

Phytochemical Analysis and Antioxidant Activity of *Amorphophallus paeoniifolius*, *Colocasia antiquorum* and *Colocasia esculenta* Leaves from the Araceae Family

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ABSTRACT

The Araceae family, comprising species like *Amorphophallus paeoniifolius*, *Colocasia antiquorum*, and *Colocasia esculenta*, is of significant interest due to their diverse medicinal and nutritional properties. These plants possess bioactive phytochemicals such as alkaloids, saponins, flavonoids, terpenoids, and tannins contributing to their therapeutic potential in treating various diseases like cancer, rheumatoid arthritis etc. Thus, exploring the phytochemicals from Araceae family plants can bring new aspects in herbal drug discovery and development. Currently herbal drugs market demand is very high as compared to other alternatives that may cause side effects. This current study objective was to explore some noteworthy bioactive compounds from leaves of *Amorphophallus paeoniifolius*, *Colocasia antiquorum* and *Colocasia esculenta*. This study confirms the presence of different phytochemical groups in Araceae family plants that may have therapeutic benefits if used in herbal drug formulations against cancer. Initially, all 3 plant (leaves) extracts were prepared with different solvents using the Soxhlet method and screened for phytochemical groups. Further phytochemical groups were determined using UV-visible spectroscopy. Further, the extracts were also explored for antioxidant activity due to their richness in antioxidant molecules. The quantitative analysis revealed that *Amorphophallus paeoniifolius* exhibited the highest crude yield of alkaloids and saponins while *Colocasia esculenta* demonstrated the highest yield of flavonoids and total phenolic total phenolic content. In UV-visible spectroscopy analysis identified some phytochemicals like unknown phenolic acids, unknown flavonoid, chlorophyll etc. The antioxidant potential using the DPPH assay showed that the ethanolic extract of *Colocasia esculenta* exhibited the highest scavenging activity, achieving 84.14% inhibition at 500µg/ml. The IC₅₀ values further confirmed the varying antioxidant capacities, as *Colocasia antiquorum* ethanol extracts showed better results then *Amorphophallus paeoniifolius*, *Colocasia esculenta*. This study shows phytochemical of these three plants are having high therapeutic importance, particularly in their antioxidant properties, supporting their use in traditional medicine and potential for further pharmacological applications.

Keywords: Araceae family, DPPH assay, IC₅₀, traditional medicine, bioactive compounds, pharmacological applications.

INTRODUCTION

Since antiquity, the family Araceae has garnered significant scholarly attention due to its numerous traditional applications; this taxonomic family of species is employed in herbal medicine owing to its antioxidant, hepatoprotective, antibacterial, anticancer, and nutritional attributes. Ranking as the fourth largest among monocotyledonous plant families, subsequent

to sedges, grasses, and orchids, this family encompasses 125 genera comprising approximately 3,750 species^[1,2]. Plants within the Araceae family represent vital contributors to agriculture, food production, and medicinal applications^[3]. Among the various genera within the Araceae family, *Colocasia* and *Amorphophallus* are recognized as sources of edible aroids. These plants thrive in rain-dependent perennial ecosystems and other moist habitats, exhibiting adaptability to fluctuating environmental conditions, such as alterations in water availability and temperature^[4,5]. According to Wei *et al.* (2008), chronic herbs, which are inclusive of the tribes within the Araceae family, such as *Amorphophallus paeoniifolius*, *Colocasia antiquorum*, and *Colocasia esculenta*, can utilize all parts of the plant for culinary, medicinal, and feed ingredient purposes; these plants are also recognized for containing phytochemicals applicable in cosmetic treatments and possess the potential to degrade plastic materials, characterizing them as annual herbaceous plants within the Araceae family^[6,7]. In addition to these clinical applications, the medicinal properties of these plants are extensively utilized in traditional medicine for the management of conditions such as acute rheumatism, tumors, pulmonary edema, asthma, emesis, and abdominal discomfort.^[8,9] The Araceae family is endowed with noteworthy medicinal properties, characterized by specialized phytochemicals that exert distinct physiological effects on the human organism, with bioactive constituents that extend beyond tannins, alkaloids, terpenoids, steroids, and flavonoids, to encompass saponins, phenolic compounds, glycosides, and essential oils, all of which contribute to its therapeutic efficacy.^[10] The investigation of these plants for their anti-inflammatory, antimicrobial, antifungal, antibacterial, and antihypertensive properties remains a promising avenue for research^[11,12]. This research endeavor primarily concentrates on the extraction and screening of phytochemicals from *Amorphophallus paeoniifolius*, *Colocasia antiquorum*, and *Colocasia esculenta*, accompanied by quantitative phytochemical analysis utilizing UV-visible spectroscopy. The extracts obtained from the aforementioned plants were also subjected to evaluation for antioxidant activity.

