

Phytochemical Composition, Antimicrobial and Antioxidant Activities of Leaves and Tubers of Three *Caladium* Species

Emmanuel E. Essien^{1*}, Imo E. Jacob², Paul S. Thomas²

¹Department of Chemistry, Faculty of Science, University of Uyo, Uyo, Nigeria

²Department of Pharmacognosy and Natural Medicine, Faculty of Pharmacy,
University of Uyo, Uyo, Nigeria

*emmaflowus1@yahoo.co.uk

Abstract: The study investigates the phytochemical composition, antimicrobial and antioxidant activities of methanol extracts of leaves and bulbs of *Caladium bicolor* (green background with white patches), *C. bicolor* (green background with pink veins) and *C. tricolor* (green background with white and pink patches). The chemical and biological analyses were performed using standard procedures. The phytochemical screening indicated varying amount of alkaloids, saponins, cardiac glycosides, terpenoids/steroids, de-oxy sugars, phenols, flavonoids, reducing sugars, phlobatannins and carbohydrates in the extracts. The samples contained high concentration of saponins and carbohydrates, especially in the tubers. Phlobatannins and anthraquinones were not detected in any of the plant extracts. The different concentrations of *Caladium* leaves and bulbs extracts exhibited moderate antibacterial and antifungal activity against selected wound pathogens (*Streptococcus pyogenes*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Candida albicans*) using disc diffusion method. The minimum inhibitory concentration (MIC) varied between 0.006 g/ml to 0.55 g/ml. In DPPH assay, leaves extract of *Caladium bicolor* (green background with white patches) demonstrated the highest free radical scavenging activity (64.7%, IC₅₀ value, 1.15 mg/ml) which could be correlated to its phenolic content. The results of the study indicate that *Caladium bicolor* and *C. tricolor* leaves and bulbs contain potential natural antimicrobial and antioxidant agents which could be exploited for possible drug development.

Keywords: *Caladium* spp., phytochemicals, antioxidants, antimicrobials, minimum inhibitory concentration.

1. INTRODUCTION

Caladium Vent, commonly known as fancy- leafed caladium, elephant's ear and heart of Jesus, is an ornamental foliage plant grown from tubers and planted extensively in landscape, especially in the southeastern U.S.A. *Caladium* is indigenous to South and Central America and belongs to the family Araceae. The colouration of this plant makes the environment in which it is found beautiful and adoring. The ornamental value of caladium use as pot or landscape plants is determined primarily by leaf characteristics. Improving leaf characteristics or generating new combinations of them has been one of the most important objectives in caladium breeding and cultivar development [1]. Species identification is from the leaf blades which possess various patterns of pink and white spots. Leaf is mostly heart, lance or arrowhead shape. They produce berry fruits with several small ovoid seeds. Cultivar development in caladium has a history of some 150 years [2]. However, about 51 cultivars of caladium have gone to extinction due to lack of conservation. For instance in 1970 only 141 cultivars were listed in exotica and also indicated in recent survey of the Florida tuber producers [3, 4].

Caladium contains oxalate crystals which can cause illness and swelling of the mouth and throat.

An infusion of fresh leaf is used for the treatment of angina. The powdered dried leaf is used to treat infected sores; powdered tuber employed to treat facial skin blemishes by the French Guiana [5]. In Nigeria, different types and species of *Caladium* are found in the wild on road sites, school premises and in farm lands; there is paucity of information on their medicinal potential. However, a number of authors have reported on the chemical composition and biological activities of some *Caladium* species [5-8]. This work is aimed at evaluating the phytochemical composition, *in vitro* antioxidant activity and antimicrobial potential of three caladium leaves and tubers against selected wound pathogens.

2. MATERIALS AND METHODS

2.1. Plants Collection and Identification

The whole mature plants of *Caladium bicolor* (green background with white patches) (GWS), *C. bicolor* (green background with pink veins) (GPS) and *C. tricolor* (green background with white and

pink patches) (GPWS) [Plate 1] were collected from a farmland in November at Ukanafun Local Government Area of Akwa Ibom State, Nigeria. The plant was identified and authenticated by a taxonomist, Dr. M. E. Bassey of the Department of Botany and Ecological Studies, University of Uyo, where voucher specimens were deposited with herbarium number UUH3345.



