

## Physicochemical analysis and anti-bacterial activity of rhizome of tumeric (*Curcuma longa* L.) vegetable plants

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### Abstract

*Curcuma longa* L (Turmeric) a rhizomatous perennial herb belonging to the ginger family Zingiberaceae, was studied to evaluate its physicochemical properties and antibacterial activity against gram-positive and gram-negative bacteria. Two solvent extractions (Aqueous and Ethanol) were prepared from the rhizomes obtained from Makurdi, Benue State, Nigeria which were dried and ground into powder of mesh size of 0.2 mm in diameter. The physicochemical analysis showed a pH of 6.95, Iodine Value of 51.39, Refractive Index of 1.44 and Specific Gravity of 1.45 which imply moderate level of unsaturated fatty acids content and high antimicrobial activity. Its antibacterial evaluation against *Staphylococcus aureus* American Type Culture Collection (ATCC) 25923 (gram +ve) and *Pseudomonas aeruginosa* ATCC 29953 (Gram -ve) were carried out with the two extracts and Chloramphenicol as the control using agar well diffusion method. The antibiotic showed significantly higher inhibitory effect on the bacterial isolates than the aqueous extract ( $P < 0.05$ ). It also showed higher effect than the ethanol extract on *S. aureus* ( $P < 0.05$ ) but statistically equal effect on *P. aeruginosa* ( $P > 0.05$ ). The ethanol extract showed higher activity against the test organisms than the aqueous extract at  $P < 0.05$ . The zones of inhibition for the aqueous extract ranged from 7.00 mm – 13.67 mm for *S. aureus* and 7.33 mm – 14.33 mm for *P. aeruginosa* and the ethanol extract, 13.33 mm – 19.00 mm and 15.00 mm – 13.00 mm for *S. aureus* and *P. aeruginosa* respectively. The Minimum Inhibitory Concentration was observed at 12.5 mg/mL in both cases. The antibacterial activity shown by *C. longa* may be due to the presence of phytochemicals such as Alkaloids, Steroids, Saponins, Flavonoids, Phenols and Tannins which were observed when the extract was analyzed phytochemically. *C. longa* showed potent activity against bacteria in this study and would make a more active and viable antibiotic.

**Keywords:** Chloramphenicol; Activity; Extraction; Aqueous; Ethanol; Rhizomatous perennial herb

### 1. Introduction

Turmeric is a rhizomatous perennial herb having primary and secondary rhizomes that can be present in different forms, from spherical to slightly conical, hemispherical, and cylindrical. The rhizomes contain a thin, slightly brown peel (peridermis layer) having an orange-yellow flesh [1]. It is used as an herbal medicine for rheumatoid arthritis, chronic anterior uveitis, conjunctivitis, skin cancer, small pox, chicken pox, wound healing, urinary tract infections, and liver ailments [2]. Turmeric has been reported to decrease blood lipid peroxides in humans [3], prevent ulcers [4] and protects the liver from chemical injury [5]. It is also used for digestive disorders; to reduce flatus, jaundice, menstrual difficulties, and colic; for abdominal pain and distension [6] and for dyspeptic conditions including loss of appetite, postprandial feelings of fullness, and liver and gallbladder complaints. It has anti-inflammatory, choleric,

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antimicrobial and carminative actions [7]. Turmeric also has been used as foodstuff and cosmetic amongst other uses and importance. It has found application as natural preservative in many foods such as canned beverages, baked products and biscuits, [8] and has been used in ice cream, yogurt, yellow cakes, orange juice, popcorn color, cereals, sauces, gelatins, etc. [9].

Natural plant products have been used throughout human history for various purposes. Having co-evolved with animal life, many of the plants from which these natural products are derived are billions of years old. Tens of thousands of these products are produced as secondary metabolites by higher plants including Tumeric as a natural defense mechanism against disease and infection. Many of these natural products have pharmacological or biological activity that can be exploited in pharmaceutical drug discovery and drug design. Medicines derived from plants have played a pivotal role in the health care of many cultures, both ancient and modern [10, 11, 12, 13, 14].

In Benue State, Nigeria, it is identified as Agbedan in Tiv and Mngboo in Etulo. The Hausas in Northern Nigeria call it Tari, the Yorubas in the West call it Ata Ile Pupa while the Ibos in the East call it Odo. In Nigeria, about 19 states are prominent in the cultivation of Turmeric (Olojede *et al.* [15] In: Ihenaecho *et al.* [16] and in view of the prevailing favourable soil and climatic conditions in Nigeria, the country can play a leading role in turmeric production [17]. This however, has not been achieved due to lack of awareness on the potentials and diversified opportunities associated with turmeric as well as the production techniques required which are poorly understood. Research into *Curcuma longa L* (Turmeric) is to come with pertinent data on the pharmaceutical activities, antimicrobial and therapeutic values so as to be used effectively in the treatment and cure of various ailments by using them against a wide range of diseases. This will create more awareness into medically active rhizomatous perennial herbs and will act as a data base in medicine. Therefore this study was conducted to assess the phytochemical and physicochemical properties of *Curcuma longa L* (Turmeric) as well as to determine its antimicrobial activity against certain organisms.