MATERIALS AND METHODS

Sample Collection and Extraction

For this investigation, the botanical materials utilized comprised the foliage of *Amorphophallus paeoniifolius*, *Colocasia antiquorum*, and *Colocasia esculenta*, which were procured from the Kalinga University greenhouse located in Raipur, India. The leaves underwent a process of fragmentation and were subjected to desiccation at ambient temperature for a duration of 3 to 4 days. Upon completion of the drying process, the plant materials were subjected to mechanical pulverization into a fine powder utilizing a mechanical blender (Model: Waring Commercial Blender HGB140). The resultant powdered samples were subsequently extracted employing the Soxhlet extraction method (Soxhlet apparatus, BUCHI) over a period of 72 hours utilizing solvents such as acetone, ethanol, and methanol, arranged in a sequence of increasing polarity (acetone: low polarity < ethanol: medium polarity < methanol: high polarity). The extracts were preserved in the refrigeration unit at a temperature of 4°C until they were necessitated for subsequent phytochemical evaluation. Additionally, the extracts underwent qualitative chemical assessments utilizing picric acid, potassium iodide, distilled water, sodium hydroxide, ferric chloride, ammonium hydroxide, among other reagents (acquired from the Biotechnology Laboratory at Kalinga University, Raipur) to ascertain the presence of diverse bioactive compounds, followed by quantitative analysis employing UV-visible spectroscopy (Model: Shimadzu UV-1800) operated at a rotational speed of 1200 rpm. Moreover, the extracts from all three botanical specimens were assessed for their potential antioxidant enzymes, which are instrumental in mitigating oxidative stress.

Phytochemical Screening

The methodology of phytochemical screening serves as a valuable tool and is extensively employed for the identification of bioactive chemical constituents found within botanical specimens. These compounds, synthesized by plant organisms, play an instrumental role in the potential treatment of a variety of ailments. The process of phytochemical screening encompasses both qualitative and quantitative approaches that facilitate the identification and assessment of the presence or absence of specific phytochemicals. Preliminary of all 3 plants were screened using crude leaf extracts prepared with solvent (acetone<ethanol<methanol) total of 3 samples were prepared for each plant using standard procedures and methods mentioned below.

Detection of Alkaloids: Each plant extracts were dissolved in dilute hydrochloric acid and passed through a filter. The Alkaloidal test: filtrates were analyzed for the presence of alkaloids.

Mayer's Test: Each filtrates were added to Mayer's reagent, formation of yellow cream precipitate indicated the existence of alkaloids.

Wagner's test: Each filtrates were treated with Wagner's reagent, and brown or reddish-brown precipitate indicated alkaloid presence ^[13].

Detection of Saponins

Foam Test: 1ml of each extract was shaken with 5ml of distilled water. Their presence was confirmed by the formation of stable, persistent foam ^[14].

Detection of Terpenoids

Salkowski Test: 0.5ml of each extract was mixed with 2ml of chloroform, then added 3ml of concentrated sulfuric acid(H₂SO₄) carefully to form layer. At the interface, a reddish-brown color formation indicated the presence of terpenoids ^[15].

Detection of Tannins: About 0.5g of each extract was dissolved in 10ml distilled water and filtered. Some drops of ferric chloride solution were added to ml of the filtrate. A green or bluish-green precipitate was formed, indicating the presence of tannins ^[16].

