

## **EVALUATION OF BIOACTIVE COMPOUNDS, ANTIOXIDANT POTENTIAL, AND ANTI-CANDIDAL ACTIVITY OF TURMERIC (*Curcuma longa* L.) RHIZOME EXTRACT**

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### **ABSTRACT**

This study evaluated the phytochemical composition, antioxidant potential, and antifungal activity of the ethanolic extract of *Curcuma longa* (turmeric) rhizomes. The dried rhizomes were obtained from Warri, Delta State, Nigeria, authenticated and extracted using 70% ethanol by maceration. The phytochemical screening revealed the presence of some secondary metabolites, including alkaloids (high), flavonoids, phenols, and terpenoids (moderate), as well as low levels of glycosides, saponins, and tannins. The antioxidant activity was determined by the DPPH radical scavenging assay, and the results showed that the extract exhibited a dose-dependent increase in inhibition, ranging from 39.2% at 62.5 mg/mL to 69.9% at 1000 mg/mL, comparable to the standard antioxidant (71.87%). The antifungal activity was assessed using the poisoned food technique while the Minimum inhibitory concentration was determined using the agar well diffusion method. The antifungal assay demonstrated that *C. longa* extract inhibited the growth of *Candida albicans* in a concentration-dependent manner, with inhibition zones ranging from 7.25 mm at 5 mg/mL to 16.10 mm at 30 mg/mL. In comparison, fluconazole (5 mg/mL) produced a larger zone of inhibition (22.18 mm). The Minimum Inhibitory Concentration (MIC) of the extract was 6.25 mg/mL, showing remarkable antifungal potential. These findings substantiate the ethnomedicinal use of turmeric and highlight its potential as a natural source of bioactive compounds for the development of novel antioxidant and antifungal agents. This study recommends further research to isolate and characterise the specific active constituents responsible for these effects.

**Keywords:** Antioxidant, *Candida albicans*, Turmeric, Bioactive compounds

### **Introduction**

The rising threat of multidrug-resistant pathogens is reducing the clinical efficacy of many orthodox antibiotics (Uddin *et al.*,

2021). This increasing failure of chemotherapeutics and the widespread issue of antimicrobial resistance have strengthened the quest for novel antimicrobial agents, leading to the screening of numerous

medicinal plants for their potential antimicrobial activity (Abass *et al.*, 2022). The use of plant extracts in disease treatment has consequently gained significant scientific The same here. Medicinal plants contain a diverse array of compounds that may serve as natural antibacterial and antifungal agents for treating common infections (Mbah-Omeje, 2019).

Fungal infections have been a major health concern, especially in tropical regions. Their spread is intensified by factors such as poor environmental hygiene, inadequate sanitation, and unhealthy lifestyles. Although, synthetic antibiotics and preservatives have been used to combat these infections, their efficacy is often compromised over time as pathogens develop resistance (Hartiwi *et al.*, 2019; Cruz *et al.*, 2024). This development of microbial resistance has spurred research into discovering new antibiotics, either through laboratory synthesis or extraction from natural compounds, with plants being a major source (Saleem *et al.*, 2010).

African plants are rich in a wide variety of secondary metabolites, including tannins, terpenoids, alkaloids, and flavonoids, which have demonstrated antimicrobial properties *in vitro*. The application of plant extracts and their identified phytochemicals in therapeutic treatments is therefore of great significance. In recent years, numerous studies across different countries have focused on proving the efficacy of turmeric for this purpose (Arutselvi *et al.*, 2012).

Turmeric (*Curcuma longa* Linn.) is a monocotyledonous plant that belongs to the

Zingiberaceae family (Jilani *et al.*, 2012). This plant is valued for its underground rhizomes, which contain a yellow phenolic pigment called curcumin (Moulick *et al.*, 2025). This compound serves as a natural colouring agent in food, cosmetics, and dyes, and is also an active ingredient in various medicines (Amadi *et al.*, 2015). In Nigeria, turmeric is primarily cultivated in small-scale home gardens (Amadi *et al.*, 2013).

