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Phytochemical Analysis and Antioxidant Activity of *Colocasia esculenta* (L.) Leaves

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Abstract*—Colocasia esculenta* leaves and roots are widely used in Asian countries, such as, India, Srilanka and Pakistan, as food and feed material. The root is high in carbohydrates and rich in zinc. The leaves and stalks are often traditionally preserved to be eaten in dry season. Leaf juice is stimulant, expectorant, astringent, appetizer, and otalgia. Looking at the medicinal uses of the plant leaves; phytochemicals were extracted from the plant leaves and were characterized using Fourier-transform infrared spectroscopy (FTIR) to find the functional groups. Phytochemical analysis of *Colocasia esculenta* (L.) leaf was studied using three solvents (methanol, chloroform, and ethanol) with soxhlet apparatus. Powder of the leaves was employed to obtain the extracts, which was qualitatively and quantitatively analyzed for phytochemical content using standard methods. Phytochemical constituents were abundant in the leave extract. Leaf was found to have various phytochemicals such as alkaloids, glycosides, flavonoids, terpenoids, saponins, oxalates and phenols etc., which could have lot of medicinal benefits such as reducing headache, treatment of congestive heart failure, prevent oxidative cell damage etc. These phytochemicals were identified using UV spectrophotometer and results were presented. In order to find the antioxidant activity of the extract, DPPH (2,2-diphenyl-1-picrylhydrazyl) method was employed using ascorbic acid as standard. DPPH scavenging activity of ascorbic acid was found to be 84%, whereas for ethanol it was observed to be 78.92%, for methanol: 76.46% and for chloroform: 72.46%. Looking at the high antioxidant activity, *Colocasia esculenta* may be recommended for medicinal applications. The characterizations of functional groups were analyzed using FTIR spectroscopy.

***Keywords*—**Antioxidant activity, *Colocasia esculenta*, leaves, characterization, FTIR.

I. Introduction

THE complex metabolic events occurring inside the cell of an organism involves number of oxidative reactions. These reactions produce variety of free radicals which can cause cell damage. A genre of reducing agents exists in the biological world and is known as antioxidants. These antioxidants are well known to quench the free radicals generated due to numerous metabolic reactions in the cell. Hence, the activity possessed by these chemicals is termed as antioxidant activity. However, these antioxidants are nothing but a group of plant originated chemicals represented as phytochemicals. It is well known that not single antioxidant is effective in combating the various free radicals and its recovery from various forms of fruits and vegetables is a recent area of research.

The phytochemicals are the plant derived chemicals possessing numerous herbal & medicinal properties. They exhibit anti-inflammatory, antimicrobial, antifungal, antibacterial & anti-hypertensive properties [1], [2]. Eventually, they are used by plants for different sort of growth & defense mechanism against pathogens, predators & competitors. They are also said to protect plants from unfavourable environmental conditions like UV radiation, high temperatures, drought etc. Thus, other than being used as essential nutrient for growth and defense, phytochemicals have a lot of other research applications.

Taro is the common name of *Colocasia esculenta* (L.) which belongs to Araceae family and is a tropical and perennial plant. It is widely disturbed in the entire Southeast Asia, East Africa, Caribbean and Southeast America [3]. The entire plant is used as food and is a major source of carbohydrate, protein and minerals. Eating taro is good in preventing constipation and also it lessens the risk of colon cancer. The presence of 97% vitamin A content makes it useful for eyes. High content of vitamin C makes taro effective for cold, cough and even cancer.

Extraction of phytochemical is essential so that the useful phytochemical may be utilized for both medicinal and nutritional usages. The extraction can be done by various means such as soxhlet extraction, cold extraction, maceration, microwave assisted extraction etc. Conventionally extraction is carried out by soxhlet apparatus, which is a liquid liquid extraction based technology. In this regard various solvents have been tried in literature [3], [4]. Soxhlet provide an inexpensive method for recovery of phytochemical and had been proven to provide high extraction. In the present work, the perspective of medicinal and health benefits of the plant leaves of Taro had been explored.

Azubike et al. [4] stated the hepatoprotective properties of *C. esculenta* leaves due to saponin content. Antimicrobial and anticancer activity of leaves was identified in *Colocasia esculenta* leaves [5]. The antioxidant activity of *C. esculenta* leaves was evaluated in this research followed by the phytochemical analysis. Phytochemicals were extracted employing solvent extraction using various solvents. Qualitative determination of various phytochemicals was done by chemical texts. Antioxidant activity of the extraction was determined by DPPH test. The FTIR spectroscopy was performed for the identification of phytochemical groups present in the extract.