Quantitative Phytochemical Analysis

Determination of Total Saponins

To explore Saponins in each sample the method used given by of Obdoni and Ochuko.*et al*, (2001). The powdered material (20g) was mixed with 100ml of 20% aqueous ethanol. This mixture was stirred and kept in a hot water bath at around 55°C for 4hours. The filtrate was then subjected to filtration and the residue re-extracted with 200ml of 20% ethanol. The combined extracts were evaporated in a water bath at approximately 90°C, until concentrated to 40ml, and moved to a 250ml separation funnel followed by the addition of 20ml of diethyl ether and vigorous shaking. The aqueous layer was cut off and the ether layer was discarded. This purification step was repeated. Then, 60ml of n-butanol was added to the aqueous extract. The aggregated n-butanol phases were washed twice with 10ml of 5% aqueous NaCl. When the solvent evaporated, the remaining solution was dried in an oven until reached to a constant weight or dried in a water bath. The saponin content was subsequently assessed ^[17].

Determination of Flavonoid by using Bohm and Kocipai Abvazan (1994) method

Ten grams of each plant sample was extracted several times with 100ml of 80% aqueous methanol at room temperature. The final solution was filtered by Whatman filter paper number

42(125mm). Then the filtrate was evaporated to dryness in a water bath after transferring it to a crucible. The residue was weighed when it was at a constant weight ^[18].

Determination of Alkaloid using Harborne (1973) method

A 5g of each plant sample was conditioned into 250ml beaker, and 200ml 10% acetone in ethanol was dropped in the beaker. The beaker was then covered, and the mixture was allowed to stand for 4hours. The solution was filtered, and the extract was concentrated to a quarter of its original volume in a water bath. The extract was precipitated with dropwise addition of a concentrated ammonium hydroxide solution. The mixture was let to sit, the precipitate filtered, washed with dilute ammonium hydroxide, and dried. The alkaloid was obtained and then dried and weighed ^[19].

Determination of Total Phenolic Content (TPC)

The total phenolic content of the sample was determined using Folin-Ciocalteu reagent described by Biradar SR.*et al.*(2013), with slightly modifications. Briefly, 10mg of the crude extract was dissolved in 10ml of ethanol (1mg/ml) and vortex till becomes homogenous stock solution ^[20]. Various dilutions were prepared from this stock solution. Then, the solution was treated with the Folin-Ciocalteu reagent. After 3minutes of incubation, 20%(w/v) sodium carbonate was added to the solution and allowed to stand for 1hour at room temperature before the absorbance was measured at 765nm using UV-visible spectrophotometer. The total phenolic content was determined using a calibration curve based on a gallic acid standard solution and expressed as milligrams of gallic acid equivalents (GAE) per gram of sample (mg/g). Further results were analyzed.

Analytical Studies

UV-Visible Spectroscopy

Identification of phytochemicals in all 3 plants were performed using UV-visible spectroscopy (Shimadzu UV-1800). UV-visible spectroscopy is a technique that involves measuring a substance's ability to absorb ultraviolet light, which can be related to its electronic structure and the presence of certain functional groups ^[21]. Some natural phytochemical, such as alkaloids, flavonoids, and polyphenols, have unique UV absorption spectra. The concentration of a given compound in a sample can be quantified by building a calibration curve which correlates concentration to absorbance at a given wavelength. UV-visible spectroscopy was performed on the diluted each plant samples (diluted to 1:10). The readings & data thus obtained from UV-visible spectroscopy were documented.

Antioxidant Activity using DPPH scavenging assay

Antioxidants are molecules that play a crucial role in protecting the body from oxidative stress, a condition caused by an imbalance between free radicals and the body's ability to neutralize them. DPPH is a free radical that appears red in color but turns yellow upon scavenging, which corresponds to a specific absorbance. The degree of discoloration reflects the scavenging potential of antioxidant compounds, particularly their hydrogen-donating ability. DPPH is a free radical that appears red in color but turns yellow upon scavenging, which corresponds to a specific absorbance. The degree of discoloration reflects the scavenging potential of antioxidant compounds, particularly their hydrogen-donating ability.

