehiobu, J. M., & Ogu, G. I. (2018). Phytochemical content and in vitro anti-mycelial efficacy of *Colocasia esculenta* L, *Manihot esculenta Crantz* and *Dioscorea rotundata* Poir leaf extracts on *Aspergillus niger* and *Botryodiplodia theobromae*. *Journal of Horticulture and Plant Research*, *1*, 9-18. https://doi.org/10.18052/www.scipress.com/JHPR.1.1

Elmosallamy, A., Eltawil, N., Ibrahim, S., & Hussein, S. A. A. (2021). Phenolic Profile: Antimicrobial Activity and Antioxidant Capacity of *Colocasia esculenta* (L.) Schott. *Egyptian Journal of Chemistry*, *64*(4), 2165-2172. 10.21608/ejchem.2021.56495.3213

Kapcum, C., Pasada, K., Kantiwong, P., Sroysang, B., Phiwtawee, J., Suphantharika, M., ... & Wongsagonsup, R. (2022). Effects of different cooking methods on chemical compositions, in vitro starch digestibility, and antioxidant activity of taro (*Colocasia esculenta*) corms. *International Journal of Food Science & Technology*, *57*(8), 5144-5154. https://doi.org/10.1111/ijfs.15823

Karigidi, K. O., Adetuyi, F. O., Akintimehin, E. S., Dada, I. O., & Olugotun, A. F. (2019). Effect of cooking methods on polyphenol, antioxidant, and inhibition of key enzymes linked to carbohydrate metabolism of cocoyam (*Colocasia esculenta* L. Schott). *Annals. Food Science and Technology, 20*(3), 543-552.

Kasote, D. M., Bhalerao, B. M., Jagtap, S. D., Khyade, M. S., & Deshmukh, K. K. (2011) Antioxidant and alpha-amylase inhibitory activity of methanol extract of *Colocasia esculenta* corm. *Pharmacologyonline* 2: 715-721.

Keshav, A., Sharma, A., & Mazumdar, B. (2019). Phytochemical analysis and antioxidant activity of *Colocasia esculenta* (L.) leaves. *International Journal of Chemical and Molecular Engineering*, *13*(1), 20-23. doi.org/10.5281/zenodo.3607765

Kim, Y. S., Adeyemi, D., Korovulavula, P., Jang, D. W., & Park, M. K. (2019). Effect of steaming on the functional compounds and antioxidant activity of Fijian taro (*Colocasia esculenta* L. Schott) corms. *Korean Journal of Food Preservation*, *26*(4), 449-454. https://doi.org/10.11002/kjfp.2019.26.4.449

Kubde, M. S., Khadabadi, S. S., Farooqui, I. A., & Deore, S. L. (2010). In-vitro anthelmintic activity of *Colocasia esculenta*. *Der Pharmacia Lettre*, *2*(2), 82-85.

Kumawat, N. S., Chaudhari, S. P., Wani, N. S., Deshmukh, T. A., & Patil, V. R. (2010). Antidiabetic activity of ethanol extract of *Colocasia esculenta* leaves in alloxan-induced diabetic rats. *International Journal of PharmTech Research*, *2*(2), 1246-9.

Kundu, N., Campbell, P., Hampton, B., Lin, C. Y., Ma, X., Ambulos, N., ... & Fulton, A. M. (2012). Antimetastatic activity isolated from *Colocasia esculenta* (taro). *Anti-cancer Drugs*, *23*(2), 200-211. https://doi.org/10.1097/CAD.0b013e32834b85e8

Kundu, N., Ma, X., Hoag, S., Wang, F., Ibrahim, A., Godoy-Ruiz, R., ... & Fulton, A. M. (2021). An extract of taro (*Colocasia esculenta*) mediates potent inhibitory actions on metastatic and cancer stem cells

by tumor cell-autonomous and immune-dependent mechanisms. *Breast Cancer: Basic and Clinical Research*, *15*, 11782234211034937. https://doi.org/10.1177/11782234211034937

Lee, M. H., Lin, Y. S., Lin, Y. H., Hsu, F. L., & Hou, W. C. (2003). The mucilage of yam (*Dioscorea batatas* Decne) tuber exhibited angiotensin-converting enzyme inhibitory activities. *Botanical Bulletin of Academia Sinica*, 44.

