PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL EXTRACTS ACTIVITY OF CALADIUM BICOLOR

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Abstract. This study investigated the phytochemical composition and antimicrobial activities of ethanol and methanol leaf, stem and root extracts of *Caladium bicolor* (Aiton) Vent against some clinical pathogens. The ethanol and methanol extracts of the plant parts were prepared by adding separately 10g, 20g, 30g and 40g of the leaf, stem and root powder of *C. bicolor* into 100ml if ethanol and methanol respectively. All extracts of the plant parts at varying concentrations showed antifungal activity against the test fungi. Ethanol leaf extract showed between slightly (4.23±0.05–19.30±0.65) and moderately effective (25.08±0.06–31.44±0.10) inhibition on the mycelial growth of all the test fungi. Ethanol stem extract had slightly effective (2.07±0.01–16.00±0.08) inhibition on the test fungi. Ethanol root extract had slightly effective (2.05±0.06–12.60±0.15) inhibition on the test fungi. Also, methanol leaf extract showed between slightly (2.30±0.06–18.10±0.11) and moderately effective (20.28±0.01–21.02±0.04) inhibition respectively. Methanol stem extract had slightly effective (4.03±0.08–14.03±0.77) inhibition on the test fungi. Methanol root extract had slightly effective (1.00±0.06–11.40±0.09) inhibition on all the test fungi. The phytochemical analysis was done using standard techniques. Data were analysed using Analysis of Variance (ANOVA) to test for significance. Means were separated using Duncan's Multiple Range Test (DMRT) at 5% level of probability using Statistical Analysis Software (SAS) package. This study showed that ethanol and methanol extracts of leaf, stem and root of *C. bicolor* were able to inhibit the pathogenic fungi hence the fungitoxic potentials of these extracts can provide an alternative to synthetic fungicides since it is less expensive, environmental friendly and easy to prepare.
Keywords: *phytochemical, antimicrobial, extracts, caladium bicolor*

Introduction

Caladium bicolor (Aiton) Vent belongs to the family Araceae. The anatomy of vascular and support tissues in the leaf and petiole in Araceae showed and inferred an interesting relationship among them (Cusimano et al., 2011; Goncalves et al., 2004; Keating, 2004; 2002; 2000). Morpho-anatomical character patterns seem to imply a major adaptive shift in the evolution of aroids (Hesse, 2006), and could be used to distinguish closely related plants (Osuji and Nwala, 2015; Kemka-Evans et al., 2014). There are over 1000 cultivar names of Caladium, and over a hundred cultivars are grown today (Wilfret, 1993). *C. picturatum* and*C. marmoratum* are now considered synonyms of *C. bicolor* (Madison, 1981). In South and Central America, other species of Caladium like *C. marmoratum* Mathieu, *C. picturatum* Koch and *C. schomburgkii* Schott including *Caladium bicolor* (Aiton) Vent have been reported (Wilfret, 1993; Hayward, 1950). Breeding of this species of plant has led to many variants (Wilfret, 1993). Also, intraspecific or interspecific hybridizations among these American species may have produced the cultivated species *C. hortulanum* and other variants of economic values (Deng and Harbaugh, 2006; Wilfret, 1993; Hayward, 1950).

The naming and identification of *Caladium bicolor* cultivars is difficult. Among the Araceae family, calcium oxalate deposits can be diagnostic, including their presence, type, diversity, occurrence and distribution have been noted to enhance the delimitation of members of this family (Osuji and Nwala, 2014; Chairiyah et al., 2013; Osuji, 2013; Saadi and Mondal, 2011; Coté, 2009) and raphide idioblasts are known as storage facilities for aroids (Okoli, 1988; Okoli and Green, 1987; Okoli and McEuen, 1986). Anderson (2004) defined phytochemicals as plant-derived chemicals, which are beneficial to human health and disease prevention. In plants, phytochemicals attract beneficial and repel harmful organisms, serve as photoprotectants and responds to environmental changes. For instance, isoflavones, anthocyanins, and flavonoids do function as phytoalexins; substances that assist a plant to resist pathogens (Agate et al., 2000). According to Liu (2004), phytochemicals are bioactive, non-nutrient plant compounds in fruits, vegetables, grains and other plant foods that have been linked to reducing the risk of major degenerative diseases. According to Stampfer et al. (1993), phytochemicals are subsets of functional foods and are chemicals that are found in plant and plant-derived foods. A phytochemical is often found in coloured variety of fruits and vegetables. However, there are also several beneficial phytochemicals in colourless or less coloured fruits and vegetables (Stampfer et al., 1993). This study investigated the phytochemical screening and antimicrobial activity of ethanol and methanol extracts of leaf, stem and root of *Caladium bicolor*.