The DPPH assay was performed according to the method of Barman BN.*et al.*(2000), with brief modifications. A solution of DPPH was prepared by dissolving 0.6mg of DPPH in 15ml of

ethanol, with a concentration of 0.1mm^[22]. The working procedure involved pipetting 2.5ml of the extract at different concentrations (300, 350, 400, 450, and 500 µg/ml) into 2.5ml of DPPH solution, resulting in a total solution volume of 5ml. The scavenging effect was measured after 20 minutes of incubation in the dark using a UV-vis spectrophotometer at 517 nm. DPPH scavenging ability was determined using the following formula:

$$\text{DPPH scavenging activity (\%)} = [(\text{Abs control} - \text{Abs sample}) / \text{Abs control}] \times 100 \quad (1)$$

[Where: Abs control= Absorbance of DPPH + absolute ethanol Abs sample = Absorbance of DPPH radical + sample or standard]

RESULTS AND DISCUSSION

The present study on leaves of *Amorphophallus paeoniifolius*, *Colocasia antiquorum* and *Colocasia esculenta* leaves revealed the presence of bioactive compounds with therapeutic importance. The phytochemical extracts prepared using Soxhlet were screened using chemical tests and samples were subjected to quantitative analysis, the results are as follow.

Phytochemical Screening and extraction

The phytochemical constituents were extracted using Soxhlet with solvents (acetone<ethanol<methanol) as per polarity level. Thus, table1 shows the results of the phytochemical screening of *Amorphophallus paeoniifolius*, *Colocasia antiquorum* and *Colocasia esculenta* leaves extracts. The findings indicate the presence of many phytochemical compounds like alkaloids, tannins, saponins, terpenoids and flavonoids in all 3 species of the Araceae family. Presence of tannins, which possess strong astringent properties, was found in all samples. Tannins are known to promote the healing of wounds and inflamed mucous membranes^[23]. The presence of alkaloids was also confirmed they are known for antiproliferative and anti-metastatic effects on various types of cancer^[24]. Additionally, alkaloids confirmation shows the plants may exhibit anti-inflammatory and antioxidant properties^[25]. In *Colocasia esculenta*, the Salkowski test for terpenoids did not indicate the presence of terpenoids in any of the extracts. However, the ferric fluoride test for tannins showed their presence in the methanol and ethanol extracts, but not in the acetone extract. These results highlight the solvent dependent extraction efficiency of phytochemicals.

Quantitative analysis

The detected phytochemical group present in table 1 are present in varying quantity in all 3 plants *Amorphophallus paeoniifolius*, *Colocasia antiquorum* and *Colocasia esculenta*. Thus, the yield of all 3 plants were explored and data is shown in table2. In results among these plants, *Amorphophallus paeoniifolius* exhibited the highest crude yield of alkaloids, while *Colocasia antiquorum* had the lowest yield of alkaloids. Also, the highest crude yield of flavonoid is present in *Colocasia esculenta* as compared with other plants. The results are shown in Figure1.

Table 1: Results of phytochemical screened from of leaves from *Amorphophallus paeoniifolius*, *Colocasia antiquorum* and *Colocasia esculenta*.

Sr.No	Name of test	Test/Reagent used	<i>Amorphophallus paeoniifolius</i>			<i>Colocasia antiquorum</i>			<i>Colocasia esculenta</i>		
			ME	EE	AE	ME	EE	AE	ME	EE	AE
1	Alkaloids	Mayer's reagent	+	+	-	-	-	-	-	+	-
		Wagner's reagent	+	+	+	+	-	+	+	+	-
2	Saponins	Foam test	+	+	+	+	+	-	+	-	-
3	Flavonoids	Lead acetate test	+	+	-	+	+	-	+	+	+
		Alkaline reagent test	+	+	+	+	-	-	+	+	+
4	Terpenoids	Salkowski test	-	+	-	-	+	+	-	-	-
5	Tannins	Ferric chloride test	+	-	-	+	+	-	+	+	-

Key note: ME = methanol extract, EE= ethanol, AE=acetone, +(presence) and -(absence) of phytochemical.