Li, H. M., Hwang, S. H., Kang, B. G., Hong, J. S., & Lim, S. S. (2014). Inhibitory effects of *Colocasia esculenta* (L.) Schott constituents on aldose reductase. *Molecules*, *19*(9), 13212-13224. https://doi.org/10.3390/molecules190913212

Lim, T. K. (2015). *Colocasia esculenta*. Edible Medicinal and Non-Medicinal Plants: Volume 9, Modified Stems, Roots, Bulbs, 454-492. https://doi.org/10.1007/978-94-017-9511-1

Liu, Q., Donner, E., Yin, Y., Huang, R. L., & Fan, M. Z. (2006). The physicochemical properties and in vitro digestibility of selected cereals, tubers, and legumes grown in China. *Food Chemistry*, 99(3), 470-477. https://doi.org/10.1016/j.foodchem.2005.08.008

Maron, D. M., & Ames, B. N. (1983). Revised methods for the Salmonella mutagenicity test. *Mutation Research/Environmental Mutagenesis and Related Subjects*, *113*(3-4), 173-215. https://doi.org/10.1016/0165-1161(83)90010-9

Masui, H., Kondo, T., & Kojima, M. (1989). An antifungal compound, 9, 12, 13-trihydroxy-(E)-10-octadecenoic acid, from *Colocasia antiquorum,* inoculated with *Ceratocystis fimbriata*. *Phytochemistry*, *28*(10), 2613-2615. https://doi.org/10.1016/S0031-9422(00)98051-8

Matejić, J. S., Džamić, A. M., Mihajilov-Krstev, T. M., Ranđelović, V. N., Krivošej, Z. Đ., & Marin, P. D. (2013). Total phenolic and flavonoid content, antioxidant and antimicrobial activity of extracts from *Tordylium maximum*. *Journal of Applied Pharmaceutical Science*, 3(01), 055-059 10.7324/JAPS.2013.30110

Matthews, P. J., & Ghanem, M. E. (2021). Perception gaps that may explain the status of taro (*Colocasia esculenta*) as an "orphan crop". *Plants, People, Planet*, *3*(2), 99-112. https://doi.org/10.1002/ppp3.10155

Mitharwal, S., Kumar, A., Chauhan, K., & Taneja, N. K. (2022). Nutritional, phytochemical composition and potential health benefits of taro (*Colocasia esculenta* L.) leaves A review. *Food Chemistry*, *383*, 132406. https://doi.org/10.1016/j.foodchem.2022.132406

Mitsuda, H., Yasumoto, K., & Iwami, K. (1966). Antioxidative action of indole compounds during the autoxidation of linoleic acid. *Eiyo to Shokuryo* 19: 210–214.

Mortelmans, K., & Zeiger, E. (2000). The Ames Salmonella/microsome mutagenicity assay. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*, *455*(1-2), 29-60. https://doi.org/10.1016/S0027-5107(00)00064-6

Nogodula, J. N., Draug, J. M. D., Jamero, M. S., & Suyom, C. L. E. (2012). Phytochemical and antibacterial action of taro (*Colocasia esculenta*, Araceaea) aqueous-ethanolic leaf extract against selected bacterial strains. *UIC Research Journal*, *18*(1), 221-236.

Nur-Hadirah, K., Arifullah, M., Nazahatul, A. A., Klaiklay, S., Chumkaew, P., Norhazlini, M. Z., & Zulhazman, H. (2021). Total phenolic content and antioxidant activity of an edible Aroid, *Colocasia esculenta* (L.) Schott. In *IOP Conference Series: Earth and Environmental Science, 756*(1), 012044. https://doi.org/10.1088/1755-1315/756/1/012044

Onwueme, I. (1999). Taro cultivation in Asia and the Pacific. RAP publication, 16, 1-9.

