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PHYTOCHEMICAL ANALYSIS AND ACUTE TOXICITY STUDY OF METHANOLIC LEAF EXTRACT OF *COLOCASIA GIGANTEA* IN ADULT MALE WISTAR RATS

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ABSTRACT: Background: *Colocasia gigantea* is a large, stemless, tuberous, frost-tender perennial of the arum family, which typically grows tall and wide. It is found abundantly in tropical countries, and in Southeast Asia. **Methodology and Results:** Phytochemical analysis using gas chromatography and acute toxicity testing was done on methanolic leaf extract of *Colocasia gigantea*. The adult male wistar rats used for acute toxicity testing were 13 in number. The results showed that at 5000mg/kg, of administration of methanolic leaf extract *Colocasia gigantea*, no death occurred among the wistar rats used for the study. Phytochemical analysis results on methanolic leaf extract of *Colocasia gigantea* showed the presence of flavonoids (flavon – 3-ol and flavone), alkaloids, tannins, nobiletin, artemetin. **Conclusion:** Methanolic leaf extract of *Colocasia gigantea* contains substances of significant medicinal values and acute administration of methanolic leaf extract is relatively non-toxic.

INTRODUCTION: Plants are a very important source of nutrients and very important component in human diet⁴. All plants contain biologically active compounds called phytochemicals⁴. These phytochemicals are derived from all parts of the plant; roots, stem, leaves, flowers, seeds, etc.⁴. *Colocasia gigantea*, is called ‘ede-nwokuko’ or ‘isi-apupa’ in some parts of Igboland. It is a large, stemless, tuberous, frost-tender perennial of the arum family which typically grows tall and as wide and features huge, heart shaped to arrowhead-shaped, conspicuously veined, downward pointing, peltate, dull green to gray green leaves on stout, succulent stems¹².

In Eastern Nigeria, the roots of *Colocasia gigantea* are consumed locally and believed to be good for people on some dietary restrictions, like diabetes mellitus and the leaves have also been shown to have some antidiabetic to have some antidiabetic properties¹⁴. The roots are believed to contain lower calories than yams and cassava tubers¹⁴. Some ethnic groups use the leaf stalk of *C. gigantea* as food⁶.

The cooked leaves are eaten in Hawaiian Iuaus and the corms are mashed into poi⁶. “Found abundantly in tropical countries in Southeast Asia, *Colocasia gigantea* is an edible plant, with a shoot, stout stem and large heart shaped leaves characteristic to the genus *Colocasia*²³. Phytochemical analysis is a process that involves the identification, extraction, and quantification of compounds in plants²⁴. It can be used to identify bioactive constituents, which are important in developing new treatments and therapies. It can also be used to evaluate therapeutic potential of a

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plant to be used medicinally, develop quality control standards, and create standard for medicinal plant materials. Phytochemical analysis can be quantitative or qualitative²⁴. Qualitative phytochemical analysis determines the presence or absence of a phytochemical while quantitative analysis determines the concentration or quantity of a phytochemical in a plant sample. Some techniques used in phytochemical analysis include; successive extraction using solvents of increasing polarity to extract a wide range of compounds; Soxhlet extraction using an organic solvent to extract dried plant material²⁴.

Phytochemical analysis can be applied to a variety of areas, including; plant biochemistry, plant cellular and molecular biology, plant biotechnology, food sciences, agriculture and horticulture, natural products research²⁴. Acute toxicology study evaluates the short-term effects of a substance on an organism¹¹. They are used to determine the lethal dose of a substance and to assess the risk of exposure to that substance¹¹. Acute toxicity tests are used to determine the lethal dose/concentration of a substance that causes death in 50% of the test population (LD/LC₅₀) during a short-term exposure¹⁰.

METHODS:

Extraction of Phytochemicals: 0.2g of dried, ground leaf sample was weighed and transferred in a test tube and 15ml of methanol was added. The test tube was allowed to react in a water bath at 60°C for 60 min. After the reaction time, the reaction product contained in the test tube was transferred to a separating funnel. The tube was washed successfully with 20ml of methanol, 10ml of cold water, 10ml of hot water and 3ml of hexane, which was all transferred to the funnel. This extracts were combined and washed three times with 10ml of 10% v/v methanol aqueous solution. The solution as dried with anhydrous sodium sulfate and the solvent was evaporated. The sample was solubilized in 1000ul of petroleum ether of which 200ul was transferred to a vial for analysis. The analysis of phytochemical was performed on a BUCK M910 Gas chromatography equipped with HP-5MS column (30 m in length × 250 µm in diameter × 0.25 µm in thickness of film). Spectroscopic detection by GC–MS involved an electron ionization system which

utilized high energy electrons (70 eV). Pure helium gas (99.995%) was used as the carrier gas with flow rate of 1 mL/min. The initial temperature was set at 50–150 °C with increasing rate of 3 °C/min and holding time of about 10 min. Finally, the temperature was increased to 300 °C at 10 °C/min. One microliter of the prepared 1% of the extracts diluted with respective solvents was injected in asplitless mode. Relative quantity of the chemical compounds present in each of the extracts was expressed as percentage based on peak area produced in the chromatogram¹⁸. Bioactive compounds extracted from different extracts were identified based on Gas Chromatography (GC) retention time on HP-5MS column and matching of the spectra with computer software data of standards (Replib and Mainlab data of GC–MS systems).