Materials and Methods

Collection of samples

The plant, *Caladium bicolor*, used in this study was collected from the wild at Ukpo, Dunukofia Local Government Area, Anambra State, Nigeria. The analysis was carried out at Central Service Laboratory, National Root Crops Research Institute, Umudike, Abia State, Nigeria. The botanical identity of the plant was authenticated by the Horticulture Unit of the institute.

Preparation of samples for analysis

The fresh plant leaf, stem and root were washed with tap water and oven dried at a temperature of 60°C for 72 hours. The samples were separately ground into fine powder using grinding machine after which the ground samples were sieved to obtain powdered processed sample used for the extraction.

Ethanol and methanol extractions

Using cold solvent extraction method (Doughari et al., 2007; Junaid et al., 2006; Harborne, 1973). 10g, 20g, 30g and 40g portion of each processed samples were mixed with 100ml of each solvent (ethanol and methanol) separately in a bottle to produce 10%, 20%, 30% and 40% extract concentrations respectively. The extracts were sieved through with four layers of sterile cheese cloth and stored in sterile conical flask which were later used for mycelial growth inhibition.

Qualitative determination of phytochemicals in leaf, stem and root of C. Biocolor

Qualitative tests were conducted to evaluate the presence or absence of phytochemical of interest. It was conducted using standard methods described below. The extracts were screened for the following phytochemical compounds: alkaloids, saponins, flavonoids, tannins and terpenoids (Ezebo et al., 2021).

Determination: Alkaloid, saponin, tannin, flavonoid and terpenoid

The presence of alkaloid in the samples were investigated using Meyer's colourimetirc method described by Harborne (1973). Ethanolic extract of the samples were obtained by shaking 2g of the samples in 20ml of ethanol for 30mins before filtrating over the funnel using Whatman no 1 filter paper in 100ml beaker. 2.5ml of each filtrate was taken and poured in the test tube labelled A, B, C and D, placed in a test tube rack. Few drops of Meyer's reagent were added to each of the test tube respectively. Formation of orange precipitate/colour shows the presence of alkaloid.

In saponin, the froth test and emulsion test described by Harborne (1973) were used to determine the presence of saponin. 5ml of distilled water was used to dissolve 1g of powdered samples in 250ml conical flask. Each of them was shaken and placed in water bath for 5mins. They were filtered hot over the funnel using Whatman no 1 filter paper in 100ml beaker. 2.5ml of each cooled filtrate was poured into the test tube labelled A, B, C and D, and placed in a test tube rack. 10ml of distilled water was used to dilute each of the tube respectively.

The presence of tannins was determined using the Harborne (1973) method. 1g of powdered samples were boiled with 5ml of distilled water in a water bath for 5 minutes. They were filtered hot with Whatman no 1 filter paper folded over a funnel in 100ml beakers. Four test tubes labelled A, B, C and D, was positioned in a test tube rack. 1ml of the cooled filtrates was added to each test tube accordingly. 10ml of ferric chloride was added to each of the test tube and observed. A greenish brown precipitate was observed which indicates the presence of tannin.

The presence of flavonoid in the samples was determined using the Harborne (1973), Sofowora (1996) methods. 1g of powdered samples were dissolved with 10ml of distilled water in 250ml conical flask, shaken and placed in water bath for 5mins. They were filtered hot using Whatman no 1 filter paper folded over the funnel in 100ml beaker. The filtrates were allowed to cool. Two drops of 20% NaOH was added to 1ml of each of the filtrates in a test tubes labelled A, B, C and D. A yellow amber colour

was observed in tubes A and D, light yellow in B and C. To each of the test tube was also added two drops of one normal sulphuric acid and observed. No colour change was observed in all the samples after addition of two drops of sulphuric acid. It implied that flavonoid is absent in the plant due to no formation of colourless solution.

In terpenoid, 5ml of aqueous extracts from each of the samples was poured into the test tube labelled A, B, C and D. 2ml of chloroform was added to each of the test tube. 1ml of concentrated sulphuric acid was also added to each of the flask to form a layer. A reddish brown precipitate at the interface indicates the presence of terpenoid.

Test: Froth and emulsion

Each of the flask was shaken vigorously for few minutes and observed. A stable forth (foam) upon standing indicates the presence of saponin. On the other hand, Two drops of olive oil was added to the four test tubes respectively and shaken vigorously. The formation of emulsion indicates the presence of saponin.

