Evaluation of bioactive content and pharmacological properties of *Caladium bicolor* extracts

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Abstract

The investigation looks at the phytochemical constitution, antimicrobial, and antioxidant properties of *Caladium bicolor* methanol extracts. Standard techniques were used for the chemical and biological analyses. The extracts contained varied amounts of alkaloids, flavonoids, tannins, phenols, saponins, terpenoids, carbohydrates, glycosides, and steroids, according to the phytochemical screening. Carbohydrates were found in abundance in the samples, particularly in the butanol and methanol extracts. Steroids were also found in high concentrations in all solvent extracts except ethanol extracts. Except for aqueous extracts, saponin was not found in any of the solvent extracts. Using a UV-VIS spectrophotometer, the extract of plant was scanned in the wavelength range of 248 to 420 nm, and the characteristic peaks were found. The spectra ranged from 802.93 to 3343.71 cm^{-1} in the IR results of various solvent extracts. The ethanol extracts had the strongest free radical scavenging activity in the DPPH and hydroxyl radical scavenging assays (IC_{50} values of 13.98 and 22.07 µg/ml, respectively). Using the disc diffusion method, different concentrations of *Caladium bicolor* extracts showed moderate antibacterial and antifungal activity against *E. coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Staphylococcus aureus, Bacillus subtilis, Streptococcus mutans, Aspergillus niger*, *Aspergillus flavus*, and *Candida albicans*. The study's findings suggest that *Caladium bicolor* may contain natural antibacterial and antioxidant compounds that could be used in medication development.

Keywords: antioxidants, *Caladium bicolor*, carbohydrates, DPPH and phytochemicals

1. Introduction

Essential components found in medicinal plant extracts could be used as therapeutic medications [1]. Many significant components are found in therapeutic plant extracts, including alkaloids, flavonoids, tannins, and phenolic compounds [2]. Medicinal plant extracts are high in nutrients and have few side effects, making them safe to use on a regular basis to prevent and treat a variety of ailments. Various investigations on medicinal plants' pharmacological activity have been widely publicised. Calvo et al. [3] found that approximately 25,000 plant species have pharmacological activities such as antidiabetic, anti-oxidant, anti-cancer, and anti-bacterials, but there are still many opportunities to learn more about the potential of new plants with these important traits.

Caladium, also called as elephant's ear, Jesus' heart, and fancy-leaved caladium, is a decorative foliage plant that grows from tubers and is widely used in landscapes, particularly in the Southeast. Caladium is a member of the Araceae family and is native to South and Central America. This plant's coloration enhances the beauty and adoration of the place in which it grows. Caladium leaf features determine the ornamental value of the plant whether used as a pot or landscape plant. Caladium breeding and cultivar development's top priority is to: improve leaf traits or create new combinations of them [4]. The leaf blades, which have varied patterns of white and pink dots, are used to identify the species. The most common leaf shapes are heart, lance, and arrowhead. Berry fruits with many tiny ovoid seeds are produced. Caladium cultivar development dates back over 150 years [5]. However, due to a lack of conservation, roughly 51 caladium varieties have been extinct. For example, just 141 cultivars were classified in exotica (1970), according to a recent survey of Florida tuber growers [6].

Caladium includes oxalate crystals, which can induce tongue and throat swelling and sickness. For the treatment of angina, a fresh leaf infusion is employed [7]. To treat infected wounds, use the dried leaf powder, while the tuber powder is utilized by the French Guiana to treat facial skin imperfections. However, several Caladium species' chemical composition and biological activities have been documented by a number of writers [8]. *Caladium bicolor* extracts' phytochemical composition, in vitro antioxidant activity, and antimicrobial properties are being evaluated in this study against a range of diseases.

2. Materials and methods

2.1. Plant material

In the present study, *Caladium bicolor* plant was selected based on distribution and the information collected from the literature. The plants were collected from various locations in Kanyakumari District.*C. bicolor's* taxonomic position is depicted in Figure 1.

