*Available online at* www.derpharmachemica.com

ISSN 0975-413X
CODEN (USA): PCHHAX

**Der Pharma Chemica, 2017, 9(21):17-28**
*(http://www.derpharmachemica.com/archive.html)*

# Chemical Qualities of Dried Rhizomes of *Curcuma longa* Linn. and the Antimicrobial Activities of its Extracts on Microorganisms Associated in Skin Infections

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**ABSTRACT**

*Rhizomes of Curcuma longa (Turmeric) purchased from local farmers in Ado Ekiti, Nigeria, were analysed for their nutritional, phytochemical and antimicrobial properties as well as the bioactive constituents of its essential oil. The dried rhizomes were found to be rich in energy, protein, carbohydrate and fat, with high concentrations of $Ca^{2+}$, $Fe^{2+}$, $K^{-}$, $PO_4^{2-}$ and ascorbic acid but low levels of $Zn^{2+}$, thiamine, niacin and riboflavin. Quantitative phytochemical analyses revealed high concentrations of alkaloids, tannins and saponins, moderate level of oxalate with low level of phytates and no detectable cyanogenic glycosides. Qualitative phytochemical analyses of the extracts of C. longa indicated that alkaloids, tannins and phenols were present in the acetone, methanol and ethanol extracts and saponin only in the ethanolic extract; while none of the tested phytochemicals were detected in the chloroform extract. Identification of the bio-active components of C. longa oil using GC-MS showed a total of 19 peaks. The identified compounds include beta-curcumene, Tumerone, Ar-tumerone, oleic acid and methoprene among others. The bacteria and fungi used in this study are organisms commonly found in association with skin infections. All the extracts showed high antimicrobial activities to the bacteria and fungi used. The methanol extracts however showed high superiority in antimicrobial activities, followed by the ethanol extract. In the time-kill kinetics of antimicrobial study of the extracts showed significant reduction in viable cell count within 48 hours. Results obtained in this study indicated that the extracts of C. longa rhizome possess pharmacologically active compounds that work synergistically against organisms associated with skin infections.*

**Keywords:** *Curcuma longa*, Chemical qualities, Antimicrobial activities, Skin infections

## INTRODUCTION

The human skin, the outer covering of the body, it is the largest organ of the integumentary system. The skin has multiple layers of ectodermal tissue and guards the underlying muscles, bones, ligaments and internal organs, colonized by a diverse milieu of microorganisms, most of which are harmless or even beneficial to their host. The skin is an ecosystem composed of 1.8 $m^2$ of diverse habitats with an abundance of folds, invaginations and specialized niches that support a wide range of microorganisms. The primary role of the skin is to serve as a physical barrier, protecting our bodies from potential assault by foreign organisms or toxic substances [1].

Development of bacterial resistance to the available antibiotics and increasing popularity of traditional medicine has led researchers to investigate the antimicrobial compounds in plants. *Curcuma longa* (turmeric) is a plant in the Zingiberaceae family which is a native of South Asian region [2]. The rhizomes of *C. longa* contain curcuminoids which are used as a food additive for the promotion of health as well as for the cure of various types of diseases. Curcumin and other curcuminoids present in *C. longa* exhibit a variety of physiological and pharmacological activities [3]. Curcumin 95%, a potent antioxidant is believed to be the most bioactive and soothing portion of the herb turmeric and possess antioxidant, anti-inflammatory and antiplatelet properties, is cholesterol lowering as well as having antibacterial and anti-fungal effects. It contains a mixture of powerful antioxidant phytonutrients known as curcuminoids and inhibits cancer at initiation, promotion and progression stages of tumor development. It is a strong anti-oxidant, which supports colon health, exerts neuroprotective activity and helps to maintain a healthy cardiovascular system [4].