Plate1. Picture of *Caladium bicolor* and *C. tricolor*

2.2. Sample Preparation and Extraction

The leaves and tubers of the three caladium species were cleaned, cut, oven dried at 40°C and pulverized. The powdered samples (100 g) were separately macerated in absolute methanol and extracts obtained using a rotary evaporator. All chemicals and solvents used were of analytical grade from Sigma-Aldrich GmbH, Sternheim, Germany.

2.3. Phytochemical Screening

Standard methods for phytochemical screening (alkaloids, flavonoids, saponins, tannins, carbohydrates, sterols and triterpenes) were employed. Alkaloids determination was done using Mayer's and Dragendoff's reagents following the methods of Sofowora [9]; tannins and phlobatannins [10]. The methods described by Harborne [11] and Trease and Evans [10] were used for determining flavonoids, phenol and cardiac glycosides. The persistent frothing, sodium bicarbonate and carbonate tests, as described by Trease and Evans [10] and Sofowora [9] were used for saponins. Carbohydrates, sterols and triterpenes determination were done using Fehling's reagent following the method described by Harbone [11].

2.4. Collection of Bacterial and Fungus Isolates

Clinical bacterial and fungal isolates were collected from infected wounds using sterile swab sticks at St. Lukes Hospital, Anua, Uyo and Macson's clinic, Ukanafun Local Government Area, Akwa Ibom State. These isolates were transported on slants to Microbiology Laboratory, University of Uyo. The test organisms were sub-cultured into nutrient broth and incubated for 48 hrs at 37°C. The microbes were sub cultured on nutrient agar slant for the isolation of pure culture. Isolates were identified using standard cultural, microscopic and standard biochemical methods such as motility test, gram staining, oxidase test, oxidation fermentation test, indole test, catalase test, gelatin liquefaction test, citrate utilization, esculin hydrolysis, urease activity, decarboxylase reactions and hydrogen sulphide production tests. The Gram positive bacteria, *Staphylococcus aureus* and *Streptococcus pyogenes*; fungus, *Candida albicans* were serially diluted to factor three using 10 fold dilution. Gram negative isolates (*Pseudomonas aeruginosa*, *Kiebsiella pneumonia* and *Proteus mirabilis*) were serially diluted to factor five using 10 fold dilution. The isolates were sub-cultured into their selective medium based on their exhibited morphological characteristics. They were preserved at 4°C and later used for this work.

2.5. Preparation of Antimicrobial Discs

A 5 mm diameter plunger was used to punch a Whatman no.1 absorbent filter paper to obtain 5 mm diameter paper discs. The discs were properly labeled for identification purposes and then sterilized by autoclaving for 15 min at 121°C. The disc were impregnated with the plant extracts (0.1- 0.4 g/mL), dried and stored off in sterile bottles.

2.6. Evaluation of Antimicrobial Activity

Antimicrobial activity was tested using a modified discs diffusion assay (DDA) method [12]. The inoculums for each microorganism were prepared from broth cultures (10^5 CFU/mL). A loop of culture from the nutrient agar (NA) slant stock was cultured in Mueller Hinton medium overnight and spread with a sterile swab into Petri-plates. Each microbial swab was spread on separate plates. Sterile disc (5 mm in diameter) impregnated with the plant extracts were placed on the cultured plates. Control experiment was carried out using commercial antibiotics, antifungal and solvent (stock). The solvent loaded disc without extracts served as control in the study. Streptomycin (30 mg/ml) was used for bacterial isolates and Nystatin (150 mg/ml) for fungal isolates; plates were incubated for 24 hrs and 48 hrs respectively the results were recorded by measuring the zones of growth inhibition. Clear inhibition zones around the discs indicated the presence of antimicrobial activity. All data on antimicrobial activity were average of triplicate.

2.7. Determination of Minimum Inhibitory Concentration

The minimum inhibitory concentration of each crude extract was determined using tube dilution method [13, 14]. The initial concentration of each of the plant extract (100 mg/mL) was diluted using double fold dilution and standard volume of each diluted isolate (0.1ml) was aseptically inoculated into different concentrations of the extract. Control experiment was carried out without the crude extracts. All tubes were incubated at 37°C for 24 hrs. Minimum inhibitory concentrations (MIC) were determined as the lowest concentration without turbidity.

2.8. Determination of Total Phenolics

The amount of total phenols in the methanol extracts of *Caladium* leaves and tubers was determined with the Folin-Ciocalteu's reagent using the method of Meda *et al.* [15]. 2.5 ml of 10% Folin Ciocalteu's reagent was added to 0.5 ml of each concentration of the sample and then 2 ml of 2% w/v of Na_2CO_3 was introduced, incubated at room temperature (45°C) for 20 minutes. The absorbance was measured at 760 nm using a uv/vis spectrophotometer (Unisio, Shanghai-China). Total phenol values are expressed in terms of gallic acid equivalent (mg/g of extract).