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## 2. Material and methods

### 2.1. Study Area

The study was carried out in Makurdi, capital of Benue State, Nigeria. Makurdi is located at the North Eastern part of Benue State, Nigeria and it lies on latitude 7° 30' N and Longitude 8° 35'E. It shares boundaries with Gwer-West and Guma Local Government Areas including Nassarawa State [18]. Makurdi lies in the tropical guinea savannah zone of central Nigeria, experiences a typical climate with two distinct seasons. The dry season lasts from late October to March and the rainy season which begins in April to October is the period of intensive agricultural activities by the inhabitants who are mostly the Tivs, Idomas, Igedes and the Jukuns. (Meteorological Department, Nigeria Air force Base Makurdi, Unpublished data). The rainy season which lasts for seven months, has a mean annual rainfall ranging from 1,200-2000 mm [63]. High temperature values averaging 28 °C - 33°C are recorded in Makurdi throughout the year, most notable from March to April. Harmattan winds are accompanied with cooling effects mostly during the night of December and January [19].

#### 2.1.1. Sample collection

Samples of fresh turmeric rhizomes (*Curcuma longa L*) were randomly purchased on 5<sup>th</sup> of July, 2020, from High Level Market, Makurdi, Benue State, Nigeria. Samples were stored in a black polyethene bags and transported to the Department of Chemistry, Benue State University, Makurdi for analysis. Samples were authenticated by Wayas a botanist in the Department of biological sciences, Benue state University, Makurdi.

#### 2.1.2. Preparation of plant extract

The turmeric rhizomes were carefully peeled with a knife, rinsed with potable water, sliced and dried in the oven at 50 °C for 24 hours. The dried samples were milled using a Blender into powder and allowed to pass through a sieve with a nominal mesh size of 0.2 mm in diameter. Two extracts (aqueous and ethanol extracts) were prepared. Twenty grams (20.0 g) of the powdered tumeric was dissolved in 100 mL sterile distilled water and allowed to soak for 24 hours; after which the mixture was filtered through whatman's filter paper number 1 and kept for the analysis. In like manner, the dried powdered sample was dissolved in ethanol overnight, filtered and kept for further analysis.

## 2.2. Material and Chemical reagents

Mortar and Pestle, Conical flask, Beaker, Measuring cylinders, Soxhlet apparatus, Water bath, Boiling chips, Filter papers, Test tanks, Petri dishes, Auto clave, Capillary tube, Inoculation wire loop, Funnels, Syringes, Culture plates, Oven, Refrigerator, Condenser. Chemical reagent used includes Fehling solutions (A and B), Methanol, n-Hexane, Distilled

water, Ferric chloride 3.5% (3.5 mL of FeCl<sub>3</sub> in 96.5 mL of solvents), Dilute tetraoxosulphate (IV) acid (H<sub>2</sub>SO<sub>4</sub>), Sodium hydroxide (NaOH), Zinc chips, Meyer's reagent, Dragendoff's reagent, Ammonia, Chloroform (CHCl<sub>3</sub>), Nutrient agar, Ethyl acetate, Acetone, Aqueous hydrochloric acid (1.0% HCl), Ethanol, Benzene, n-hexane and methanol

## 2.3. Methods

### 2.3.1. Hexane extraction

Exactly 30.0 g of the powdered sample was weighed and poured into a white handkerchief and sealed properly. 300.0 mL of n hexane was measured and carefully poured into the soxhlet extractor connected to the round bottom flask. Boiling chips were placed in the flask to prevent superheating. The soxhlet extractor fitted unto the round bottom flask was mounted on a heating mantle supported by a retort stand. The set up was heated for 6 hours to ensure complete extraction using distilled column. The crude extract in the round bottom flask was heated in a water bath to concentrate the solvent. The crude extract was then transferred into an already weighed plastic container and allowed to evaporate to dryness. The concentrated extract was then kept for further analysis.

### 2.3.2. Methanol extraction

About 30.0 g of the powdered sample weighed and transferred into a white handkerchief and sealed properly. 300.0 mL of methanol was measured and used for extraction. The procedure was the same as the one in the extraction with n-hexane.