The use of turmeric extract as a cosmetic or skincare product in both topical and oral preparations has been reported. It is claimed to be effective in treating skin-aging induced by sun exposure, increased thickness and reduction in elasticity of skin, skin injury and other problems [5]. Also the fresh juice of rhizome of Haridra is used as an antiparastic in many skin afflictions [6]. Its rhizome powder mixed with cow's urine is taken internally in itching and dermatitis. *C. longa* leaves have good promise as an antifungal agent that could be used as a therapeutic remedy against human pathogenic fungi on account of its various *in vitro* and *in vivo* antifungal properties, long shelf-life, its tolerability of heavy inoculums

density, thermo stability, broad range of antidermatophytic activity and absence of any adverse effects [7]. This research work was carried out with the aim to assess the antimicrobial potentials of locally sourced *C. longa* on microorganisms associated with skin infection.

## MATERIALS AND METHODS

### Collection of test organisms

Seventeen microorganisms made up of 14 bacteria and 3 fungi collected from the stock culture of the Department of Microbiology, Afe Babalola University Ado Ekiti were used for this study. The bacteria were, *Escherichia coli* ATCC25922, *Staphylococcus aureus* ATCC25923, *Propionibacterium acnes, Escherichia coli, Proteus mirabilis, Corynebacterium accolens, Pseudomonas aeruginosa, Pediococcus acidilacti, Bacillus megaterium, Bacillus cereus, Brachibius cervicis, Arthrobacter mysorens, Bordetella trematum* and *Cetobacterium somarae*; while the fungi used were *Candida albicans* (clinical isolate), *Candida albicans* ATCC 10231 and *Malassezia furfur* ATCC 44349.

### Maintenance of bacterial and fungal cultures

The cultures of bacteria were grown on nutrient agar plates at 37°C for 24 h while the fungal cultures were grown on Sabouraud's dextrose agar plates at room temperature for 3 days. They were maintained and preserved on nutrient agar slants and Sabouraud's dextrose slants respectively at 4°C before use.

### Antibiotic susceptibility test

All bacterial isolates were tested for antibiotic susceptibility by Kirby-Bauer disc diffusion method on freshly prepared Muller-Hinton agar. This was carried out by making an even spread of the broth cultures adjusted to McFarland standard on prepared Mueller-Hinton agar, then using sterile swab sticks and aseptic placement of the antibiotics disc using sterile forceps. The plates were incubated aerobically at 37°C for 24 h after which the zones of inhibition were measured and interpreted according to Clinical and Laboratory Standards Institute [8]. Antibiotics used were trimethoprim/sulphamethoxazole (30 µg), chloramphenicol (30 µg), sparfloxacin (10 µg), ciprofloxacin (10 µg), amoxicillin (30 µg), augmentin (amoxicillin/clavulanate) (30 µg), Gentamycin (10 µg), pefloxacin (30 µg), ofloxacin (10 µg) and streptomycin (30 µg) for Gram-negative isolates, and pefloxacin (10 µg), gentamycin (10 µg), ampiclox (30 µg), cefuroxime (µg), amoxicillin (30 µg), ceftriaxone (25 µg), ciprofloxacin (10 µg), streptomycin (30 µg), amoxycillin-clarvulanic (30 µg), erythromycin (10 µg) for gram positive isolates.

### Plant sample collection

The rhizomes of *C. longa* used for this study were purchased from the local farmers in Ado-Ekiti, Nigeria and transported to the Microbiology Laboratory of Afe Babalola University, Ado-Ekiti (ABUAD).

### Preparation of turmeric (*C. longa*) extracts

Before extraction, the rhizomes of *C. longa* were washed to remove sand and other particules, peeled, cut into bits and air-dried over a period of one week. After air drying the rhizomes were ground into fine powder with the electric blender. 100 g of powder was placed in 4 different containers and 500 ml of acetone, ethanol, methanol and chloroform were added to each container and allowed to stay for 72 h at room temperature with occasional shaking. The resulting solutions were then filtered retaining the supernatant only. The extracts were dried by first using a rotary evaporator then a water bath and stored in air tight containers at 4°C prior to use.

### Proximate analysis of *C. longa*

The methods described in official methods of Association of Official Analytical Chemists [9] were used for proximate analysis.