Kingdom	**:**	**Plantae**
Class	**:**	**Monocots**
Order	**:**	**Alismatales**
Family	**:**	**Araceae**
Genus	**:**	***Caladium***
Species		***bicolor***

Figure 1. Taxonomic position of plant, *Caladium bicolor*

2.2. Sample preparation and Soxhlet extraction

The fresh whole plant of *Caladium bicolor* is collected. Washed in tap water and respectively distilled water was used to rinse to remove the dirt and dust particles which is present in it. The washed plant was dried using shade dried method and powdered using mortar and pestle. After the setup of Soxhlet apparatus using clamps and mounts. The round bottom flask is filled with solvent based on the nature of solvent (non-polar to polar = Chloroform > Butanol > Ethanol > Methanol > Aqueous), around 250-300ml of solvent is filled in the round bottom flask. Cotton is used to cover the hole of siphon or capillary tube in need to avoid the powdered sample to get settled in round bottom flask and also in siphon tube. The powdered

sample were rolled in a cotton cloth and placed in the soxhlet thimble. Once the condenser is filled with running tap water. Isomantle (heat source) is used to evaporate the solvent, the temperature was fixed about the heating point of desired solvents. Repeatedly fifteen times the extraction is carried out by running the soxhlet apparatus. The extract was collected and used for further analysis.

2.3. Phytochemical screening

2.3.1. Qualitative assay

Alkaloids [9], flavonoids [10], tannin, phenol, saponin, and terpenoids [11], carbohydrates [12], glycosides [13], and steroids [14] were identified in the whole plant extract using a conventional technique.

2.3.2. Quantitative assay

A standard procedure was used to determine the amounts of alkaloids, saponin, and steroids [15], flavonoids, tannin, and phenol [16], terpenoids [17], carbohydrates [18], and glycosides [19] in solvent extracts include aqueous, Chloroform, methanol, Butanol and Ethanol.

2.4. Characterization of *Caladium bicolor* extract

2.4.1. UV

The *Caladium bicolor* extract was subjected to UV-VIS spectrophotometric analysis using a UV-VIS spectrophotometer (Perkin Elmer, USA Model: Lambda 950) using a 10-mm cell and a 2 nm slit width, at ambient temperature. The extract was examined for proximate analysis using visible and UV light with a wavelength range of 300 to 800 nm. For UV-VIS spectrophotometer analysis, the extract was centrifuged at 3000 rpm for 10 minutes before being filtered through Whatman No. 1 filter paper. With the same solvent, the sample is diluted to 1:10.

2,4.2. FTIR

The characteristic functional groups in the extract were identified using Fourier transform infrared (FTIR). It provides details about a molecule's structure, which is frequently discernible from its absorption spectrum. *Caladium bicolor* extract was combined with dry potassium bromide in a little amount (KBr). In a mortar, the ingredients were properly mixed before being pressed at a pressure of 6 bars for 2 minutes to produce a KBr thin disc. The disc was then placed in a diffuse reflectance accessory sample cup. The IR was collected using a Bruker Vertex 70

infrared spectrometer from Germany. From 4000 to 400 cm^{-1}, the sample was scanned. Peak values for FTIR and UV-VIS were noted.

2.5. Antioxidant activity

The diphenyl picrylhydrazyl (DPPH) and hydroxyl radical scavenging methods were used to assess the antioxidant activity of the various solvent fractions [20].

2.6. Antimicrobial activity

2.6.1. Test organisms

Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Streptococcus mutans, Bacillus subtilis and *Staphylococcus aureus*, were used as antimicrobial test microorganisms. Fungi including *Aspergillus niger, Aspergillus flavus,* and *Candida albicans* were cultured on Nutrient Agar using strains of bacteria and fungi that were received from the Microbial Type Culture Collection and Gene Bank (MTCC) in Chandigarh (NA). The medium used is saboured dextrose agar (SDA).

2.6.2. Preparation of Nutrient Broth

In five different solvents, the method, disc diffusion is employed to determine the plant's efficacy. A Nutrient Agar plate was infected with the pure culture from the plate. The inoculum was made by aseptically mixing 2 ml of sterile 0.145 mol/l saline into 2 ml of fresh culture and incubating at 37°C for 24 hours. The cell density is adjusted to 1.5108 CFU/ml of bacterial suspension is provided by meeting the 0.5 McFarland turbidity criterion. In the antimicrobial test, standard microorganisms were used.

2.6.3. Antibacterial activity

After being dissolved in 1000 ml of distilled water, 38 g of Mueller-Hinton Agar Medium was autoclaved for 15 minutes at 121°C, pH 7.3, and 15 Lbs of pressure. The autoclaved medium was distributed into 25 mL petri dishes after being cooled and well mixed. Bacteria were swabbed off the plates. The plates were then filled with samples and incubated at 37°C for 24 hours before the disc was put on top of Mueller-Hinton Agar substrate. A clean ruler was used to measure the zone of inhibition's dimensions in millimetres after it was thoroughly checked all around the disc. It was assumed that if there was no zone inhibition, there was no activity [21].