In Africa turmeric has been used to treat a range of ailments, including gastrointestinal and liver disorders, kidney inflammation, gallstones, hemorrhoids, rheumatism, high cholesterol, and menstrual issues, and to stimulate appetite and breast milk production (Obeta *et al.*, 2023). Furthermore, the rhizome is widely used as a culinary spice. Turmeric is a natural remedy with a broad spectrum of biological activities, including anti-inflammatory, antioxidant, anticarcinogenic, antimutagenic, anticoagulant, antidiabetic, antibacterial, and antifungal properties (Amadi *et al.*, 2015; Tian *et al.*, 2025). Its primary active components are a group of polyphenols known as curcuminoids, which possess strong antioxidant functions. Curcumin (diferuloylmethane), the main bioactive constituent, has been extensively studied for its wide range of biological actions, making it a candidate for various therapeutic applications (Moulick *et al.*, 2025). Another significant compound, aromatic-turmerone, has recently shown promise in the regeneration of neural tissues (Mbah-Omeje, 2019). The aim of this study is to investigate the phytochemical composition, antioxidant properties, and antifungal activity of the ethanolic extract of *Curcuma longa* rhizome.

## Materials and Methods

### Plant Material Collection and Authentication

Dried rhizomes of *Curcuma longa* were obtained from Warri, Delta State, Nigeria and were authenticated in the Herbarium Unit, Department of Botany, Delta State university, Abraka. The voucher number of the plant sample is DELSUH-131.

### Sample Preparation

The sample was prepared using the methods of Harborne *et al.* (1998) with slight modifications. The rhizomes were sun-dried for fourteen days to reduce moisture content. Subsequently, they were pulverized into a fine powder using a sterilized electric blender. The resulting powder was sieved through a 2.0mm mesh and stored in an

$$\text{Percentage yield (\%)} = \frac{\text{Weight of Dry Extract}}{\text{Weight of Dry Sample}} \times 100$$

The concentrated extract was stored in a refrigerator at 4°C for subsequent analysis.

### Phytochemical Screening

The Qualitative phytochemical analysis of the ethanolic extract was conducted using standard procedures by Sharififar *et al.* (2013) to identify the presence of major bioactive compounds, as outlined below:

#### Determination of Alkaloids

0.5 g of the extract was stirred with 5 ml of 1% aqueous HCl on a steam bath and filtered. The filtrate was treated with a few drops of

airtight, sterile container until required for extraction.

#### Extract Preparation

The extraction was performed using the maceration method. 100g of the dried *Curcuma longa* powder was soaked in 400 ml of 70% ethanol in a sterile conical flask. The flask was sealed with cotton wool, wrapped in aluminum foil to prevent light degradation, and shaken vigorously. The mixture was then left to stand for 24 hours in a shaking water bath maintained at 40°C. The resulting mixture was filtered, and the filtrate was concentrated using a rotary evaporator attached to a vacuum pump (Pandey and Tripathi, 2014; Sulaymanov *et al.*, 2024). The percentage yield of the crude extract was calculated using the formula:

$$\text{Percentage yield (\%)} = \frac{\text{Weight of Dry Extract}}{\text{Weight of Dry Sample}} \times 100$$

Dragendorff's reagent. Turbidity or precipitation indicated a positive result (Sharififar *et al.*, 2013).

#### Determination of Saponin Content

0.5 g of the extract was dissolved in 5 ml of distilled water and shaken vigorously. The formation of a persistent froth upon warming, which formed an emulsion with olive oil, was taken as a positive test (Sharififar *et al.*, 2013).

#### Determination of Tannin Content

0.5 g of the extract was boiled in 10 ml of water and filtered. A few drops of 0.1% ferric chloride were added to the filtrate. A brownish-green or blue-black colouration confirmed the presence of tannins (Sharififar *et al.*, 2013).