Quantitative determination of phytochemicals in leaf, stem and root of C. Biocolor

Determination of alkaloid

2g of each sample was analysed in accordance with the alkaline precipitation gravimetric method (Harborne, 1973). The weighed samples were soaked in 100ml of 10% acetic acid solution in ethanol and allowed to stand for 4 hours at room temperature before filtering using Whatman no 1 filter paper. The filtrates were reduced to a quarter of their original volume by evaporation over a steam bath. Alkaloids in the extracts were precipitated by drop wise addition of concentrated NH_4OH solution until full turbidity was obtained. The precipitate was recovered by filtration using weighed filter papers and then washed with 1% NH_4OH solution, dried in the oven at 100^oC for an hour. They were cooled in desiccator and reweighed. By difference, the weight of alkaloids present in the samples were determined and expressed as percentage for the samples and analysed using the formula:

$$\% \text{ Alkaloid} = W2\text{-}W1\times 100/\text{Weight of sample} \qquad \text{Eq (1)}$$

Where, W1 refer to weight of empty filter paper; and W2 refer as weight of paper plus alkaloid precipitate.

Determination of saponin

Saponin content of the samples were determined by double solvent extraction gravimetric method (Harborne, 1973). 2g of the powdered samples were mixed with 50ml of 20% aqueous ethanol solution. The mixtures were heated with periodic agitation in water bath for 30mins at 55^oC. They were filtered, the residues were extracted with 50ml of ethanol and both extracts were put together. The combined extracts were reduced to about 40ml at 90^oC and transferred to a separating funnel where 40ml of diethyl ether was added and shaken vigorously. Separation was by partition during which the ether layer was discarded and the aqueous layer reserved. Reaction was carried out until the aqueous layer became clear. The saponins were extracted with 60ml of normal butanol. The combined extracts were washed with 5% aqueous NaCl solution and evaporated to dryness in a pre-weighed evaporating dish.

They were dried at 60^{o}C in the oven and reweighed. The saponin content was calculated as percentage of original sample as:

$$\% \text{ Saponin} = W2\text{-}W1\times100/\text{Weight of sample} \quad \text{Eq. (2)}$$

Where, W1 is the weight of evaporating dish; and W2 is the weight of dish plus sample.

Determination of tannin

The Follins-Dennis spectrophotometric (Pearson, 1976) was used. With 2g of the powdered samples were dispensed into 50ml of distilled water in a conical flask and shaken for 30mins in a shaker. The mixtures were filtered. 5ml of the filtrates were measured into 50ml volumetric flask and then diluted with 35ml of distilled water. Also, 5ml of standard tannic acid solution and 5ml of distilled water were measured with separate flasks to serve as standard and blank respectively. They were diluted with 35ml of distilled water separately. 1ml of Follins-Dennis Reagent was added to each of the flask followed by 2.5ml of saturated sodium carbonate solution. The content of each flask was filled to mark level with distilled water and incubated for 90mins at room temperature. The absorbance of the developed colour was measured at 76nm wavelength with the reagent blank at zero. The tannin content was calculated as shown below:

$$\% \text{ Tannin} = 100/w\times AU/As\times VF/VA\times D \quad \text{Eq. (3)}$$

Where, W is the weight of the sample analysed; AU is the absorbance of test sample; AS is the concentration of standard in mg/ml; C is the total volume of extract; VF is the volume of filtrate analysed; and D is the dilution factor (where applicable).

Determination of flavonoid

Flavonoids determination was done using BohamandKocipai method. 10g of the plant samples were extracted repeatedly with 100ml of 80% aqueous methanol in conical flask at room temperature. The whole solution was filtered using a weighed Whatman no 1 filter paper. The filtrates were transferred into crucible and dryness in the oven, cooled in the desiccator and weighed. The percentage flavonoid was expressed as the weight of sample analysed using the formula;

$$\% \text{ Flavonoid} = W2\text{-}W1\times100/\text{Weight of sample} \quad \text{Eq. (4)}$$

Where, W1 is the weight of empty filter paper; and W2 is the weight of filter paper plus flavonoid precipitate.

Microbial analysis

Using a wire loop, colonies of the test organisms (*Aspergillus flavus* and *Candida albican*) were collected from the Central Service Laboratory, National Root Crops Research Institute, Umudike, Abia State, Nigeria.