Acute Toxicity of Methanolic Leaf Extract of *Colocasia gigantean*: The median lethal dose (LD₅₀) of methanolic leaf extract of *Colocasia gigantean* was determined using a method of Lorkes' (1983), which was divided into two phases. It was conducted in the Department of Human Anatomy, Faculty of Basic Medical Sciences, College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus. In this study, 13 experimental rats were used each, and extract was given through oral gavage in the two phases.

Phase I: Nine (9) rats were used and they were grouped into three groups of three rats each. Group 1 received 10mg/kg of Methanolic leaf extract of *Colocasia gigantea*. Group 2 received 100mg/kg of Methanolic *Colocasia gigantean*. Group 3 received 1000mg/kg of Methanolic *Colocasia gigantean*. The animals were observed over a period of 24hrs for mortality. From the result of phase 1, the second phase was carried out. In this phase, 4 rats were used and they were grouped into four groups of one animal each per group.

Phase II: Group 1 received 1200mg/kg of Methanolic *Colocasia gigantean*. Group 2 received 1600mg/kg of Methanolic *Colocasia gigantean*. Group 3 received 2900mg/kg of Methanolic *Colocasia gigantean*. Group 4 received 5000mg/kg of Methanolic *Colocasia gigantean*. The animal were monitored over a period of another 24 hrs for mortality:

The formula for calculating LD₅₀:

$$LD_{50} = \sqrt{a \times b}$$

A = maximum dose with 0% mortality, B = minimum dosed with 100% mortality. There was no mortality in the study and the LD₅₀ of *Colocasia gigantea* was estimated to be greater than 5000mg/kg.

RESULTS:

TABLE 1: RESULTS OF GAS CHROMATOGRAPHY QUANTITATIVE PHYTOCHEMICAL ANALYSIS

Name of Phytochemical	Concentration (ppm)
Genistein	3.13
Epicatechin	3.88
Flavone	4.70
Daidzein	4.10
Epigallocatechin	1.47
Lunamarin	3.99
Naringi	.74
Flavon-3-ol	4
Butein	5.37
Artemetin	2.91
Vannillic acid	25.50
Naringenin	5.50
Luteolin	4.25
Kaempferol	1.87
Qercetin	3.12
Gallocatechin-3-gallate	5.27
Robinetin	ND
Myricetin	D
Nobiletin	N
Tangeretin	15.25
Baicalin	4.68
Cinnamic acid	ND
Ferrulic acid	1.58
Piperic acid	1.66
Gentisic acid	2.04
Cinnamic acid	ND
Syringic acid	ND
Rosemaric acid	ND

Key: ND= Not detected

TABLE 2: RESULTS FOR THE ACUTE TOXICITY STUDY

S. no.	Dose	Death	Observation
1.	10mg/kg of <i>Colocasia gigantea</i>	0/3	The animals were calm.
	100mg/kg of <i>Colocasia gigantea</i>	0/3	
	1000mg/kg of <i>Colocasia gigantea</i>	0/3	
2.	1200mg/kg of <i>Colocasia gigantea</i>	0/1	The animals were calm.
	1600mg/kg of <i>Colocasia gigantea</i>	0/1	
	2900mg/kg of <i>Colocasia gigantea</i>	0/1	
	5000mg/kg of <i>Colocasia gigantea</i>		Weak but survived after 24hrs

DISCUSSION: GC-MS (Gas Chromatography – Mass Spectrometry) has been regarded as a gold standard for forensic substance identification because of its ability to chromatographically separate and analyze components in mixtures⁵. It is able to isolate and analyze different components in unknown mixtures. It does not require any substantial method development for each new sample⁵. GC-MS is one of the very few techniques able to qualitatively identify the actual nature of chemicals in the sample⁹. The main phytochemical components present in medicinal plants are tannins, alkaloids, saponins, cardiac glycosides, steroids, terpenoids, flavonoids, phlobatannis, anthraquinones and reducing sugars¹.