2.6.4. Antifungal Activity

Antibiotic susceptibility was assessed using the agar disc diffusion technique [22]. Fungi strains was swabbed with sterilized cotton swabs on the SDA agar plate. Up to 50 µl of each concentration of the extract was pipetted into the sterile disc using sterile pipettes. The chemical was then given five minutes to diffuse on top of the SDA medium before being cultured for 48 hours at 22°C. The zone of inhibition surrounding the disc was examined and calculated in millimetres with a clean ruler at the end of the incubation period.

3. Results and Discussion

Table 1 and Figure 2 shows the findings of phytochemical screening of *Caladium bicolor* extracts. The extracts contained varied amounts of alkaloids, flavonoids, tannin, phenols, terpenoids, glycosides, steroids, saponins, and carbohydrates, according to the phytochemical screening. Except for ethanol extracts, all of the extracts had a significant content of carbohydrates and steroids. Except for aqueous extracts, saponins were not found in any of the solvent extracts. Phytochemical content and concentration in extracts can be linked to the species type and the components' inherent character. Ekanem et al. [8] studied the phytochemical profile of certain *Caladium species'* stems, bulbs, leaves, and roots, finding that the leaves have higher quantities of flavonoids, alkaloids, and saponins than other portions of the plant. The phytochemical constituents and antibacterial activity of methanol and ethanol leaf, stem, and root extracts of *Caladium bicolor* (Aiton) against several clinical infections were investigated by Ezebo et al. [23].

Table 1. Qualitative phytochemical constituents of *C. bicolor*

Phytochemical Qualitative Tests	**Extracts of *Caladium bicolor***				
	Ethanol	**Methanol**	**Chloroform**	**Butanol**	**Aqueous**
Alkaloids	+	+	-	+	-
Flavonoids	-	-	++	+	-
Tannin	+	+	-	-	+
Phenol	+	+	-	+	+
Saponins	-	-	-	-	+
Terpenoids	-	-	+++	++	+
Carbohydrates	-	+	-	+	+
Glycosides	+	+	+	+	-
Steroids	-	+	-	++	+

+++: High; ++: Moderate; +: Trace; -: absent

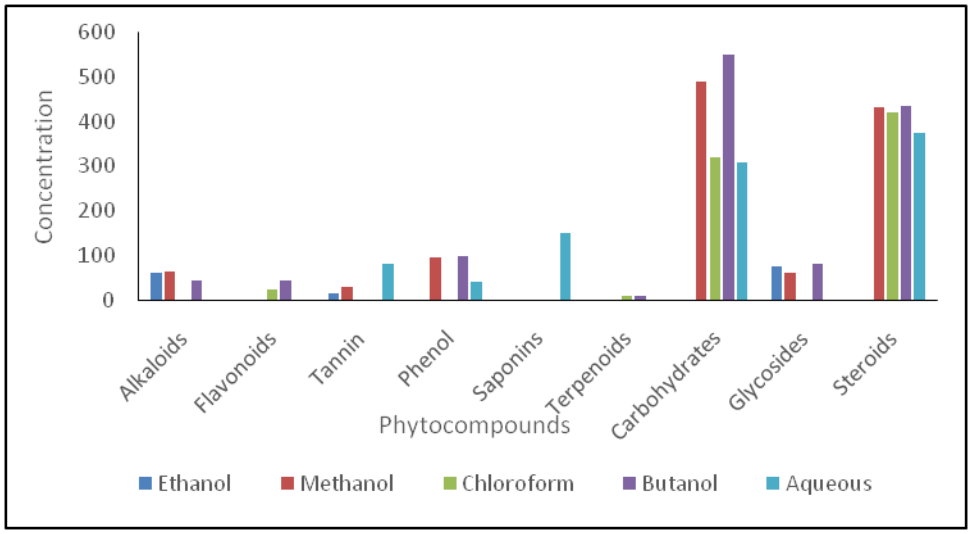


Figure 2. Quantitative phytochemical constituents of *C. bicolor*

3.1. UV

Caladium bicolor extracts were subjected to UV-VIS analysis in order to identify phytoconstituents (Fig. 3). To detect compounds with σ-bonds, π-bonds and lone pair of electrons, chromophores, and aromatic rings, UV-visible spectra were used. Due to the sharpness of the peaks and adequate baseline, the qualitative UV-VIS profile of all *Caladium bicolor* extracts was collected at a wavelength of 248 to 420 nm. Unsaturated groups and heteroatoms like S, N, and O are indicated by the appearance of one or more peaks in the UV-VIS spectra between 200 and 400 nm [24]. Though, the application of UV-visible spectrophotometery in the investigation of complex media is limited due to the inherent challenges of assigning absorption peaks to specific elements in the system. To enable proper extract characterization and ingredient identification, The results of UV-VIS analysis must be validated by additional analytical methods like GC/MS, etc [25].