Susceptibility test of fungi pathogens to plant extracts

Effect of plant extracts on mycelial growth with test fungi was studied using the food poisoning techniques (Sangoyomi, 2004). One millitre of each plant extract concentrations (10%, 20%, 30% and 40%) was dispensed per Petri dish and 9 ml of the media (molten PDA) was added to each of the Petri dish containing extracts and carefully spread evenly over the plates. These were used for the inhibition of mycelia growth. The plates were gently rotated to ensure even dispersion of the extracts. The agar extract mixture were allowed to solidify and then inoculated at the centre with a 4mm diameter mycelia disc obtained from the colony edge of 7-day old pure cultures of the test fungi. Each treatment was duplicated. The control set up consists of blank agar plate (no extract) inoculated with the test fungi.

All the plates were inoculated at 27 ± 2^0C for 5 days and examined daily for growth and presence of inhibition. Colony diameter was taken as the mean growth along two directions on two pre-drawn perpendicular lines on the reverse side of the plates. The effectiveness of the extract was recorded in terms of percentage inhibition, which was calculated according to the method described by Whipps (1987).

$$\text{Percentage inhibition} = R_1\text{-}R_2/R_1\times 100/1 \qquad \text{Eq. (5)}$$

Where; R_1 is the farthest radial distance of pathogen in control plates, while $R_2$is the farthest radial distance of pathogen in extract incorporated agar plates. The percentage inhibition was determined as a guide in selecting the minimum inhibition concentration (MIC) that will be effective in controlling the rot-causing fungi. Extracts were rated for their inhibitory effects using the scale of Sangoyomi (2004): $\leq$ 0% = no inhibition; > 0-20% = slight inhibition; > 20-50% = moderate inhibition; > 50-100% = effective inhibition; and 100% = high inhibition.

Statistical analysis

All data were analysed with Analysis of Variance (ANOVA) to test for significance. Means were separated using Duncan's New Multiple Range Test (DNMRT) at 5% level of probability using Statistical Analysis Software (SAS) package.

Results and Discussion

Qualitative determination of phytochemical composition of ethanol and methanol extraction in C. Bicolor leaf, stem and root

The results of the qualitative phytochemical analyses of the ethanol and methanol leaf, stem and root extracts of *C. bicolor* showed the presence of alkaloids, saponins, flavonoids, tannins and terpenoids. The ethanol extract of the leaf showed the presence of all the phytochemicals assayed while the methanol extract of leaf showed moderate and excessive presence of tannin and flavonoid respectively (*Table 1*). The ethanol and methanol extracts of the stem showed the presence of all the phytochemicals assayed (*Table 2*). Also, the ethanol and methanol extracts of the root showed the presence of all the phytochemicals assayed (*Table 3*).

Table 1. *Qualitative phytochemical composition of ethanol and methanol leaf extracts of C. Bicolor.*

Phytochemicals	Ethanol	Methanol

Alkaloid	+	+
Saponin	+	+
Tannin	+	++
Flavonoid	+	+++
Terpenoid	+	+

Notes: + means present; ++ means moderately present; +++ means excessively present.

***Table 2**. Qualitative phytochemical composition of ethanol and methanol stem extracts of C. Bicolor.*

Phytochemicals	Ethanol	Methanol
Alkaloid	+	+
Saponin	+	+
Tannin	+	+
Flavonoid	+	+
Terpenoid	+	+

Notes: + means present; ++ means moderately present; +++ means excessively present.

***Table 3**. Qualitative phytochemical composition of ethanol and methanol root extracts of C. Bicolor.*

Phytochemicals	Ethanol	Methanol
Alkaloid	+	+
Saponin	+	+
Tannin	+	+
Flavonoid	+	+
Terpenoid	+	+

Notes: + means present; ++ means moderately present; +++ means excessively present.

Quantitative determination of phytochemical composition of ethanol and methanol etraction in C. Bicolor leaf, stem and root

The results of the quantitative phytochemical analysis of the leaf extract of *C. bicolor* revealed that ethanol extract had the highest composition of saponin (11.86±4.31) while the lowest was alkaloid (0.07±0.13). The methanol extract showed the highest composition of saponin (9.74±2.27) while the lowest was tannin (0.20±0.11) (*Table 4*).

***Table 4**. Quantitative phytochemical composition of ethanol and methanol leaf extract of C. Bicolor.*

Plant extracts	Alkaloids	Saponins	Flavonoids	Tannins	Terpenoids
Ethanol	0.07±0.13	11.86±4.31	0.67±0.89	0.36±0.17	0.74±0.09
Methanol	0.31±0.06	9.74±2.27	0.46±0.55	0.20±0.11	0.64±0.07

Notes: ± is mean±standard deviation.