#### **Determination of Anthraquinones**

0.5 g of the extract was boiled with 10 ml of sulphuric acid and filtered. The filtrate was shaken with 5 ml of chloroform, and the chloroform layer was separated. The addition of 1 ml of 10% ammonia solution resulting in a color change indicated the presence of anthraquinones (Sharififar *et al.*, 2013).

#### **Determination of Flavonoid Content**

Five (5) ml of a dilute aqueous filtrate of the extract was treated with 1 ml of concentrated sulphuric acid. The appearance of a yellow coloration that disappeared on standing was considered a positive test (Sharififar *et al.*, 2013).

#### **Determination of Cardiac Glycosides (Keller-Killiani Test)**

0.5 g of the extract was diluted with 5 ml of water. Then, 2 ml of glacial acetic acid containing one drop of ferric chloride solution was added. This was underlaid with

1 ml of concentrated sulphuric acid. A brown ring at the interface indicated the presence of deoxysugar characteristic of cardenolides (Sharififar *et al.*, 2013).

#### **Determination of Phenolic Contents**

Total phenolic content was determined using the Tannic Acid Equivalent (TAE) assay, which measures the relative astringency of the extract (Sharififar *et al.*, 2013).

#### **Antioxidant Activity (DPPH Radical Scavenging Assay)**

The free radical scavenging activity of the extract was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method (Qader *et al.*, 2011) with slight modifications. A 0.0024% (w/v) methanolic solution of DPPH was prepared. One milliliter (1 ml) of the extract at varying concentrations (1000, 250, 125, and 62.5 mg/ml) was mixed with 3 ml of the DPPH solution. The mixture was shaken, and then incubated in the dark at room temperature for 30 minutes, and the absorbance was measured at 515 nm using a spectrophotometer. A blank control containing only DPPH and methanol was also measured. All tests were performed in triplicate (Qader *et al.*, 2011). The percentage of DPPH radical scavenging activity was calculated as follows:

$$\% \text{ DPPH Scavenging} = \frac{A_0 - A_1}{A_0} \times 100$$

Where  $A_0$  is the absorbance of the control and  $A_1$  is the absorbance of the sample. A standard curve was prepared using Trolox.

#### **Antifungal Activity**

#### **Microorganism and Inoculum Preparation**

A clinical isolate of *Candida albicans* was obtained from Maiestas Biomed Laboratory,

Abraka, Delta State. The culture was maintained on Potato Dextrose Agar (PDA) slants at 4°C. Before testing, the organism was sub-cultured in nutrient broth and incubated for 24 hours to achieve active growth (Liu *et al.*, 2002).

### **Antifungal Susceptibility Testing (Poisoned Food Technique)**

The antifungal activity was assessed using the poisoned food technique (Aondo, 2018). Two milliliters (2 mL) of the plant extract were dispensed into sterile Petri dishes,

followed by the addition of 15-20 mL of molten PDA (Aondo, 2018). The mixture was gently swirled to ensure even dispersion and then allowed to solidify. A 4 mm mycelial disc from a five-day-old culture of *C. albicans* was inoculated at the centre of the plate. Two replicates were maintained for each treatment. Control plates contained PDA without extract, and a positive control plate contained fluconazole (5 mg/ml). All plates were incubated at 27-30°C for 5-7 days in a completely randomized design. The percentage inhibition of fungal growth was calculated as:

$$\% \text{ Growth Inhibition} = \frac{L_1 - L_2}{L_1} \times 100$$

Where  $L_1$  is the radial growth in the control and  $L_2$  is the radial growth in the treatment.