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II. Materials and Methods

Solvents: Ethanol, methanol and chloroform were of analytical grade and obtained from Thermo Fisher Scientific India Pvt. Ltd. India. DPPH reagent was obtained from Himedia Laboratories Pvt. Ltd. Extraction was carried out in Soxhlet apparatus made from Borosil glass.

Fresh leaves were procured from the local market of Raipur (India). The leaves were rinsed to remove the physical impurities and dirt. The leaves of *C. esculenta* were dried for 2 days and then grinded to make powder which was further screened by mesh to get a uniform particle size. About 10 g of powdered sample was taken in thimble and was mixed with 100 ml of respective solvent in the Soxhlet apparatus. Extraction was carried out for 12 hours. The extract was then filtered by Whatman filter paper no. 1. The solvents were removed by drying at room temperature to get the crude extracts, stored at refrigerated condition for further analysis.

Phytochemicals present *C. esculenta* were qualitatively determined by standard procedures available in literature [6] as given below [6].

Test for tannins: $FeCl_3$ (0.1%) was added to 5 ml of the extract and observed for brownish green or a blue-black coloration, which shows the presence of tannins.

Test for phlobatannins: 10 ml of aqueous extract sample was boiled with 2 ml 1% HCl acid in a test tube. Deposition of a red precipitates indicated the presence of phlobatannins.

Test for saponins: 20 ml of the extract sample was measured into test tube and boiled in a water bath, then filtered. Half of above sample after filteration was shaken vigorously for consistent froth formation. To this froth olive oil (few ml) was added. Presence of saponins is ascertained if emulsion forms.

Test for flavonoids: Few drops of 1% NH_3 solution were added to the aqueous solution of extract in a test tube. A yellow coloration was observed which indicated the presence of flavonoid compounds.

Test for terpenoids: 5 ml of aqueous solution of extract sample was mixed with 2 ml of $CHCl_3$ in a test tube. 3 ml of concentrated H_2SO_4 was carefully added to the mixture to form a layer. An interface with a reddish-brown coloration was formed which showed the presence of terpenoids.

UV-VIS Spectroscopy (Shimadzu UV-1800) and FTIR Spectroscopy (Bruker) were used for the identification and characterization of phytochemicals. The crude samples were diluted to the ratio of 1:10 before the UV Spectroscopy. The KBr thin disc was formed for FTIR analysis, which was made by mixing small amount of *C. esculenta* extract with dry potassium bromide. Further, the disc was placed over the sample cup of diffuse reflectance accessory. The extracts were analyzed using FTIR Spectrophotometer where IR spectrum was within 4000-400 cm^{-1}. The results thus obtained from UV VIS & FTIR were recorded.

The antioxidant activity was evaluated by DPPH assay [7]. About 3.8 ml of freshly prepared DPPH solution was taken and 200µL of extract was added for each solvent viz. ethanol, methanol and chloroform. Further the reaction mixture was incubated in dark for 1 hour at room temperature. The measurement of absorbance was done at 517 nm wavelength by UV-VIS spectrometer. Ascorbic acid was used for the positive control. The DPPH activity was calculated by:

$$DPPH\ activity = \frac{Control\ absorbance - Sample\ absorbance}{Control\ absorbance} * 100$$

III. Results & Discussion

The sample was subjected to chemical tests for identification of various phytochemicals and the results are presented in Table I. It was observed that the methanol extraction was quantified with all the phytochemicals like tannins, phlobatannins, saponins, flavonoids and terpenoids. However, ethanol extract could not provide identification of saponins. Chloroform extraction was unable to predict both phlobatannins and saponins in the chemical tests. The UV VIS Spectroscopy gave the peak values of λ_{max} for each solvent extract thus depicting the presence of phytochemicals quantified by the chemical tests. The λ_{max} peak values for ethanolic extracts were observed at 874, 827, 665, 610, 537, 442 & 248 nm representing the presence of phenolic acids, terpenoids, flavonoids, chlorophyll, taraxerol and xanthophylls (Table II). Similarly, at 975, 934, 835, 823, 663, 607, 536, 478 and 238 nm λ_{max} peaks were observed for methanolic extracts and hence signifying presence of tannins, oxalates, chlorophyll, phenolic acids, terpenoids, taraxerol and xanthophylls (Table III). The extracts of chloroform showed presence of tannins, oxalates, chlorophyll, phenolic acids, terpenoids, taraxerol and carotenoids at 962, 895, 659, 611, 539, 491, 260 and 222 nm (Table IV). Thus the appearance of peak at the corresponding λ_{max} ascertains the presence of particular phytochemical, as obtained by chemical tests.