As proposed by WHO, the primary health care of most population of developing countries depend on traditional medicines mostly natural plant products¹. Methanolic leaf extract of *Colocasia gigantea* is rich in nutrients and found to be useful due to nutritive and health promoting contents as the corm, for instance is relatively low in protein (1.5%) and fat (0.2%) as noted in other tuber crops, but it is a great starch source (70–80 g/100 g dry taro), fiber (0.8%), ash (1.2%), thiamine, riboflavin, iron, phosphorus, zinc, Vitamin B6, vitamin C, niacin, potassium, copper, and manganese^{21, 22}. The leaves when used as leafy vegetables have been noted as being rich in nutrients including minerals and vitamins such as calcium, phosphorous, iron, vitamin C, thiamine, riboflavin and niacin^{16, 20}.

Different traditional medicinal plant species are studied by different researchers in the world¹. The medicinal power of traditional plants species lies in phytochemical components that cause definite pharmacological action on the human body¹. Based on their metabolism activity in the plant, phytochemical components can be mainly divided into two groups, which are primary; which has mainly sugared, amino acids, chlorophyll and proteins, and secondary constituents which consists of alkaloids, flavonoids, saponins, tannins, phenolic compounds^{1, 15}. Phytochemical analysis of the leaf extract on *Colocasia. gigantea*, using gas chromatography, showed the presence of flavonoids (flavon – 3-ol and flavone), alkaloids, tannins, nobiletin, artemetin; among others and absence of proteins and phenols.

The term tannin is widely applied to a complex large biomolecule of polyphenol nature having sufficient hydroxyls and other suitable groups such as carboxyl to form strong complexes with various macromolecules¹. Tannins are used in the tanning process and as healing agents in inflammation, burn, piles and gonorrhea¹. Flavonoids consist of a large group of polyphenol compounds having a benzoul-y-pyrone structure and are present in plants¹. They are synthesized by the phenylpropanoid pathway¹. Alkaloids are one of the main and largest components produced by plants. They are metabolic byproducts that are derived from the amino acids^{1,17}. 'Colocasia leaf extract contains several phytochemical compounds which show active compounds in the form of catechins, anthraquinones, cinnamic, isovitexin, acid derivatives, viexin and acid derivatives which are useful because they are biological'¹⁹.

The findings simulate the findings by Nurul Husna (2023)¹⁹ carried out in Indonesia in which *Colocasia gigantea* was reported to contain tannins, flavonoids, steroids, and phenolic, which had potential as antibacterial specialists¹⁹. *Colocasia gigantea* also contains high dietary fiber, pyridoxine and nicotinamide within the leaf stalk¹⁹. *Colocasia gigantea* leaf extract contain alkaloids, saponins, tannins, triterpenes, terpenes, flavonoids, flobatamines, anthraquinone, cardiac glycosides, and polyphenols¹⁹. This finding is similar to the phytochemical compounds reported in this study. Another study on the phytochemical screening of *Colocasia gigantea* by Alam (2020)² reported that the plant contained flavonoids, mixture of α -amyrin and β -amyrin, penduletin, monoglyceride of stearic acid².

Acute toxicity evaluates the adverse effects that occur following exposure of organisms to a single or multiple doses of a test substance within 24hours by a known route⁷. The route may be oral, dermal or inhalation⁷. After administration the test substance is absorbed and distributed to various parts of the body before it elicits systemic adverse effect⁷. The regulatory body requires the acute toxicity test report for labelling and classification of substances for human use⁷. The LD₅₀ (median Lethal Dose) test was introduced in 1927 by W. Trevan to estimate the dose of a test substance that produces 50% death in a given species of animals

(Erhirhie 2018). It is usually the first test conducted for every chemical before further toxicity tests are carried out (Erhirhie 2018)⁷. Although its major end point is death, non – lethal acute effect may occur as signs of toxicity depending on the chemical being tested⁷. Assessment of the acute toxic potential of substances is needed to determine their adverse effects that might occur due to accidental or deliberate short-term exposure⁷. Results from acute toxicity test serve as a guide in dosage selection for long term toxicity studies as well as other studies that involve the use of animals⁷. From the result of an acute toxicity test, a conclusion can be made on the toxicity status of the test substance⁷.

From the acute toxicity study, carried out earlier, the index of acute toxicity; that is the value for LD₅₀ was not calculated. This is because no death was recorded during the period of the study. 'The LD₅₀ was estimated to be greater than 5000mg/kg, since according to Lorke, LD₅₀ values greater than 5000mg/kg are of no practical toxicological interest'¹³. The animals also showed no obvious behavioral or biochemical changes except for weakness; for which recovery was seen within 24 hours. 'Toxicological manifestations of an administered substance maybe detected from behavioral and / or biochemical changes elicited in animals'¹³. Methanolic leaf extract of *Colocasia gigantea* appeared safe for consumption as acute toxicity measured was more than 5000mg/kg.

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