Table 2 summarizes the results of the quantitative phytochemical analysis of *Amorphophallus paeoniifolius*, *Colocasia antiquorum* and *Colocasia esculenta*. This experiment aligns with the findings of Longbap, B.D.*et al*,(2018) and presents the percentage yield of total crude phytochemicals. Among the tested species, *Amorphophallus paeoniifolius* exhibited the highest crude yield of alkaloids, and saponin while flavonoids showed the lowest yield. In contrast, *Colocasia esculenta* demonstrated the highest yield of total phenolic compounds and flavonoids compared to *Amorphophallus paeoniifolius* and *Colocasia antiquorum*. And *Colocasia esculenta* content the lowest yield of saponins. These variations in phytochemical yield highlight species-specific differences and the diverse potential applications of these plants [15].

Table 2: Results of quantitative phytochemical analysis of *Amorphophallus paeoniifolius*, *Colocasia antiquorum* and *Colocasia esculenta* leaf extract.

	Sr. No	Name of Phytochemical Test	Sample Weight (G)	Dried Filtrate Weight (G)	% Calculated For Total Crude Yield
<i>Amorphophallus paeoniifolius</i>	1	Alkaloids	5	0.5	10
	2	Flavonoids	10	0.7	7
	3	Saponins	20	1.9	9.5
	4	Total phenolic content	10	1.4	14
<i>Colocasia antiquorum</i>	1	Alkaloids	5	0.3	6
	2	Flavonoids	10	0.9	9
	3	Saponins	20	1.1	5.5
	4	Total phenolic content	10	1.5	15
<i>Colocasia esculenta</i>	1	Alkaloids	5	0.4	8
	2	Flavonoids	10	1.5	15
	3	Saponins	20	0.5	2.5
	4	Total phenolic content	10	2.0	20