### 2.3.3. Determination of Physicochemical Properties

Analyses of specific gravity, refractive index, iodine value, pH and Colour were carried out using the methods of AOAC [20].

### 2.3.4. Determination of specific gravity

A clean 50.0 mL specific gravity bottle was weighted as (W<sub>0</sub>). The bottle was then filled to the brim with water and a stopper inserted until extra water spilled out. The water on the stopper and bottle was carefully wiped off and reweighed as (W<sub>1</sub>). Same process was repeated, but using tumeric extract sample instead of water and weighed again as (W<sub>2</sub>). The specific gravity of the extract was then calculated using the following formula by AOAC, [20].

$$\text{Specific gravity of test sample} = \frac{W_2 - W_0}{W_1 - W_0} \dots \dots \dots (1)$$

Where W<sub>0</sub> = Weight of empty specific gravity bottle

W<sub>1</sub> = Weight of water + specific gravity bottle

W<sub>2</sub> = Weight of test sample + specific gravity bottle.

### 2.3.5. Iodine value determination

0.1 g of the extract was weighed into a conical flask. 5.0 mL of carbon tetrachloride and 10.0 mL of the Wij's solution were added to the flask and the solution was kept in dark for 30 min at room temperature. 7.5 mL of 10 per cent potassium iodide solution with 50.0 mL of distilled water was added to the flask. The resulting solution was titrated against 0.1 M sodium thiosulphate (Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>), using starch as indicator till the end point where the blue black coloration became colorless. A blank titration was carried out at the same time starting with 5.0 mL carbon tetrachloride. Iodine value was then calculated by the following formula by AOAC, [20].

$$\text{Iodine number} = \frac{(B - S) \times N \times 12.69}{\text{Weight of the sample}} \dots \dots \dots (2)$$

Where B = 0.1 N sodium thiosulfate required (mL) by blank

S = 0.1 N sodium thiosulfate required (mL) by sample

N = Normality of sodium thiosulfate solution.

### 2.3.6. Refractive index

The refractive index of the extract was determined using a refractometer. The refractometer was connected to a thermostatically controlled water bath and the temperature maintained at 40 °C. The sample of the extract was spotted onto the slide of the refractometer and viewed by rotating the knobs while the refractive index was recorded.

## 2.4. Phytochemical Analysis

The turmeric extract was screened for the detection of phyto-constituents such as alkaloids, steroids, saponins, hydrogen cyanide, flavonoids, phenols, tannins, carbohydrate. Starch and protein using the procedures by Pearson [21], Harborne [22], AOAC [20], Onwuka [23], Udeozo *et al.* [24] and Srivastav *et al.* [25] as described below.

### 2.4.1. Test for alkaloid (Picric Acid Test)

1.0 mL of Picric Acid was added to about 2.0 mL of the extract. A yellow precipitate was observed which indicated the presence of alkaloid.

### 2.4.2. Test for steroids (Salkowski Test)

The extract (1.0 mL) was dissolved in 2.0 mL of chloroform in a test tube, and then 1.0 mL of concentrated sulfuric acid was also added. The formation of reddish brown colour at the inter-phase indicated the presence of steroids.

### 2.4.3. Test for saponins (Froth Test)

1.0 mL of the filtrate was diluted in 1.0 mL of water and shaken vigorously. Persistence foam indicated the presence of saponins.

### 2.4.4. Test for hydrogen cyanide (Spot Paper Test)

2 - 3 drops of toluene solution was added to 1.0 mL of the extract. A change from the yellow colour of the paper to brick red colour indicated a positive result for hydrogen cyanide.

### 2.4.5. Test for flavonoid sample (Sodium Hydroxide Test)

The extract (1.0 mL) was diluted in 2.0 mL of 10% NaOH. A yellow precipitate was formed and following the addition of dilute HCl, the yellow colour turned colourless. This indicated the presence of flavonoids.

### 2.4.6. Test for phenols (Ferric Chloride Test)

1.0 mL of 10% Ferric chloride was added to the extract (1.0 mL). The formation of a greenish brown precipitate indicated the presence of phenols.

### 2.4.7. Test for tannins (Ferric Chloride Test)

5.0 mL of the extract was added to 2.0 mL of 1.0% HCl. Deposition of a red precipitate showed the presence of tannins.

### 2.4.8. Test for carbohydrate (Fehling's Test)

2.0 mL extract was taken in a test tube and equal amount of Fehling solution A and B added and then boiled for sometimes. A Red brick precipitate indicated the presence of Carbohydrate.

### 2.4.9. Test for starch

Few drops of iodine solution were added to the sample extract taken in a test tube. A blue black color indicated the presence of starch.