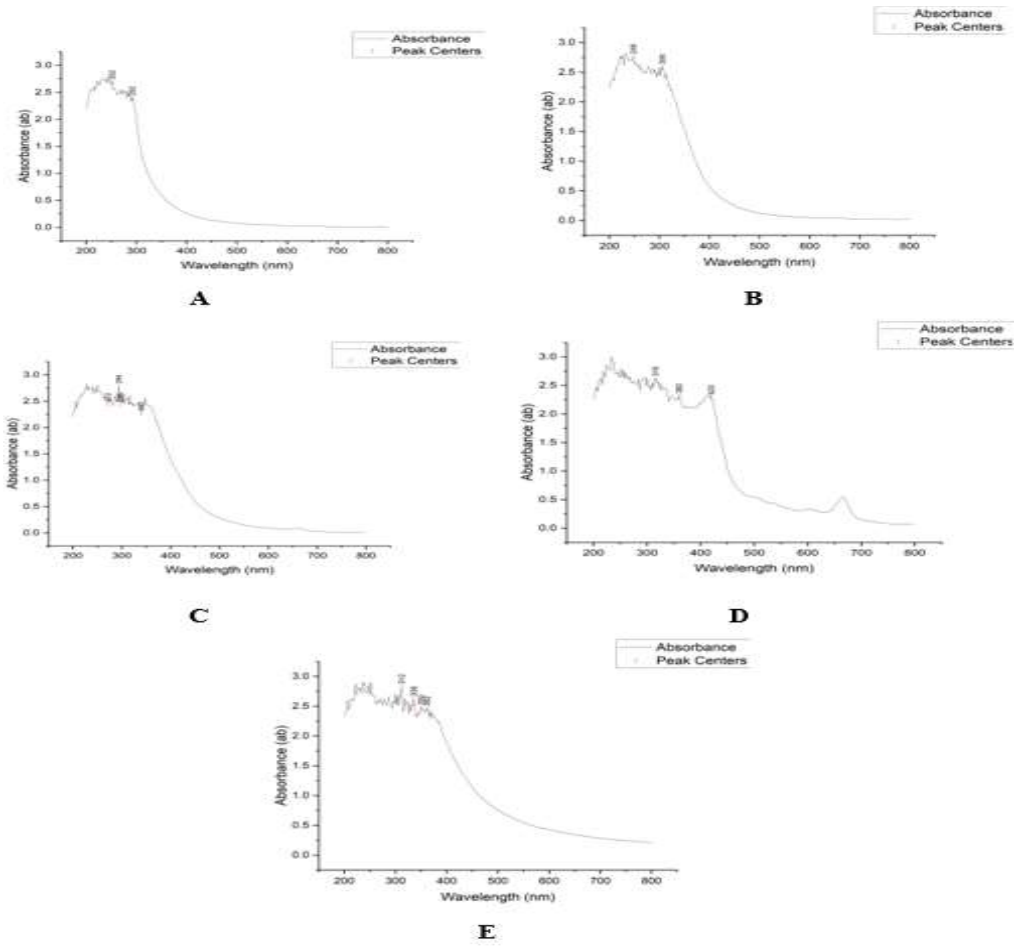


Figure 3. UV-VIS spectra of *C. bicolor* extracts, A. Ethanol; B. Methanol, C. Chloroform; D. Butanol; E. Aqueous

3.2. FTIR

The spectrum ranged from 802.93 to 3343.71 cm^{-1} in the IR results of various solvent extracts (Fig. 4). The functional groups phenol, carboxylic acid, alkanes, and alkyl halides are responsible for antibacterial activity in the IR spectrum range of various solvent extracts of *Caladium bicolor*. Bioactive compounds in various solvents and aqueous crude extracts have the ability to form complexes with proteins and bacterial membranes. These findings indicate that *Caladium bicolor* extract has antibacterial efficacy against intestinal microorganisms. The presence of phenolic chemicals, flavonoids, saponins, tannins, and glucose as significant functional groups was found by FTIR analysis of *Caladium bicolor* preparations [26]. Ragavendran et al. [27] looked at the medicinal properties of the functional groups of amines,

carboxylic acids, amides, polysaccharides, organic hydrocarbons, sulphur derivatives, and halogens in Aervalanata.