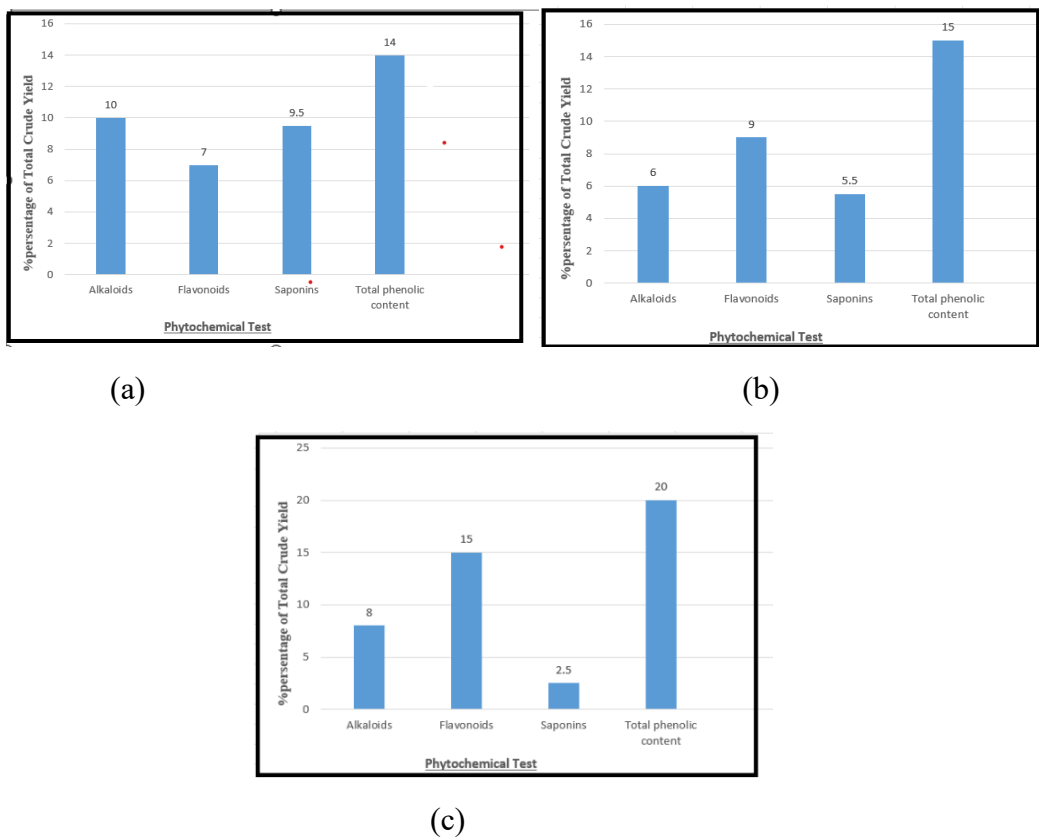


Figure 1: The histograms display the quantification analysis of phytochemicals (1 = Alkaloids, 2 = Flavonoids, 3 = Saponins, 4 = Total phenolic content) present in varying amounts in the leaves of: (a) *amorphophallus paeoniifolius*, (b) *Colocasia antiquorum* (c) *Colocasia esculenta*.

UV-Visible Spectroscopy Analysis for *Amorphophallus paeoniifolius*, *Colocasia antiquorum* and *Colocasia esculenta* leaf extract

Further extracts of 3 plants were investigated to identify and quantify the components of the sample by analyzing the pattern of absorption and transmission of light. The UV-visible spectroscopy analysis determined the λ_{max} peak values for each solvent extract, which correlate with the presence of phytochemicals identified through chemical tests. Based on Jain PK, *et al*,(2016) analysis preformed for identification of phytochemicals present in methanolic extract of *M. spicata*. The λ_{max} peak values for methanolic, ethanolic and acetone extracts of *Amorphophallus paeoniifolius*, *Colocasia antiquorum* and *Colocasia esculenta* were observed representing the identification of some phytochemicals. The obtained data of UV-visible spectroscopic analysis is given as follows in table3.

Antioxidant Activity of *Amorphophallus paeoniifolius*, *Colocasia antiquorum* and *Colocasia esculenta*.

Antioxidant activity when explored using DPPH assay, the result of these study are shown in table 4, ethanolic extract of *Colocasia esculenta* has shown highest percentage of inhibition across all different samples concentrations (100-500 $\mu\text{g/ml}$). At 500 $\mu\text{g/ml}$ concentration, the ethanolic extract of *Colocasia esculenta* achieved 84.14% inhibition, while the ethanolic extracts of *Colocasia antiquorum* and *Amorphophallus paeoniifolius* showed lower inhibition percentages data shown in (figure 2).

Table 3: UV-Visible spectroscopy results of *Amorphophallus paeoniifolius*, *Colocasia antiquorum* and *Colocasia esculenta* leaf extracts.

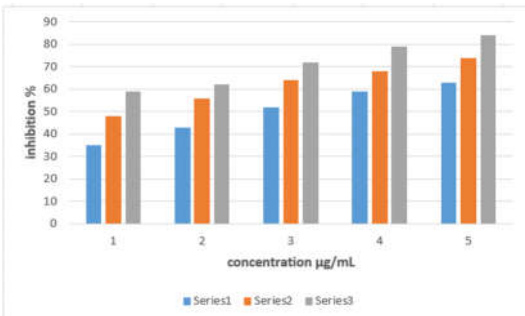
<u>Phytochemical</u>	<u><i>Colocasia esculenta</i></u>			<u><i>Amorphophallus paeoniifolius</i></u>			<u><i>Colocasia antiquorum</i></u>		
	ME	EE	AE	ME	EE	AA	ME	EE	AA
Unknown phenolic acids	880	875	892	884	872	889	886	863	883
Unknown flavonoids	825	813	802	823	833	821	829	817	829
chlorophyll b	650	665	648	660	659	653	663	657	669
chlorophyll a	610	617	623	616	615	618	607	611	612
Taraxerol	530	548	559	538	543	558	536	543	533
β carotene	435	415	428	431	433	434	423	429	413

Key note: Unit value of [λmax] = nm. ME = methanol extract, EE= ethanol extract, AE=acetone extract.