### **Determination of Minimum Inhibitory Concentration (MIC)**

The Minimum Inhibitory Concentration (MIC) was determined by the agar well diffusion method (Obaji *et al.*, 2020). The extract was subjected to a two-fold serial dilution to obtain concentrations of 12.5, 6.25, 3.125, and 1.5625 mg/mL. A standardized inoculum (0.1 ml of 0.5 McFarland standard) of *C. albicans* was spread onto PDA plates. Wells (6 mm in diameter) were bored in the agar using a sterile cork borer and filled with 0.1 ml of each extract concentration. The plates were incubated at 27°C for 24 hours, and the zones of inhibition were measured. The MIC was defined as the lowest concentration that

completely inhibited the visible growth of the test organism (Obaji *et al.*, 2020).

### **Statistical analysis**

The results obtained from the study were analysed using SPSS version 31 and Graph Pad Prism 9. The results were presented as Mean±Standard deviation, with statistical significance set at  $p<0.05$ .

### **Results**

#### **Phytochemical screening of ethanolic extract of *Curcuma longa* rhizomes**

The results of the phytochemical screening (Table 3.1) show that the ethanolic extract of the rhizomes of *C. longa* contains many active phytochemicals. The extract has a high amount of alkaloids, moderate amounts of flavonoids, phenols, and terpenoids, as well

as a low amount of glycosides, saponins and tannins.

**Table 3.1:** Phytochemical screening of ethanolic extract of *Curcuma longa* rhizomes

Phytochemicals	Inferences
Alkaloids	+++
Glycosides	+
Flavonoids	+++
Phenols	++
Saponins	+
Tannins	+
Terpenoids	++

Keys: + = low, ++ moderate, +++ = High

**Antioxidant activity of the rhizomes of *Curcuma longa* (Turmeric)**

The ethanolic extract of *Curcuma longa* rhizomes shows a significant and dose-dependent antioxidant activity. As the concentration of the turmeric extract increases from 62.5 mg/mL to 1000 mg/mL,

the percentage of inhibition also increases steadily, from 39.2% to 69.9%. The activity of the turmeric extract is comparable to the standard antioxidant (Vitamin C) used in the test, particularly at the mid-range concentration of 62.5 mg/ml, where their inhibition percentages are similar.

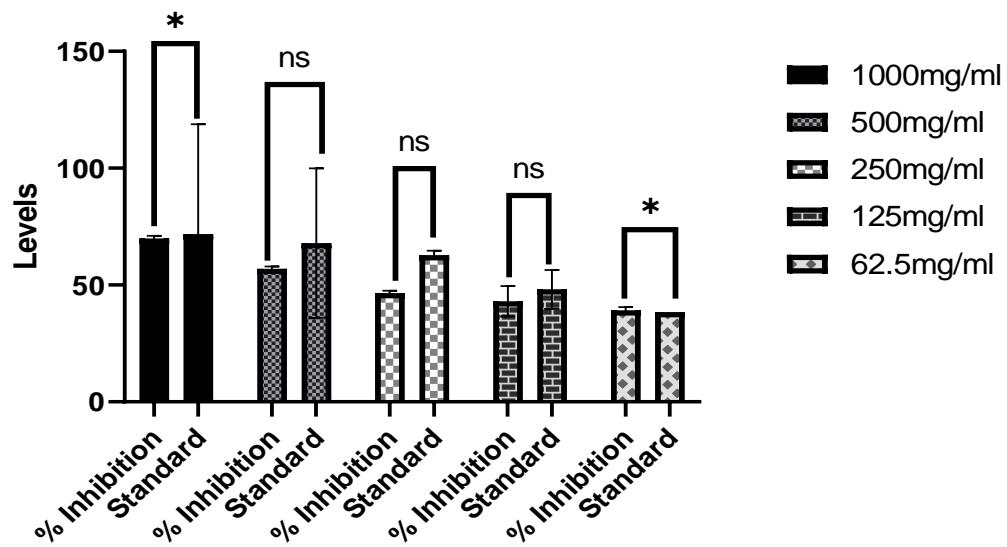
**Tables 2.** Antioxidant activity of *Curcuma longa* rhizomes