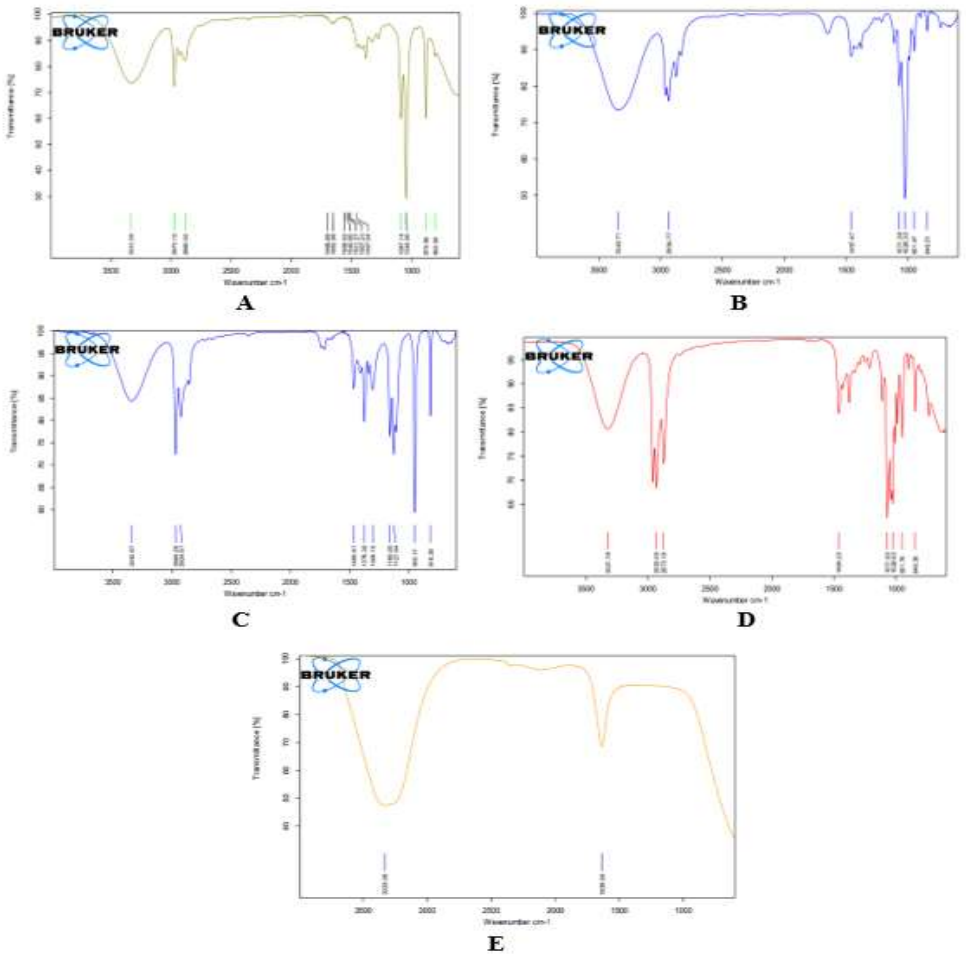


Figure 4. FTIR spectra of *C. bicolor* extracts, A. Ethanol; B. Methanol, C. Chloroform; D. Butanol; E. Aqueous

3.3. Antioxidant activity

3.3.1. DPPH radical scavenging activity

Figure 5 shows the anti-oxidant activity of several *Caladium bicolor* extracts as measured by the DPPH assay. The extracts' free radical scavenging potentials were determined to be in the following order: ethanol > methanol > butanol > aqueous > chloroform. In this study, the percentage inhibition DPPH scavenging ability of ethanol extract (13.98 µg/ml) was higher than others. Methanol (27.38 µg/ml), butanol (158.28 µg/ml), aqueous (434.53 µg/ml) and chloroform (567.57 µg/ml) extracts have inhibition values of <50% ascorbic acid that used as positive

control showed the lowest DPPH scavenging ability (642.77 µg/ml).All of the extracts had significant antioxidant properties when compared to the standard. The finding also implies that the plant includes phytoconstituents capable of giving hydrogen to protect the cell from harm.