2.9. DPPH Radical Scavenging Activity

DPPH radical scavenging activity of each extract was determined according to the method of Blois [16]. 3 mls of different concentrations of sample and standard drug BHA were added to 1 ml of 0.3 mM DPPH in methanol. The mixture was vortexed and then incubated in a dark chamber for 30 minutes, after which the absorbance was measured (in triplicates) at 517 nm against a control containing DPPH (0.03 mM) in methanol without the sample. The DPPH radical scavenging activity was calculated using the following formula:

$$\% \text{ inhibition} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

3. RESULTS AND DISCUSSION

The percentage yield of *C. bicolor* (green background with white patches) bulbs extract was found to be 0.82%, *C. tricolor* (green background with white and red patches (2.40%) and *C. bicolor* (green background with red veins) (0.83%) for the bulbs while that of the leaves were (15.50%), (11.30%), and (8.30%) respectively. The results of phytochemical screening of *Caladium* leaves and tuber methanol extracts are presented in Table 1. The phytochemical screening indicated varying amount of alkaloids, saponins, cardiac glycosides, terpenoids/steroids, de-oxy sugars, phenols, flavonoids, reducing sugars, phlobatannins and carbohydrates in the extracts. The samples contain high concentration of saponins and carbohydrates, especially in the tubers. Phlobatannins and anthraquinones were not detected in any of the plant extracts. Tannins were exclusively present in the leaf extract of GWS and alkaloids in all tuber extracts. The variation in the presence and concentration of phytochemicals in extracts may be attributed to the specie type and the inherent nature of the constituents. Ekanem *et al.* [6] reported on the quantitative phytochemical profile of some species of *Caladium* stem, leaves, bulbs and roots; significant levels of flavonoids, alkaloids and saponins were detected in the leaves than other parts of the plant.

Table1. Phytochemical screening of *Caladium* leaves and tuber extracts

Test	GWS		GPWS		GPS	
	Leaves	Tuber	Leaves	Tuber	Leaves	Tuber
Alkaloids	-	+++	-	+++	-	+++
Tannins	++	-	-	-	-	-
Saponins	+++	+	+++	++	+++	+++
Cardiac glycosides	+++	+	-	+++	+++	++
Flavonoids	++	+	++	+	++	+
Terpenes/steroids	-	-	++	-	++	+++
Phlobatannins	-	-	-	-	-	-
Anthraquinones	-	-	-	-	-	-
Deoxy sugars	++	++	-	-	++	+++
Carbohydrates	++	+++	++	+++	+	+++

+++ : High; ++ : Moderate; + : Trace; - : Not detected; GWS = *C. bicolor* (green background with white patches); GPS = *C. bicolor* (green background with pink veins); GPWS = *C. tricolor* (green background with white and pink patches).

The antimicrobial activity of the *Caladium* extracts were also assayed against pathogenic bacteria and fungus at varied concentrations (0.1-0.4 g/ml), presented in Table 2a & Table 2b. The results revealed that all the extracts tested showed varying degree of antimicrobial activities (6-16 mm) against the test microbial strains. The zones of inhibition varied with the extract and the organism tested. It was observed that the zones of inhibition increased with increase in concentration as improved antimicrobial activity is concentration dependent. However, *S. aureus* and *P. mirabilis* displayed the most resistance to the test extracts, excluding bulb extracts of GPS and GWS with clear zone of 8 mm and 10 mm (400 mg/ml) respectively. *Pseudomonas aeruginosa* showed more susceptibility to the antimicrobial agents in the leaves extracts compared with the tuber extracts; *S. pyogenes* was significantly inhibited by the tuber extracts compared with the leaves extracts. GPS leaves extract exhibited the widest zone of inhibition (16 mm) against *S. pyogenes* relative to the standard drug, streptomycin (17 mm). *Pseudomonas aeruginosa*, *K. pneumonia* and *C. albicans* were also significantly inhibited by GPS leaves extracts.