### 2.4.10. Test for protein

1.0 mL of distilled water was added to sample extract taken in test tube, after which 5.0–6.0 mL Millon's reagent was added. White precipitates which turned red after boiling confirmed Protein.

The above procedures were also used by Srivastav *et al.* [25] and Hosea *et al.* [26] in their respective studies.

## 2.5. Microbial Analysis

### 2.5.1. Bacteria strains

The bacteria strains used for this study were of the gram-positive and gram-negative origins collected in the American Type Culture Collection (ATCC). These include the strains of *Staphylococcus aureus* ATCC 25923 (gram-positive) and *Pseudomonas aeruginosa* ATCC 29953 (gram-negative). They were obtained from the Bacteria section, Department of

Microbiology and Biotechnology (MB and BT), National Institute for Pharmaceutical Research and Development (NIPRD), Idu Layout, Abuja. They were stored in a refrigerator at 40°C after they were obtained.

### 2.5.2. Preparation of media

Nutrient Agar (NA) powder was collected and prepared according to the manufacturer's instruction in Microbiology Laboratory, Department of Biological Sciences, Benue State University Makurdi. 11.2 g NA was weighed on analytical weighing balance and dissolved in 400 mL of distilled water, shook and heated for 10 minutes. 20 mL was dispensed into sterilization bottles (McCartney bottle) using a sterile syringe and autoclaved at 121°C for 15 mins (15 PSI). After sterilization each bottle containing NA was dispensed onto sterile Petri dish in biosafety cabinet (BSC) and allowed to set.

### 2.5.3. Organism preparation

0.1 mL overnight culture of each test organism (*Pseudomonas aeruginosa* ATCC 29953 and *Staphylococcus aureus* ATCC 25923) were transferred onto 5.0 mL of nutrient broth and incubated for 3hrs at 37 °C and then compared with 0.5 McFarland Standard. The 0.5 McFarland Standard was prepared by dissolving 1.0 g of Barium Chloride in 99.0 mL of distilled water to give 1.0% of BaCl<sub>2</sub> solution. 1.0 mL of concentrated sulfuric acid was diluted in 99.0 mL of distilled water to give 1.0% of H<sub>2</sub>SO<sub>4</sub>. 9.95 mL of Barium Chloride was then diluted in 0.05 mL of the sulfuric acid to form precipitate. The isolates of the test organisms were then dissolved in 10 mL of normal saline solution to give McFarland standard. This helped to give the population of test organisms as 10<sup>6</sup> colony forming unit (cfu/mL).

## 2.6. Determination of antimicrobial activity using agar well diffusion method

0.1mL of each of the 3 hours culture of the test organisms was aseptically transferred onto the Petri dishes containing 20 mL of the molten NA (maintained at 45 °C - 55 °C). The inoculums were spread around the plate using a sterile cotton swab to give uniform distribution of the test organisms in the agar and allowed to set. Wells were aseptically bored into the plates containing each of the organisms using a cork borer of 5 mm in an inoculating chamber. The bottom of the Wells was sealed with 100.0 mg, 50.0 mg, 25.0 mg and 12.5 mg of different concentrations of the extracts and the plates were incubated at 37 °C for 18-24 hours. The diameter of the zones of bacteria inhibitions around each Well was measured using a meter rule. The experiment was performed in three replicates.

### 2.6.1. Determination of minimum inhibitory concentration (MIC)

Minimum inhibitory concentrations (MIC) were determined by agar dilution assay using Nutrient Agar respectively. The concentration of the extract test ranged from 100.0 mg/mL, 50.0 mg/mL, 25.0 mg/mL and 12.5.0 mg/mL. In this case, agar diffusion techniques were performed in agar plate containing various concentrations of the extracts. The plate were inoculated with 100 µL of the test organisms containing 10<sup>5</sup> cfu/mL Log phase bacteria and incubated at 37 °C for 18-24 hrs, the lowest concentration of the extract that inhibited the growth of the bacteria was determined as the MIC.

### 2.6.2. Determination of minimum bactericidal concentration (MBC)

The preparation was made by sub culturing 100 µL of Nutrient broth from each well on MHA Plate after 24 hrs of initial incubation. Nutrient Agar plates were incubated for 18-24 hrs at 37 °C. The lowest concentration of extracts that resulted in no bacteria growth was considered as MBC, the experiment was performed in four replicate.

### 2.6.3. Positive control

250.0 mg of Chloramphenicol was used as positive control. Serial dilution was made to give concentrations of 25.0 mg/mL, 12.5 mg/mL, 6.25 mg/mL and 3.125 mg/mL. Different concentrations of the control were appropriately dispensed in the well of Nutrient agar containing the tested organisms. The plates were also incubated at 37 °C for 24 hrs and the diameter of the zones of bacteria inhibition around the wells of each concentration was measured.