The results of the quantitative phytochemical analysis of the stem extract of *C. bicolor* showed that ethanol extract had the highest composition of saponin (8.67±0.03) while the lowest was tannin (0.21±0.01). The methanol extract showed the highest composition of tannin (6.02±0.20) while the lowest was alkaloid (0.06±0.01) (*Table 5*).

***Table 5**. Quantitative phytochemical composition of ethanol and methanol stem extract of C. Bicolor.*

Plant extracts	Alkaloids	Saponins	Flavonoids	Tannins	Terpenoids
Ethanol	0.53±0.03	8.67±0.03	0.83±0.12	0.21±0.01	0.77±0.04

Methanol	0.06±0.01	3.04±0.55	0.12±0.64	6.02±0.20	0.89±0.36

Notes: ± is mean±standard deviation.

The results of the phytochemical analysis of the root extract of *C. bicolor* indicated that ethanol extract had the highest composition of flavonoid (0.60±0.10) while the lowest was alkaloid (0.02±0.11). The methanol extract showed the highest composition of flavonoid (0.33±0.21) while the lowest was terpenoid (0.03±0.01) (*Table 6*).

***Table 6**. Quantitative phytochemical composition of ethanol and methanol root extract of C. Bicolor.*

Plant extracts	Alkaloids	Saponins	Flavonoids	Tannins	Terpenoids
Ethanol	0.02±0.11	0.13±0.28	0.60±0.10	0.04±0.22	0.09±0.45
Methanol	0.06±0.20	0.12±0.08	0.33±0.21	0.18±0.03	0.03±0.01

Notes: ± is mean±standard deviation.

Susceptibility test of fungal pathogens to leaf, stem and root extracts of C. Bicolor

All the plant extracts showed varying degrees of inhibition on the test fungi, this was dependent on the concentration of the extracts. The ethanol leaf extract at 40% extract concentration showed the highest inhibitory effect (31.44±0.10) (moderately effective) on *A.flavus*, this was significantly higher than other interactions, while the lowest inhibitory effect (4.23±0.05) (slightly effective) was observed on *C. albican* at 10% extract concentration (*Table7*). The methanol leaf extractat 40% extract concentration showed the highest inhibitory effect (21.02±0.04) (moderately effective) on *C. albican* while the lowest inhibitory effect (2.30±0.06) (slightly effective) was noticed on *A. flavus* at 10% extract concentration (*Table 8*).

***Table 7**. Effect of different concentrations of ethanol leaf extraction on the growth of test fungi.*

Organism	10%	20%	30%	40%
A.flavus	12.20±0.12	13.10±0.13	25.08±0.06	31.44±0.10
C.albican	4.23±0.05	8.01±0.12	10.00±0.03	19.30±0.65

Notes: ± is mean±standard deviation.

***Table 8**. Effect of different concentrations of methanol leaf extraction on the growth of test fungi.*

Organism	10%	20%	30%	40%
A.flavus	2.30±0.06	11.00±0.02	18.10±0.11	20.28±0.01
C.albican	3.40±0.08	5.13±0.03	8.10±0.05	21.02±0.04

Notes: ± is mean±standard deviation.

The ethanol stem extract at 40% extract concentration showed the highest inhibitory effect (16.00±0.08) (slightly effective) on *A. flavus* while the lowest inhibitory effect (2.07±0.01) (slightly effective) was noticed on *C. albican* at 10% extract concentration (*Table 9*). The methanol stem extractat 40% extract concentration showed the highest inhibitory effect (14.03±0.77) (moderately effective) on *C. albican* while the lowest inhibitory effect (4.03±0.08) (slightly effective) was observed on *A. flavus* at 10% extract concentration (*Table 10*).

***Table 9**. Effect of different concentration of ethanol stem extract on the growth of test fungi..*

Organism	10%	20%	30%	40%
A.flavus	7.12±0.04	11.30±0.90	15.40±0.23	16.28±0.08
C.albican	2.07±0.01	5.62±0.06	8.11±0.05	12.55±0.01

Notes: ± is mean±standard deviation.

***Table 10**. Effect of different concentration of methanol stem extract on the growth of test fungi.*

Organism	10%	20%	30%	40%
A.flavus	4.03±0.08	8.55±0.04	10.10±0.12	13.00±0.13
C.albican	5.04±0.09	7.48±0.22	9.45±0.67	14.03±0.77

Notes: ± is mean±standard deviation.