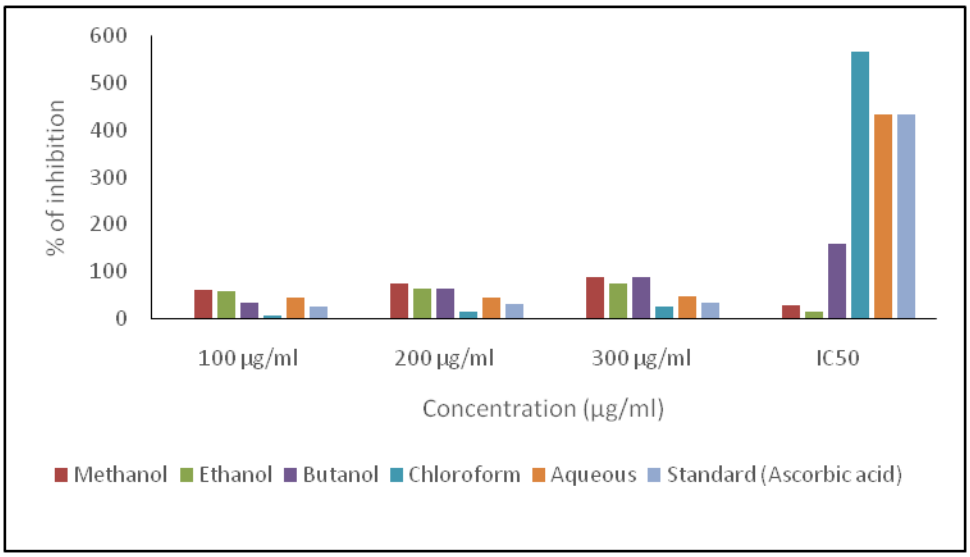


Figure 5. The antioxidant DPPH scavenging ability of *C. bicolor* extracts

3.3.2. Hydroxyl Radical Scavenging Activity

The ability of several *Caladium bicolor* extracts to scavenge hydroxyl radicals is shown in Figure 6. All of the extracts had significant hydroxyl radical scavenging activity. The butanol, ethanol and chloroform extracts had the highest Hydroxyl Radical scavenging activity, with an IC_{50} of 22.07, 23.07 and 24.06 µg/ml, followed by methanol with an IC_{50} of 113.37 µg/ml and aqueous with 196.48 µg/ml. Plant phenolics found in fruits and vegetables, such as flavonoids, phenolic acids, and tannins, may have biological activities including anti-atherosclerotic, anti-cancer, and anti-inflammatory properties, which may be linked to their antioxidant activity [28]. Plant phenolics with hydroxyl groups have a high capability for scavenging [29]. The results showed that the extract contains phenolic chemicals, which suggest that hydroxyl groups are present.