## 2.7. Statistical analysis

Analysis of Variance of antibacterial activities of Plant extracts of *C. longa* was performed using the Turkey HSD method at p<0.05.

### 3. Results

The analysis of the phytochemical composition of the aqueous and ethanol extracts of tumeric (*Curcuma longa L*) obtained from High Level market Makurdi, Benue State, Nigeria showed the presence of Alkaloids, Steroids, Saponins, Flavonoids, Phenols, Tannins, Carbohydrate and Protein, Table 1.

**Table 1** Phytochemical composition of aqueous and ethanol extracts of turmeric (*Curcuma longa L*)

S/No	Phytoconstituent	Reagent used	Inference	
			Aqueous Extract	Ethanol Extract
1.	Alkaloids	Picric Acid	+	+
2.	Steroids	Chloroform	+	+
3.	Saponins	Distilled water	+	+
4.	Hydrogen cyanide	Toluene	-	-
5.	Flavonoids	10% NaOH	+	+
6.	Phenols	10% Ferric chloride	+	+
7.	Tannins	1% HCl	+	+
8.	Carbohydrate	Fehling solns. A and B	+	+
9.	Starch	Iodine solution	-	-
10.	Protein	Millon's reagent	+	+

+ = phytochemical is Present; - = phytochemical is absent

The physico-chemical analysis of the tumeric extract revealed pale brown and yellowish colours of the fresh and prepared samples, spicy bitter taste, an aromatic odour, insolubility in water but miscibility with chloroform. The pH of 6.95, specific gravity of 1.45, refractive index of 1.44 and iodine value of 51.39 were also observed, Table 2.

**Table 2** Physicochemical properties of extract of tumeric (*Curcuma longa L*) rhizome

S/No.	Characteristics	Value in sample of <i>C. longa L</i>
1.	Colour of Fresh Sample	Pale Brown
2.	Colour of Prepared Sample	Yellow
3.	Taste	Spicy Bitter
4.	Odour	Aromatic
5.	Miscibility and Solubility	Insoluble in water but miscible in Chloroform
6.	pH (at 29 °C)	6.95
7.	Specific Gravity	1.45
8.	Refractive Index (at 20 °C)	1.44
9.	Iodine Value	51.39

The evaluation of the Mean inhibitory effect of the aqueous extract of *C. longa L* showed a potent effect on all the test organisms, Table 3.

**Table 3** Mean Inhibitory Effect of Aqueous Extract of *C. longa* L on Test Organisms

S/No.	Concentration	Zones of Inhibition (mm)	
		<i>S. aureus</i>	<i>P. aeruginosa</i>
1.	100 mg/mL	13.67±1.2	14.33±1.5
2.	50 mg/mL	10.67±0.6	11.33 <sup>b</sup> ±0.6
3.	25 mg/mL	7.66 <sup>a</sup> ±0.6	9.33 <sup>bc</sup> ±0.6
4.	12.5 mg/mL	7.00 <sup>a</sup> ±1.0	7.33 <sup>c</sup> ±0.6
	HSD (P<0.05)	1.00	1.00

Means tagged with the same alphabets are not significant; Results are in Mean ± Standard deviation of triplicates

The result of the mean inhibitory effect of the ethanol extract of *C. longa* L showed active inhibitory activity against the test bacterial isolates as shown (Table 4).

**Table 4** Mean Inhibitory Effect of Ethanol Extract of *C. longa* L on Test Organisms

S/No.	Concentration	Zones of Inhibition (mm)	
		<i>S. aureus</i>	<i>P. aeruginosa</i>
1.	100 mg/mL	19.00 <sup>a</sup> ±1.0	23.00 <sup>c</sup> ±1.0
2.	50 mg/mL	18.00 <sup>a</sup> ±1.7	20.67 <sup>c</sup> ±0.6
3.	25 mg/mL	16.00 <sup>ab</sup> ±1.0	17.00 <sup>d</sup> ±1.0
4.	12.5 mg/mL	13.33 <sup>b</sup> ±2.3	15.00 <sup>d</sup> ±1.0
	HSD (P<0.05)	0.181	0.55

Means tagged with the same alphabets are not significant; Results are in Mean ± Standard deviation of triplicates

The standard antibiotic (Chloramphenicol) used in this study showed active inhibitory effect on the test organisms at all concentrations as shown (Table 5).