Minimum inhibitory concentration (M.I.C.) which is the lowest concentration of an extract that inhibits completely the growth of micro-organism in 24 hours ranged from 0.006 g/ml to 0.55 g/ml on tested bacteria and fungus for various extracts (Table 3). GWS tuber extract showed the least MIC value (0.006 g/ml) against *S. pyogenes* and *C. albicans*; also GPS leaves extract against *S. pyogenes* (0.006 g/ml). Phytochemicals which are secondary metabolites present in various extracts may be responsible for the demonstration of antibacterial and antifungal activity against gram negative, gram positive bacteria and fungus, *C. albicans*. The moderate antimicrobial activity of the extracts in this study is consistent with findings by Obi [17] for antifungal potency of *C. hortiulanum* aerial extract against paddy kernel phyto-pathogens, *in vivo* and *in vitro*. However, Biswas *et al.* [5] also reported relatively potent and moderate antimicrobial activity for whole plant of *C. bicolor* against several antimicrobial strains with *S. aureus* most susceptible (20 mm clear zone for chloroform soluble fraction). The resistance of *S. aureus* in the present study (Table 2a and 2b) may be due to the source of the bacterium (infected wound) and the part of the plant studied. It has been suggested that Gram-positive bacteria are more sensitive to chemical compounds than Gram negative bacteria due to the relative thickness of their cell walls [18].

Table2a. Zones of inhibition of *Caladium* leaves and tuber extracts in millimeters (mm)

Organism	GWS (Tuber) g/ml				GPWS (Tuber) g/ml				GPS (Tuber) g/ml			
	0.1	0.2	0.3	0.4	0.1	0.2	0.3	0.4	0.1	0.2	0.3	0.4
<i>S. aureus</i>	-	-	-	-	-	-	-	-	-	-	-	8
<i>S. pyogenes</i>	10	12	13	15	6	10	11	12	-	-	-	8
<i>P. aeruginosa</i>	-	-	-	10	6	8	10	12	-	-	8	10
<i>P. mirabilis</i>	-	-	-	-	-	-	-	10	-	-	-	-
<i>K. pneumoniae</i>	-	-	-	10	10	10	12	14	-	-	-	6
<i>C. albicans</i>	10	11	15	20	8	10	11	14	-	-	-	-

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Table 2b. Zones of inhibition of *Caladium* leaves and tuber extracts in millimeters (mm)

Organism	GWS (leaves) g/ml				GPWS (leaves) g/ml				GPS (leaves) g/ml				Strepto- mycin	Nysta- tin
	0.1	0.2	0.3	0.4	0.1	0.2	0.3	0.4	0.1	0.2	0.3	0.4	0.08	0.15
<i>S. aureus</i>	-	-	-	-	-	-	-	-	-	-	-	-	19	NT
<i>S. pyogenes</i>	-	-	6	10	-	-	-	-	10	15	15	16	17	NT
<i>P. aeruginosa</i>	10	11	12	14	6	7	8	10	6	8	9	10	19	NT
<i>P. mirabilis</i>	-	-	-	-	-	-	-	-	-	-	-	-	10	NT
<i>K. pneumoniae</i>	-	-	10	10	6	8	9	11	6	6	8	10	16	NT
<i>C. albicans</i>	6	8	10	12	-	-	-	-	6	7	8	8	NT	22

No observed inhibition; NT: Not tested; GWS = *C. bicolor* (green background with white patches); GPS = *C. bicolor* (green background with pink veins); GPWS = *C. tricolor* (green background with white and pink patches).

Table 3. Minimum inhibitory concentrations (g/ml) of *Caladium* leaves and tuber extracts

Organisms	GWS (Tuber)	GPWS (Tuber)	GPS (Tuber)	GWS (Leaves)	GPWS (leaves)	GPS (Leaves)
<i>S. aureus</i>	0.50	0.45	0.35	0.45	0.55	0.50
<i>S. pyogenes</i>	0.006	0.025	0.35	0.25	0.45	0.006
<i>P. aeruginosa</i>	0.350	0.050	0.025	0.013	0.050	0.050
<i>P. mirabilis</i>	0.450	0.350	0.500	0.500	0.500	0.550
<i>K. pneumoniae</i>	0.350	0.013	0.300	0.250	0.050	0.025
<i>C. albicans</i>	0.006	0.013	0.500	0.025	0.500	0.025

The concentration of polyphenol components are shown in Table 4. Polyphenols content showed concentration dependence. The total phenolic content ranged from 6.423 (GPS leaves) to 10.192 mg GAE/g (GWS leaves) at 2.0 mg/ml. Variation in the phenolic contents of extracts may be attributed to the plant part studied as well as the chemical nature of the endogenous extractable compounds. Mirdha *et al.* [7] found polyphenols content (1.27-12.35 mg GAE/g) in close range with this study, for methanol, petroleum ether, carbon tetrachloride and chloroform soluble fractions of whole plant of *C. bicolor*. Phenolics exert their antioxidant activity mainly by free radical scavenging.