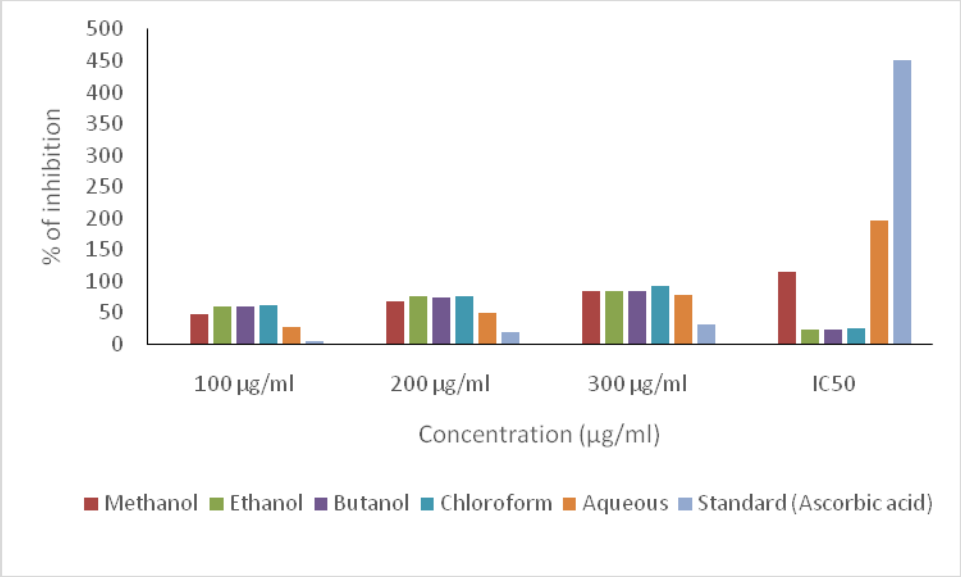


Figure 6. Hydroxyl radical scavenging activity of *C. bicolor* extracts

3.4. Antimicrobial activity

3.4.1. Antibacterial activity

Table 2 and Figure 7 indicate the antibacterial efficacy of several *Caladium bicolor* extracts against six harmful microorganisms. Antibacterial activity of aqueous extracts against *E. coli* and *K. pneumoniae* bacteria. Except for *B. subtilis*, ethanol extracts have antibacterial activity against all microorganisms tested. Methanol, chloroform, and butanol extracts, on the other hand, have antibacterial activity against all of the bacteria examined. Streptomycin was utilized as a positive control, and it had the most antibacterial activity of all the bacteria studied. The negative control (DMSO) has no influence on bacterial growth, indicating that DMSO had no effect on the experiment.*In vitro* tests revealed that the various extracts inhibited Gram-positive and Gram-negative bacteria, implying that *Caladium bicolor* extract possesses a broad spectrum of antibacterial action. Rosa et al. [30] discovered that the antibacterial potentials of *Irpexlacteus* culture extracts were identical to those found in *Irpex lacteus* culture extracts. *Caladium bicolor* has distinct antibacterial potentials due to the presence of several bioactive chemicals [31]. The antibacterial activity of the extracts was determined by the dose/concentration of bacteria utilized as a chemical constituent in the extracts, as well as the type of bacteria used. The higher the concentration of an antibacterial material, the more antibacterial chemicals it contains and the more potent its antibacterial effect [3].

Table 2. Antibacterial activity of *C. bicolor* extracts

Sample	Bacterial strains (mm)					
	E. coli	***K.pneumoniae***	***P.aeruginosa***	***S.aureus***	***S.mutans***	***B.subtilis***
CB-A	8	7	NZ	NZ	NZ	NZ
CB-M	9	8	9	10	10	11
CB-C	9	13	12	10	9	11
CB-E	8	9	9	9	10	NZ
CB-B	11	11	8	11	10	14
+ve	16	18	20	21	18	19
-ve	NZ	NZ	NZ	NZ	NZ	NZ

NZ- No Zone

CB -A - *C. bicolor*(Aqueous); CB -M - *C. bicolor*(Methanol); CB -C - *C. bicolor*(Chloroform); CB -E - *C. bicolor*(Ethanol); CB -B - *C. bicolor*(Butanol); + ve - Positive control (Streptomycin); - ve -Negative control

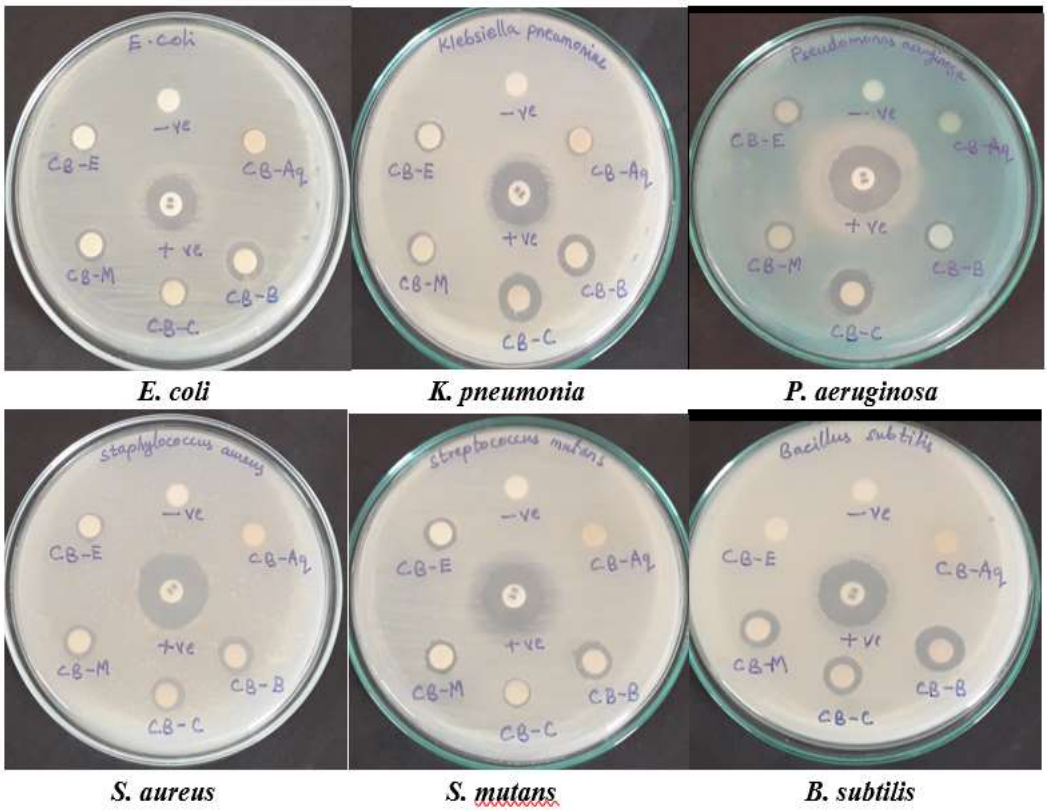


Figure 7. Antibacterial activity of *C. bicolor* extracts

3.4.2. Antifungal activity

The *C. bicolor* extracts of methanol, ethanol, and butanol had a better inhibitory impact (Table 3; Fig. 8). The extracts may have antifungal activity against the test fungi's spore