**Table 5** Mean Inhibitory Effect of Antibiotic (Chloramphenicol) on Test Organisms

S/No.	Concentration	Zones of Inhibition (mm)	
		<i>S. aureus</i>	<i>P. aeruginosa</i>
1.	100 mg/mL	24.00 <sup>a</sup> ±1.7	24.00 <sup>d</sup> ±1.0
2.	50 mg/mL	21.67 <sup>ab</sup> ±0.6	22.33 <sup>d</sup> ±0.6
3.	25 mg/mL	19.33 <sup>bc</sup> ±0.6	19.67 <sup>e</sup> ±0.6
4.	12.5 mg/mL	17.67 <sup>c</sup> ±0.6	18.33 <sup>e</sup> ±0.6
	HSD (P<0.05)	0.081	0.78

Means tagged with the same alphabets are not significant; Results are in Mean ± Standard deviation of triplicates

The comparative mean inhibitory effect of the aqueous and ethanol extracts of turmeric and the antibiotic (Chloramphenicol) used in this study as Control shows that the effect of the antibiotic on the bacterial isolates was significantly higher than the effect of the aqueous extract on both organisms (P<0.05). It was also significantly higher than the ethanol extract on *S. aureus* (P<0.05) while it was insignificant on *P. aeruginosa* (P>0.05), Table 6.

**Table 6** Comparative Mean Inhibitory Effect of Extracts of *C. longa L* (Aqueous and Ethanol) and the Standard Antibiotic (Chloramphenicol) on the Test Organisms

S/No.	Extract	Mean Inhibitory Effect		HSD (P<0.05)
		<i>S. aureus</i>	<i>P. aeruginosa</i>	
1.	Aqueous	13.67	14.33	NS
2.	Ethanol	19.00	23.00 <sup>b</sup>	1.00
3.	Chloramphenicol	24.00	24.00 <sup>b</sup>	NS
	HSD (P<0.05)	0.985	0.985	

Means tagged with the same alphabets are not significant; NS means Not Significant

#### 4. Discussion

In this study, the physicochemical and phytochemical analysis of the rhizome of turmeric (*Curcuma longa L*) and its antibacterial potential against the Gram-positive and Gram-negative bacteria were investigated.

The phytochemical investigations revealed the presence of Alkaloids, Steroids, Saponins, Flavonoids, Phenols, Tannins, Carbohydrate and Protein (Table 1). Previous phytochemical investigations by Kadem *et al.* [27], Gupta *et al.* [28], Ahamefula *et al.* [29], Srivastav *et al.* [25] and Hosea *et al.* [26] also reported the presence of Alkaloids, Steroids, Saponins, Flavonoids, Phenols, Tannins as well as Terpenes and Resins in tumeric. Carbohydrate and Protein were reported by Balalcrishnan, [30], Ifesan *et al.* [16], Ahamefula *et al.* [29] and Srivastav *et al.* [25] in tumeric in their separate studies. The presence of Carbohydrate and protein in turmeric may be an indication that, it is a good source of Carbohydrate and protein [16, 29]. The presence of phytochemicals particularly Alkaloids and Flavonoids [31]. Tannins and Saponins, account for the efficient antibacterial and medicinal activities of tumeric and other higher plants [27, 25, 26]. They serve as defense mechanisms against many microorganisms, insects and herbivores [32, 33].

The physicochemical analysis of the tumeric extract revealed pale brown and yellowish colors of the fresh and prepared samples, spicy bitter taste, an aromatic odour, insolubility in water but miscibility with chloroform. The pH of 6.95, specific gravity of 1.45, refractive index of 1.44 and iodine value of 51.39 were observed in this study (Table 2).

The yellow color of prepared tumeric extract is due to the presence of Curcumin – one of the bioactive substances found in its rhizomes [34].

The pH of turmeric in this study shows that it is weakly acidic (Table 2). Variable pH values have been reported for tumeric extracts and oil. Dash *et al.* [35] reported the pH range of 5.8 – 6.9 of the selected 10 germplasms they studied, Mane *et al.* [36] reported 5.7 (acidity level of 0.70%), Akram *et al.* [37] reported a range of 5.8 – 6.7 in the varieties of tumeric they studied, Oladimeji *et al.* [38] reported the range of 6.7 – 7.0 and Ifesan *et al.* [39] reported 7.3 in their study. Generally, the pH of turmeric sample depends on the maturity of the plant, soil type, harvesting conditions and freshness of the sample [40]. The refractive index (1.44) observed in this study was compatible with the one reported by Paul *et al.* [41] in turmeric oil (1.43 – 1.465) and that of Ifesan *et al.* [42] who reported 1.45, while the specific gravity was higher than the ones reported by Paul *et al.* [41] and Ifesan *et al.* [39]. A refractive index within the above range indicates that the oil contained certain amount of long chain unsaturated fatty acids [41]. The iodine value observed was slightly higher than that of Ifesan *et al.* [12] who reported 46.95 in Nigeria but lower than the one of Paul *et al.* [41] who reported a range of 75.53 – 90.47 in oil extracted from tumeric in Bangladesh (Table 2). Iodine value is the measure of the degree of unsaturation of fatty acids content of any fat or oil. The higher the iodine value, the higher the degree of unsaturation [43]. The result of this study implies a moderate level of unsaturation and reasonably good level of saturated fatty acids. As such, their use with other substances to prepare food ingredients is not out of place since they can keep foods stable against oxidation. It also accounts for their antimicrobial activity.