The ethanol root extract at 40% extract concentration showed the highest inhibitory effect (12.60±0.15) (slightly effective) on *C. albican* while the lowest inhibitory effect (2.05±0.06) (slightly effective) was observed on *A. flavus* at 10% extract concentration (*Table 11*). The methanol root extract at 40% extract concentration showed the highest inhibitory effect (11.40±0.09) (slightly effective) on *A. flavus* while the lowest inhibitory effect (1.00±0.06) (slightly effective) was noticed on *C. albican* at 10% extract concentration (*Table 12*).

***Table 11**. Effect of different concentration of ethanol root extract on the growth of test fungi.*

Organism	10%	20%	30%	40%
A.flavus	2.05±0.06	6.11±0.06	8.15±0.32	11.42±0.17
C.albican	4.00±0.09	6.12±0.20	8.13±0.04	12.60±0.15

Notes: ± is mean±standard deviation.

***Table 12**. Effect of different concentration of methanol root extract on the growth of test fungi.*

Organism	10%	20%	30%	40%
A.flavus	4.53±0.11	6.07±0.42	8.22±0.13	11.40±0.09
C.albican	1.00±0.06	3.94±0.33	6.25±0.10	10.52±0.01

Notes: ± is mean±standard deviation.

The result of this study showed that plant extracts showed antifungal activity against the test organisms at various concentrations of the plant extracts. Biswas et al. (2013) reported the antimicrobial activity of *C. bicolor* against several antimicrobial strains and the most susceptible one was *Staphylococcus aureus*. Essien et al. (2015) has studied the antimicrobial activity of *C. bicolor* and *C. tricolor*. The ethanol and methanol extracts of leaf, stem and root of *C. bicolor* at all concentrations were inhibitory on the test organisms *in vitro*, with ethanol extract being the most potent. This is in line with observations made by Okigbo and Odurukwe (2009) as well as Sangoyomi (2004). The inhibitory effect on the test fungi differs with the plant materials and solvent of extraction. Generally, the ethanol extracts were more effective than their corresponding methanol extracts of the plant samples. This can be attributed to the fact that ethanol is an organic solvent and will dissolve organic compounds better, hence, extract the active compounds required for antimicrobial activities (Ekwenye and Elegalam, 2005). The difference in the fungitoxic potential between extractions medium can also be a result of the different susceptibility of each of the test organisms to different concentrations of the extracts. This also agrees with the findings of some workers (Okigbo and

Odurukwe, 2009; Okigbo and Nmeka, 2005; Amadioha, 2000; Onifade, 2000). As the concentration of the extracts increased, the level of inhibition on the mycelial growth of the fungi increased. This agrees with the reports of Ekwenye and Elegalam (2005), Okigbo and Igwe (2007). This is also similar to the results obtained by Suleiman (2010) who stated a significant difference between mycelial growth value recorded on the various plant extracts concentration.

According to Srinivauson et al. (2001), the presence of bioactive substances has been reported to confer resistance to fungi, bacteria and pests. This therefore, explains the demonstration of antifungal activities by the extracts used in this study. Thus, the antifungal properties of the plant extracts are probably due to the presence of phytochemicals which are antimicrobial agents (Okwu and Joshia, 2006), and inhibitory to the growth of these pathogens (Okigbo et al., 2009). Phytochemical analysis of the plant extracts showed that ethanol leaf extract of *C. bicolor* contained more phytochemicals when compared to stem and root extracts which justifies its highest inhibition (31.44$\pm$0.10) on *A. flavus* at 40% extract concentration. However, the methanol leaf extract showed highest inhibition (21.02$\pm$0.04) on *C. albican* at 40% extract concentration. The study revealed the fungitoxic potentials of ethanol and methanol extracts of leaf, stem and root of *C. bicolor* at different concentrations could be further developed to produce natural fungicides in the control of pathogenic microorganisms that cause damage to agricultural crops.

Conclusion

This study demonstrated that leaf, stem and root extracts of *C. bicolor* showed antifungal activity against the test organisms at various concentrations of the plant extracts. The phytochemical analysis of the plant extracts revealed that ethanol leaf extract of *C. bicolor* contained more phytochemicals when compared to stem and root extracts. This finding is crucial from the point of view of controlling diseases associated with *A. flavus* and *C. albican* that affect plant and animal without the use of chemicals which cause environmental pollution.

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Conflict of interest

The author(s) confirm that there is no conflict of interest involved with any parties in this research study.

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