TABLE I
Identified Phytochemicals with Respective Solvents

Phytochemical	Ethanol extract	Methanol extract	Chloroform extract
Tannins	+	+	+
Phlobatannins	+	+	-
Saponins	-	+	-
Flavonoids	+	+	+
Terpenoids	+	+	+

TABLE II
Λ_{MAX} of Identified Phytochemicals in Ethanol Extract

S. No.	Phytochemical	Ethanol extract
		λ_{max} (nm)
1	Unknown phenolic acid	874
2	Unknown flavonoid	827
3	Chlorophyll b	665
4	Chlorophyll a	610
5	Taraxerol	537
6	β carotene	442
7	Sitosterol	248

FTIR spectroscopy was used to identify the functional groups of phytochemicals present in the extracts of *C. esculenta* leaves. The FTIR spectrum confirms the presence of different functional group at respective wave number, in the extracts of ethanol, methanol and chloroform of *C. esculenta*

leaves. Phenolic group was identified in all the extracts at 3347, 3350 & 3395 cm^{-1} wavenumber. Alkyl methyl and alkyl methylene groups were detected at 2969, 2916, 2847, 1378, 2921, 2850 & 1377 cm^{-1}. Aromatic groups were identified at 1734, 1645, 1451, 1736, 1657, 839 & 878 cm^{-1} in ethanol and chloroform extracts. Carboxylic acids were detected in ethanol and chloroform extracts at 1271 & 1551 cm^{-1}. The methanolic extracts also exhibited C=C stretch at 1624 cm^{-1} and vinyl group at 921 cm^{-1}. Similarly, chloroform extracts also gave detection for organophosphorus aromatic group at 1462 cm^{-1} & aliphatic amine at 1216 cm^{-1}. The functional groups thus detected in the different extracts leads to the assumption that a variety of phytochemicals are present in the *C. esculenta* leaves. Figs. 1 and 2 represent the functional group peaks identified for ethanol and methanol extracts, similar graph was obtained for chloroform extracts. Beta carotene, a precursor of vitamin A, plays a vital role in prevention of age-related eye diseases. Taraxerol is well known for its anti-inflammatory properties [8]. Sitosterol is a plant sterol having the potential to reduce the blood cholesterol levels [9]. Tannins have been reported to have anti-carcinogenic properties. Phenolic acids and flavonoids have cardio-protective, anti-diabetic and neuroprotective abilities [10].

DPPH test is useful to determine the radical scavenging activity of extraction. The method relies on the decrease in absorption of DPPH solution after addition of antioxidant. The standard for this test is done using ascorbic acid. DPPH has red colour and degree of discoloration indicates the scavenging potential of the antiodixant. DPPH radical scavenging activity was calculated using the absorbance values obtained by spectrophotometer. The standard for ascorbic acid was 84%. The ethanol extracts showed 78.92% of activity, while it was 76.46% and 72.46 % for methanol and chloroform, respectively. Thus *C. esculenta* leaves was observed to have significant antioxidant potential.

TABLE III
Λ_{MAX}OF IDENTIFIED PHYTOCHEMICALS IN METHANOL EXTRACT

S. No.	Phytochemical	Methanol extract
		λ_{max} (nm)
1	Unknown tannin	975
2	Unknown oxalate	935
3	Unknown phenolic acid	835
4	Unknown flavonoid	823
5	Chlorophyll b	663
6	Chlorophyll a	607
7	Taraxerol	536
8	β carotene	478
9	Sitosterol	238

TABLE IV
Λ_{MAX} OF IDENTIFIED PHYTOCHEMICALS IN METHANOL EXTRACT

S. No.	Phytochemical	Chloroform extract
		λ_{max} (nm)
1	Unknown tannin	962
2	Unknown phenolic acid	895
3	Chlorophyll b	659
4	Chlorophyll a	611
5	Taraxerol	539
6	β carotene	491
7	Sitosterol	242