<i>Curcuma longa</i>	Concentration (mg/ml)	% Inhibition	Control (Vitamin C)%
	1000	69.90 ± 1.20	71.87 ± 47.00
	500	56.90 ± 1.05	67.96 ± 32.00
	250	46.50 ± 1.00	62.82 ± 1.81
	125	43.10 ± 6.50	48.16 ± 8.30
	62.5	39.20 ± 1.30	38.49 ± 2.60

\*Values are presented as Mean ± Standard deviation (n=5)

Figure 1 shows that there was no significant difference in mean inhibition between most concentration pairs (500, 250 and 125mg/mL) of the extract. However, a

significant difference ( $p = 0.02$ ) was observed between the highest concentration (1000 mg/mL) and the lowest concentration (62.5 mg/mL).



**Figure 1:** Antioxidant activity of the rhizome of *C. longa* (Tumeric). Bars represent Mean± Standard deviation (n=5), and the means were separated using Turkey's post Hoc test. Bars with (\*) mean there is a significant difference, and bars with (ns) mean no significant difference.

### Antifungal activity of the crude extract of the rhizomes of *Curcuma longa* (Turmeric)

The crude extract of *Curcuma longa* rhizomes exhibits a dose-dependent antifungal activity against *Candida albicans*. The zone of inhibition increases with the concentration of the extract, from 7.25 mm at

5 mg/mL to 16.10 mm at 30 mg/mL, indicating that a higher dose produces a stronger antifungal effect. While the turmeric extract is effective, its inhibition zones at all tested concentrations are smaller than the 22.18 mm zone produced by the standard antifungal drug Fluconazole at a much lower concentration of 5 mg/ml.

**Table 3.3:** Antifungal activity of the crude extract of *Curcuma longa* rhizomes

Isolate	Zone of Inhibition in mm at different concentration (mg/ml)					
	30mg/ml	20mg/ml	10mg/ml	5mg/ml	Fluconazole 5mg/ml	
<i>Candida albicans</i>	16.10 ± 0.18	14.00 ± 0.25	10.08 ± 0.18	07.25 ± 0.18	22.18 ± 0.18	

\*Values are presented as Mean ± Standard deviation (n=5)

### **Minimum inhibitory concentration of *Curcuma longa* rhizome extract against *Candida albicans***

The results in Table 4 definitively show that the Minimum Inhibitory Concentration (MIC) of the *Curcuma longa* rhizome extract against *Candida albicans* is 6.25 mg/ml. This value means that 6.25 mg/ml is the lowest

concentration of the turmeric extract required to completely prevent the visible growth of the fungus, as indicated by the absence of growth (-) at this and all higher concentrations. The presence of fungal growth (+) at the next lower concentrations of 3.125 mg/ml and 1.56 mg/ml confirms that concentrations below the MIC are ineffective.

**Table 3.4:** Minimum inhibitory concentration of the ethanolic extract of *Curcuma longa* rhizomes

Test organisms	25	12.5	6.25	3.125	1.56	MIC (mg/ml)
<i>Candida albicans</i>	-	-	-	+	+	6.25

#### **Interpretation**

+ = Growth

- = No growth

#### **Discussion**

Medicinal plants have served as a cornerstone of healthcare since ancient times and continue to be a vital source of chemotherapeutic agents in both developing and developed nations (Mohmoh *et al.*, 2015). *Curcuma longa* (turmeric), widely consumed as a food spice, is also prominent in traditional medicine due to its extensive medicinal properties, which include documented bacteriostatic and bactericidal effects (Mbah-Omeje *et al.*, 2019).