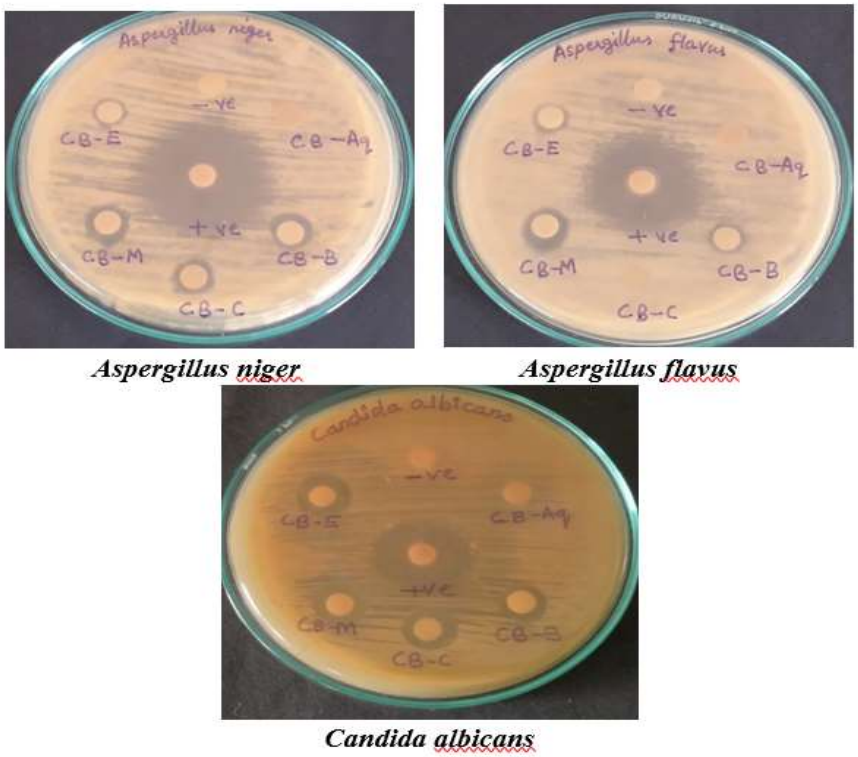
germination. Others have reported on the antifungal effect of extracts against a variety of food-borne infections, including *Botryodiplodia theobromae, Fusariu moxysporum, Aspergillus niger, Aspergillus flavus, Penicillium oxalicum* and *Verticillium theobromae,* of widespread food crops [32, 33, 34, 35].

Table 3. Antifungal activity of *C. bicolor* extracts

Sample	Fungal strains (mm)		
	Aspergillus niger	***Aspergillus flavus***	***Candida albicans***
CB-A	NZ	NZ	NZ
CB-M	9	10	11
CB-C	10	NZ	11
CB-E	8	9	11
CB-B	10	8	10
+ve	28	26	16
-ve	NZ	NZ	NZ

NZ- No Zone

CB -A - *C. bicolor*(Aqueous); CB -M - *C. bicolor*(Methanol); CB -C - *C. bicolor*(Chloroform); CB -E - *C. bicolor*(Ethanol); CB -B - *C. bicolor*(Butanol); + ve - Positive control; - ve -Negative control



Aspergillus niger ***Aspergillus flavus***

Candida albicans

Figure 8. Antifungal activity of *C. bicolor* extracts

4. Conclusion

Using UV-VIS and FTIR techniques, this work has provided preliminary information for determining the chemical composition of *Caladium bicolor*. *Caladium bicolor* extracts contain DPPH and hydroxyl radical inhibitory properties. All of the extracts, with the exception of aqueous and ethanol, demonstrated antibacterial activity against all of the bacteria tested. All of the fungal infections studied demonstrated that methanol, ethanol, and butanol had antifungal action. *Caladium bicolor* plants contain bioactive chemicals, indicating that it has been used by humans. It also applies to the development of new pharmaceuticals based on the isolation of specific molecules. *Caladium bicolor* is thought to contain a variety of bioactive chemicals. As a result, it is recommended as a phytopharmaceutical plant. To fully comprehend its bioactivity, toxicological profile, and effects on the environment and agricultural products, more research is nonetheless required.

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