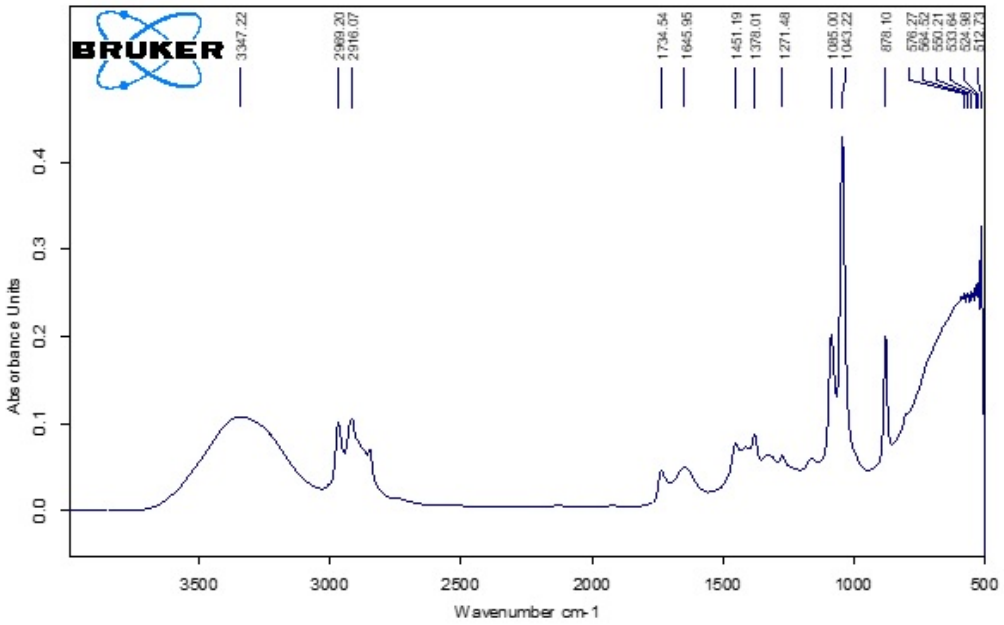

Fig. 1 FTIR peaks of functional groups identified in ethanol extracts

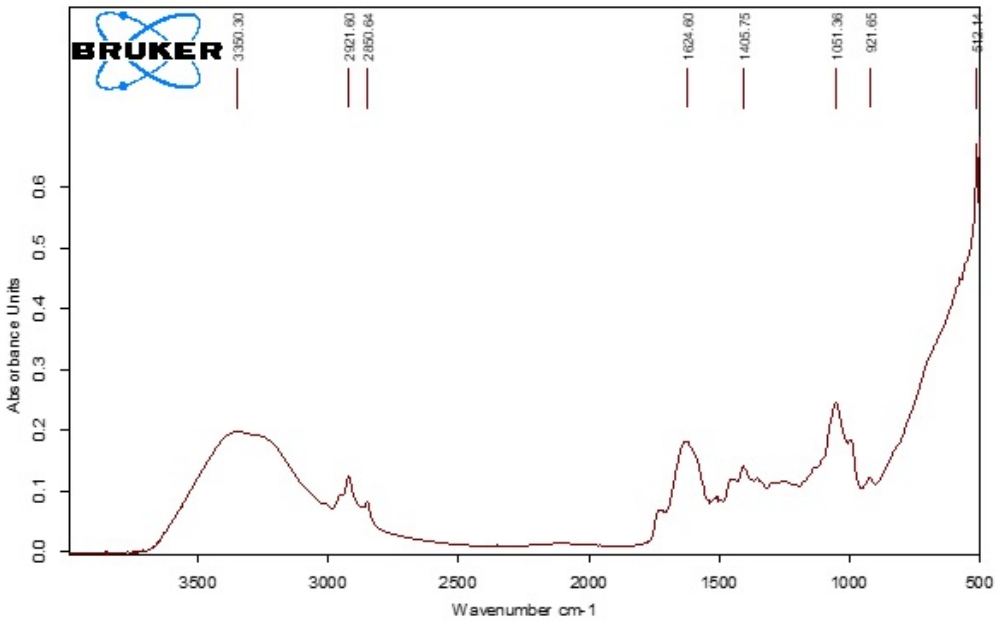

Fig. 2 FTIR peaks of functional groups identified in methanol extracts

IV. Conclusion

This research depicts the presence of various phytochemical of *Colocasia esculenta* leaf extracts through chemical test, which were further qualitatively determined using UV-VIS and FTIR spectroscopy. Extraction studies were performed using different solvents (ethanol, methanol and chloroform). All the extractions were found to show the presence of various phytochemical groups found in Taro leaf extract. Ethanol extracts proved to be better than methanol and chloroform in terms of DPPH radical scavenging activity. The results provide the antioxidant profile for combating stress and prevalent diseases and hence have medicinal usage.

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