The antioxidant activity of *C. longa* rhizomes shows a dose-dependent increase in percentage inhibition. At the highest concentration (1000 mg/mL), *C. longa* exhibited a strong inhibition of  $69.9 \pm 1.20\%$ , which is comparable to the control (71.87

$\pm 4.7\%$ ). This similarity in activity at higher concentrations suggests that the extract possesses potent radical-scavenging abilities, which is likely due to its rich content of phenolic compounds, flavonoids, and curcuminoids, particularly curcumin, demethoxycurcumin, and bisdemethoxycurcumin, which are known for their redox properties (Prasad *et al.*, 2014). At 62.5 mg/mL, the inhibition reduced to  $39.2 \pm 1.30\%$ , showing that the activity is concentration-dependent and requires higher doses to match the effectiveness of the standard antioxidant (Vitamin C).

Previous studies had indicated similar findings in the antioxidant activity of *C. longa*. For example, Singh *et al.* (2010) observed that methanolic extracts of *Curcuma longa* showed over 70% DPPH

radical scavenging activity at 1000 µg/mL and this aligns closely with the present data. Similarly, Ravindran *et al.* (2016) reported that the extract showed high antioxidant potential due to their capacity to donate hydrogen atoms or electrons to free radicals. Gandhi *et al.* (2018) also found that ethanolic extracts of *C. longa* exhibited DPPH inhibition values ranging from 35% to 68% as concentration increased, again confirming the dose-dependent trend. The comparable performance of the rhizome extract with the standard antioxidant supports its potential application in phytotherapeutic formulations and functional foods (Ak and Gülcin, 2008; Prasad *et al.*, 2014).

The antifungal activity of the crude extract of *Curcuma longa* (Turmeric) rhizomes against *Candida albicans* showed a dose-dependent increase, as shown in Table 3.3. The inhibition zones increased gradually with extract concentration, from  $7.25 \pm 0.18$  mm at 5 mg/mL to  $16.10 \pm 0.18$  mm at 30 mg/mL. The positive control, fluconazole (5 mg/mL), showed the highest inhibition zone ( $22.18 \pm 0.18$  mm), indicating that while the *C. longa* extract possesses antifungal properties, its strength is comparatively lower than that of the standard drug. The progressive increase in inhibition zone with concentration suggests that the antifungal activity is likely linked to the concentration of bioactive constituents such as curcumin, demethoxycurcumin, and bisdemethoxycurcumin, which have been studied and known to disrupt fungal cell membrane integrity and inhibit the biosynthesis of ergosterol (Murugesh *et al.*, 2019).

The results obtained in this study are consistent with those from previous studies. Sharma *et al.* (2022) reported that essential oil extracted from *C. longa* leaves produced inhibition zones ranging from 11.5 to 13.0 mm against *C. albicans*, while fluconazole showed higher activity ( $>20$  mm). Correspondingly, Siddique *et al.* (2021) observed that *C. longa* extract exhibited antifungal effects that increased with concentration but remained consistently lower than the standard drug, fluconazole. These studies, together with the present findings, confirm that although *C. longa* has antifungal potential, it is less potent than standard azole-based antifungal agents. The slight variations in the inhibition zones among studies can be attributed to differences in solvent extraction methods, the concentration of active phytochemicals, and the strain of *C. albicans* that was used in testing.

The observed bioactivity can be attributed to the rich phytochemical profile of the extract. Qualitative analysis revealed the presence of secondary metabolites, including tannins, flavonoids, alkaloids, saponins, and phenolic compounds. This finding aligns with previous phytochemical studies on *Curcuma longa* (Oghenejobo *et al.*, 2017; Mbah-Omeje, 2019). The antifungal activity is likely mediated by these compounds, especially tannins and saponins, which are well-documented for their ability to disrupt microbial cell membranes and inhibit their growth.

## Conclusion

In conclusion, this study supports the traditional use of *Curcuma longa*. The findings confirm that the ethanolic extract of turmeric rhizomes is a rich source of bioactive phytochemicals, exhibiting potent, dose-dependent antifungal activity against *Candida albicans* and significant free radical scavenging ability. These results underscore the potential of *Curcuma longa* as a promising natural source for developing novel antifungal and antioxidant agents. Further research to isolate and identify the specific active compounds responsible for these effects is highly recommended.

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