The antibacterial activity of the extracts (aqueous and ethanol) of *C. longa* on both Gram-positive and Gram-negative bacteria was also evaluated in this study using the Agar Well Diffusion Method. The result of the antibacterial activity of the aqueous extract of turmeric shows a potent effect on all the test organisms (Table 3). The highest zone of inhibition for *S. aureus* was observed at 100 mg/mL (13.67 mm) and the effect was significantly higher than all the other concentrations at P<0.05. The effect at 50 mg/mL was significantly higher than 25 mg/mL and 12.5 mg/mL (P<0.05) but insignificant when compared between 50 mg/mL and 12.5 mg/mL at P>0.05. The highest zone of inhibition for *P.*



*aeruginosa* was also observed at 100 mg/mL (14.00 mm). This had a significant effect when compared with the other concentrations at  $P < 0.05$ . The effect at 50 mg/mL was insignificant when compared with 25 mg/mL at  $P > 0.05$  while it was significant compared with 12.5 mg/mL at  $P < 0.05$ . The effect at 25 mg/mL and 12.5 mg/mL when compared was however insignificant ( $P > 0.05$ ).

The result of the mean inhibitory effect of the ethanol extract of *C. longa L* shows active inhibitory activity against the test bacterial isolates (Table 4). The highest zone of inhibition for *S. aureus* was (19.00 mm) at 100 mg/mL. Its effect at this concentration was however insignificant when compared with 50 mg/mL and 25 mg/mL at  $P > 0.05$ . The effect at 25 mg/mL and 12.5 mg/mL when compared was also insignificant ( $P > 0.05$ ). The highest inhibitory activity of the ethanol extract on *P. aeruginosa* was also observed at 100 mg/mL (23.00 mm). Its effect at this concentration was insignificant ( $P > 0.05$ ) when compared with its effect at 50 mg/mL but significant ( $P < 0.05$ ) when compared with the effect at 25 mg/mL and 12.5 mg/mL. It was also significant at 50 mg/mL when compared with 25 mg/mL and 12.5 mg/mL at  $P < 0.05$ .

Several antibacterial studies by Negi *et al.* [44], Unphaiboon *et al.* [45], Tajbaksh *et al.* [46], Niamsa and Sittiwet [47], Lawharinit *et al.* [48], Hosny *et al.* [49], Ahamefula *et al.* [29], Gul and Bakht [50], Gupta *et al.* [51] and Hosea *et al.* [26] also reported antibacterial activity of the different extracts of turmeric on *S. aureus* and a host of other bacteria such as: *Vibrio harveyi*, *V. alginolyticus*, *V. vulnificus*, *V. parahaemolyticus*, *V. cholerae*, *Bacillus subtilis*, *B. cereus*, *Aeromonas hydrophila*, *Streptococcus agalactiae*, *S. intermedius*, *S. epidermidis*, *Edwardsiella tarda*, *Klebsiella pneumoniae*, *Salmonella typhimurium*, *Salmonella typhi*, *Listeria monocytogenes* and *B. Coagulans*. While the potent effect of turmeric extracts on *P. aeruginosa* was reported by Negi *et al.* [52], Tajbaksh *et al.* [53], Hosny *et al.* [46] and Ahamefula *et al.* [24].

The result of the mean inhibitory effect of the Chloramphenicol the antibiotic used in this study shows that it exhibited very active effect on the test organisms at all concentrations (Table 5). The highest zone of inhibition for *S. aureus* (24.00 mm) was observed at the highest concentration of 25 mg/mL. It had significant effect at this concentration compared with the effect at 6.25 mg/mL and 3.125 mg/mL ( $P < 0.05$ ) but insignificant ( $P > 0.05$ ) compared with 25 mg/mL. The effect at 12.5 mg/mL and 6.25 mg/mL was insignificant when compared, likewise the effect at 6.25 mg/mL and 3.125 mg/mL when compared at  $P > 0.05$  respectively. The highest zone of inhibition (24.00 mm) was also observed for *P. aeruginosa* at 25 mg/mL. The effect was significantly higher than the effect at 6.25 mg/mL and 3.125 mg/mL ( $P < 0.05$ ) but statistically the same when compared with 12.5 mg/mL at  $P > 0.05$ . Its effect at 6.25 mg/mL and 3.125 mg/mL was also statistically the same ( $P > 0.05$ ). Hosea *et al.* [61] used tetracycline as a control in their study and also observed significant result at  $P < 0.05$  on all the test organisms. Ahamefula *et al.* [24] used Ampicillin and also reported a significant result on the test organisms.