Patel, A., & Singh, J. (2023). Taro (*Colocasia esculenta* L): Review on its botany, morphology, ethno-medical uses, phytochemistry, and pharmacological activities. *The Pharma Innovation Journal*, *12*(2), 5-14. https://doi.org/10.22271/tpi.2023.v12.i3a.18908

Prajapati, R., Kalariya, M., Umbarkar, R., Parmar, S., & Sheth, N. (2011). *Colocasia esculenta*: A potent indigenous plant. *International Journal of Nutrition, Pharmacology, Neurological Diseases*, 1(2), 90-96. https://doi.org/10.4103/2231-0738.84188

Prathibha, S., Nambisan, B., & Leelamma, S. (1995). Enzyme inhibitors in tuber crops and their thermal stability. *Plant Foods for Human Nutrition*, *48*, 247-257. https://doi.org/10.1007/BF01088446

Rasouli, H., Farzaei, M. H., & Khodarahmi, R. (2017). Polyphenols and their benefits: A review. *International Journal of Food Properties*, *20*(sup2), 1700-1741. https://doi.org/10.1080/10942912.2017.1354017

Ribeiro Pereira, P., Bertozzi de Aquino Mattos, E., Nitzsche Teixeira Fernandes Correa, A. C., Afonso Vericimo, M., & Margaret Flosi Paschoalin, V. (2020). Anticancer and Immunomodulatory Benefits of Taro (*Colocasia esculenta*) Corms, an underexploited tuber crop. *International Journal of Molecular Sciences*, *22*(1), 265. https://doi.org/10.3390/ijms22010265

Sheikh, M. A., & Tembhre, M. (2016). Preliminary phytochemical screening, in vitro antioxidant activity, total phenolic and total flavonoid contents of *Colocasia esculenta* leaf extract. *Asian Journal of Experimental Sciences*, *30*, 39-43.

Sheikh, M. A., & Tembhre, M. (2018). Liver Toxicity and its Amelioration by Natural Antioxidants-A Review. *Asian Journal of Experimental Sciences*, *32*(1), 35-43.

Sheth, A., Mitaliya, K. D., & Joshi, S. (2005). The herbs of Ayurveda. *Ahmedabad: AK Sheth Publishers*, *356*.

Singh, S., Singh, D. R., Salim, K. M., Srivastava, A., Singh, L. B., & Srivastava, R. C. (2011). Estimation of proximate composition, micronutrients and phytochemical compounds in traditional vegetables from Andaman and Nicobar Islands. *International Journal of Food Sciences and Nutrition*, *62*(7), 765-773. https://doi.org/10.3109/09637486.2011.585961

Slinkard, K., & Singleton, V. L. (1977). Total phenol analysis: automation and comparison with manual methods. *American Journal of Enology and Viticulture*, *28*(1), 49-55. https://doi.org/10.5344/ajev.1977.28.1.49

Sudhakar, P., Thenmozhi, V., Srivignesh, S., & Dhanalakshmi, M. (2020). *Colocasia esculenta* (L.) Schott: Pharmacognostic and pharmacological review. *Journal of Pharmacognosy and Phytochemistry*, *9*(4), 1382-1386. 10.22271/phyto.2020.v9.i4s.11937

Thepouyporn, A., Kwanbunjan, K., Pooudong, S., & Changbumrung, S. (2006). Mutagenicity study of weeds and common plants used in traditional medicine and for animal feed. *Southeast Asian Journal of Tropical Medicine and Public Health*, *37*, 195.

Tungmunnithum, D., Thongboonyou, A., Pholboon, A., & Yangsabai, A. (2018). Flavonoids and other phenolic compounds from medicinal plants for pharmaceutical and medical aspects: An overview. *Medicines*, *5*(3), 93. https://doi.org/10.3390/medicines5030093

Tuti, M. D., Pal, R. S., Arun Kumar, R., Bist, J. K., & Bhatt, J. C. (2015). Colocasia-based cropping systems affects the antioxidant properties and productivity of colocasia [*Colocasia esculenta* (l.) schott] tuber. *The Bioscan an International Quaterly Journal of Life Sciences*, *10*(1), 117-123.