Table 4. Total phenols content in *Caladium* leaves and bulb methanol extracts

Concentration (mg/ml)	GWS (Bulb)	GPWS (Bulb)	GPS (Bulb)	GWS (Leaves)	GPWS (leaves)	GPS (Leaves)
0.25	0.677	0.038	1.077	1.769	1.115	1.308
0.50	1.192	1.808	2.115	2.731	2.077	1.321
1.00	3.000	4.769	2.500	5.246	3.154	5.538
1.50	4.808	5.577	4.462	7.231	4.077	5.962
2.00	7.192	9.577	8.654	10.192	6.885	6.423

The result of the antioxidant activity (DPPH assay) is displayed in Fig. 1. It was observed that *Caladium* extracts (0.25-2.0 mg/ml) significantly scavenged the DPPH radical in a concentration dependent manner. The plots in Fig. 1 also showed that at 2.0 mg/ml dose, GWS leaves extract inhibited DPPH radical by 64.7% compared to the standard drugs-BHA (69.7%). The antioxidant activity correlates with the amount of polyphenols in the different extracts (Table 4). GWS leaves extract shows pronounced antioxidant activity than GWS tuber extract; GPWS and GRS tuber extracts exhibited stronger antioxidant activity compared with the corresponding leaves extracts. The effectiveness of antioxidant properties is inversely correlated with IC₅₀ values. IC₅₀ values (1.15-2.5 mg/ml) were obtained for the *Caladium* extracts in this study. GWS leaves extract demonstrated the highest free radical scavenging activity with IC₅₀, 1.15 mg/ml. Mirdha *et al.* [7] reported slightly higher DPPH activity (124.21-211.48 µg/ml) for different organic fractions of *C. bicolor* whole plant. DPPH radical is commonly used as substrate to evaluate antioxidant activity; it is a useful and stable free radical that can accept an electron or hydrogen radical to become a stable molecule. In DPPH radical scavenging assay, the antioxidants react with the stable free radical DPPH and convert it to 1,1-diphenyl-2-picryl hydrazine with decoloration. All of the assessed *Caladium* extracts were able to reduce the stable, purple-coloured radical DPPH to the yellow coloured DPPH-H form.

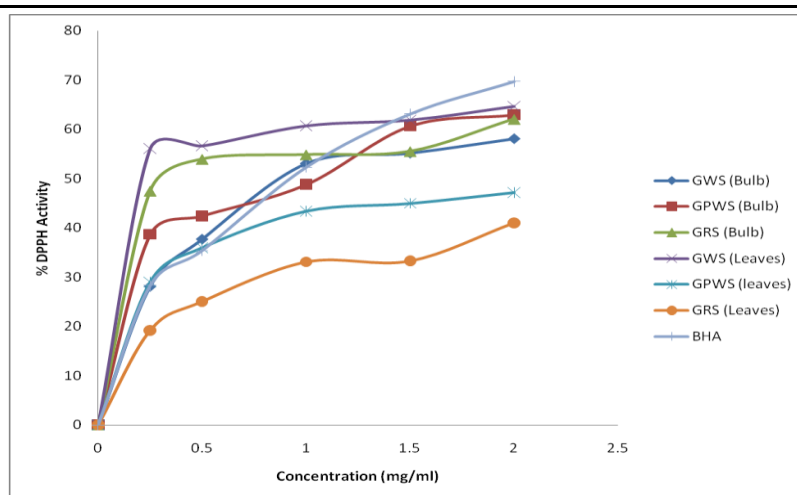


Fig1. DPPH scavenging activity of *Caladium* leaves and tuber extracts

GWS = *C. bicolor* (green background with white patches); GPS = *C. bicolor* (green background with pink veins); GPWS = *C. tricolor* (green background with white and pink patches).

4. CONCLUSION

It is clearly evident from the above findings that the samples of *C. bicolor* and *C. tricolor* have significant antioxidant activity. The plant parts also exhibited mild to moderate antimicrobial activity. Therefore, these plants are good candidates for further systematic, chemical and biological studies to isolate the active principles.

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