The comparative Mean Inhibitory Effect of the aqueous and ethanol extracts of turmeric and the antibiotic (Chloramphenicol) used in this study as Control shows that the effect of the antibiotic on the bacterial isolates was significantly higher than the effect of the aqueous extract on both organisms ( $P < 0.05$ ) (Table 6). It was also significantly higher than the ethanol extract on *S. aureus* ( $P < 0.05$ ) but insignificant on *P. aeruginosa* ( $P > 0.05$ ). This is compatible with the findings of Ahamefula *et al.* [29] who also reported insignificant effect of the ethanol extract of turmeric on *P. aeruginosa* compared with the antibiotic even though my extract and theirs were prepared at different concentrations and different antibiotics were used.

Furthermore, the ethanol extract significantly showed higher antibacterial potency against the test organisms compared with the aqueous extract of Turmeric at  $P < 0.05$ . Li *et al.* [54], Tanvir *et al.* [55] and Hosea *et al.* [26] also reported that ethanol is better in the preparation of turmeric extract compared with water. Nishiyama *et al.* [56] and Lawharinit *et al.* [46] reported that ethanol is even better than hexane for turmeric extraction. This suggests that, ethanol is a better extraction solvent for turmeric compared with water and other solvents.

The effect of the antibiotic displayed statistically equal effect on both bacterial isolates at  $P > 0.05$  respectively. The aqueous extract also showed no significant effect when compared on both organisms ( $P > 0.05$ ) while the ethanol extract showed greater effect on *P. aeruginosa* than *S. aureus* at  $P < 0.05$ . Ahamefula *et al.* [29] also reported higher inhibitory effect on *P. aeruginosa* than *S. aureus*.

Generally, *P. aeruginosa* has been reported as a multidrug resistant pathogen and has displayed low susceptibility to many antibiotics [57]. One of the reasons for its high susceptibility to many drugs is the formation of biofilms which act as a diffusion barrier to the antibiotics [58]. Its high susceptibility to turmeric extracts in this study may be due to the presence of curcumin a bioactive substance in the rhizome of *C. longa L* which has antibacterial and antibiofilm activities. Curcumin was found to restore bacterial susceptibility to antibiotics by inhibiting its biofilm mode of growth and

rendering it sensitive to antibiotics in *in vitro* studies by Karaman *et al.* [59]. Kali *et al.* [60] also reported antibacterial synergy of curcumin with antibiotics against *P. aeruginosa* and other biofilm forming bacteria.

The presence of high percentage of Flavonoids in turmeric may also be responsible for the high susceptibility of *P.aeruginosa*. Even though the mechanism of action of turmeric extract against the studied organisms was not investigated, one of the mechanisms of action of flavonoids against bacteria is to also inhibit biofilm formation, synthesis of cell envelope and nucleic acid and also inhibit electron transport chain and Adenosine Triphosphate (ATP) synthesis in bacteria [61]. Other phytochemical components of turmeric also hold good potential against bacterial as I reported earlier.

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## 5. Conclusion

The physicochemical and antibacterial analysis of *C. longa L* revealed that *C. longa L* has promising antibacterial activity against both Gram-positive and Gram-negative bacteria. This antibacterial activity is as a result of the ability of turmeric to synthesize certain secondary metabolites of high medicinal values such as alkaloids, Steroids, saponins, flavonoids, phenols and tannins. These metabolites serve as defensive mechanisms against many microorganisms. As a result of this, turmeric when extracted and purified can serve as a very effective antibiotic agent especially against biofilm forming bacteria which constitute great medical concerns for their high antibiotic resistance potential. The physicochemical analysis also showed that the turmeric oil when extracted and purified would be good for human consumption as a result of its moderate unsaturated fatty acids content. Two methods of extraction (Aqueous and Ethanol) were used in this study. The evaluation of these respective methods showed that ethanol is a better extraction method compared to water for higher potency against bacteria because ethanol showed better capacity in conserving the stability of the chemical structure of the phytochemicals contained in the plant extract than water.

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## Compliance with ethical standards

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### Disclosure of conflict of interest

The authors declared that there are no conflicting interests.

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