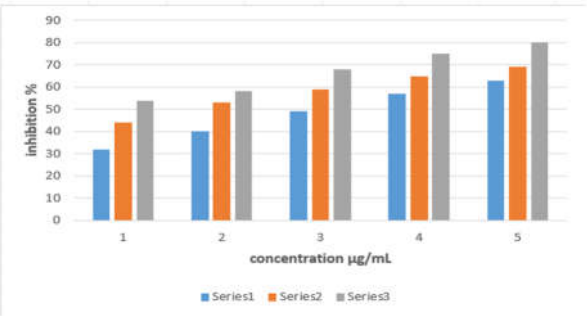
Hussain G, *et al*,(2018) noted that *Colocasia esculenta* leaves displayed the highest antioxidant activity^[27]. Plant extracts with a lower IC₅₀ value demonstrate greater antioxidant activity. In this study, the acetone extract *Colocasia antiquorum* showed the highest IC₅₀ value, suggesting it has the lowest antioxidant activity, as a higher IC₅₀ value correlates with weaker activity. The methanolic extract exhibited an IC₅₀ value between that of ethanol and acetone, indicating moderate antioxidant activity for these solvents.

Table 4: Results of DPPH radical scavenging assay for *Colocasia esculenta*, *Amorphophallus paeoniifolius* and *Colocasia antiquorum*.

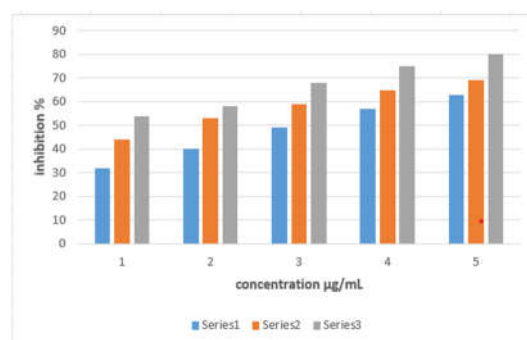
<u>Extract</u>	<u>DPPH Radical Scavenging Assay</u>		
	<u><i>Colocasia esculenta</i></u>	<u><i>Amorphophallus paeoniifolius</i></u>	<u><i>Colocasia antiquorum</i></u>
Methanol	61.71±5.77 %	54.89±5.17 %	58.26±5.24 %
Ethanol	67.91±6.34 %	59.26±5.24 %	64.76±6.19 %
Acetone	32.74±6.60 %	29.10±6.40 %	31.53±6.20 %



(a)



(b)



(c)

Figure 2: DPPH Assay comparing the inhibition (%) of the plants (a) *Colocasia esculenta* (b) *Colocasia antiquorum* (c) *Amorphophallus paeoniifolius*, Leaf extracts with different concentrations.

[Plant extract = series1 (methanol), series2 (acetone), series3 (ethanol)

Concentrations= 1(300),2(350),3(400),4(450),5(500)]

Conclusion

The Araceae family are known to play vital roles in ecosystems, particularly in tropical and subtropical areas and holds importance not only in horticulture and cuisine but also in ecological and cultural areas. This analysis was aimed to identify presence of phytochemicals in *Amorphophallus paeoniifolius*, *Colocasia antiquorum* and *Colocasia esculenta* plants shows the presence of various bioactive compounds, such as alkaloids, tannins, saponins, terpenoids, and flavonoids, in the leaf extracts. These compounds are known for their diverse pharmacological properties, including antioxidant, anti-inflammatory, and anticancer activities etc. The UV-visible spectroscopy analysis further confirmed the presence of key phytochemicals compounds, correlating with the observed antioxidant activity. The antioxidant potential of the extracts, from all the 3 plants, were evaluated through the DPPH scavenging assay, where the ethanol extracts of the plants showed the high antioxidant activity, there were many phytochemical components were found in *Colocasia esculenta* also it is rich in flavonoids as seen in yield. further antioxidant activity confirms that its potential as a natural source of antioxidant. This research highlights the therapeutic potential of the Araceae family plants and their bioactive constituents, suggesting that they could serve as a valuable source for the development of herbal products of therapeutic importance. However, further studies are necessary to explore their full pharmacological properties, toxicity, and potential clinical applications.

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