*Ash content*

A dry Ashing method was used to determine the ash content. First, the crucible was weighed before the mass of the sample and crucible was taken prior to incineration in a Muffle furnace at 400°C. The resulting material was cooled in a desiccator to constant mass, and the ash content determined.

$$\%\text{Ash} = \frac{Mass\ of\ sample\ remaining}{Mass\ of\ original\ sample} \times \frac{100}{1}$$

*Determination of moisture content*

The oven was used to dry a known weight of the sample to constant mass at 105°C and weighed. The percentage of moisture content was calculated as:

$$\%\ \text{Moisture} = \frac{\text{Moisture lost by mass}}{\text{Mass of original sample}} \times 100$$

*Determination of crude fibre*

This is the non-digestible component of food sample. This test was performed as described AOAC (2012) and the crude fibre is calculated thus:

$$\%\ \text{crude fibre} = \frac{(\text{Mass of sample} - (\text{mass of sample} + \text{mass of crucible})}{\text{Mass of sample}} \times 100$$

*Determination of protein*

The crude protein was determined using Kjeldahl Nitrogen method.

$$\%\ \text{Nitrogen} = \frac{\text{v2} - \text{v1} \times ca \times 0.014 \times 100}{\text{ms} \times 10\ (\text{ml of sample})} \times 100$$

*Mineral composition test*

The mineral contents were determined on aliquots of the solution of the dried rhizome sample by UV/Visible and atomic absorption spectrophotometry. A NOVA 400 atomic absorption spectrometer (Analytik Jena AG, Jena, Germany) with an air/acetylene flame and respective hollow-cathode lamps was used for absorbance measurements. Wavelengths, slits and lamp currents used for determination of the six elements were 213.9 nm, 0.5 nm, 4.0 mA (zinc), 422.7 nm, 1.2 nm, 4.0 mA (calcium); 248.3 m, 0.2 nm, 6.0 Ma (iron) and 766.5 nm, 0.8 nm, 4.0 mA (potassium), respectively. The results for mineral contents are expressed as mg/100 g.

**Quantitative phytochemical analysis**

Tests for the presence of the following plant secondary metabolites including alkaloids, flavonoids, steroids, saponins, phenols, tannins, glycosides and cardiac glycosides and phytates were carried out on the powdered samples following the methods of Herboone [10] and Mayuri [11].

*Test for flavonoids*

A weight of 1 g of the sample was weighed into 10 ml of 80% methanol; left to stand for 2 h, filtered into a weighed petri dish and left to dry in the oven at 40°C until it attained a constant mass after cooling in a desiccator. The mass of the petri dish was recorded.

**Determination of total flavonoids:** A volume of 0.5 ml of 2% $AlCl_3$ methanol solution was added to 0.05 ml sample solution. After 1 h at room standard calibration graph was produced using 10, 20, 30, 40, 50 mg/100 g standard solutions of tannic acid. The concentration of tannin was read off taking into consideration dilution factors.

*Alkaloids*

1 g of the sample (M) was added to 20 ml of 10% acetic acid in ethanol, shaken, allowed to stand for 4 h and filtered. The filtrate was allowed to evaporate to about a quarter of its original volume and one drop of concentrated ammonia added. The precipitate formed was filtered through a weighed (M1) filter paper. The filter paper dried in the oven at 60°C, weighed when it has attained a constant weight (M2).

$$\%\text{Alkaloid} = \frac{m2-m1}{m} \times 100$$

*Phenols/Phenolics/Phenolic acids*

A 2 ml of the extract was mixed with 0.5 ml of Folin-Ciocalteau reagent and 1.5 ml sodium carbonate (20%). Mix for 15 sec and allow standing at 40°C for 30 min to develop colour which was measured at $A_{765}$ nm and express as GAE/g (Gallic Acid Equivalent).

*Saponins*

1 g of sample was added to 5 ml of 20% ethanol in a conical flask and placed in a water bath at 55°C for 4 h. This was followed by filtering and washing the residue with 20% ethanol twice and reducing the extract to about 5 ml in the oven. The extract was further treated successively with petroleum ether, butanol and 5% sodium chloride.

*Steroids*

5 g of the sample was added to 100 ml of water and drops of 0.1 M ammonium hydroxide was added to take the pH to 9.1; then 2 ml petroleum ether, 3 ml acetic anhydride and concentrated $H_2SO_4$ were added and the absorbance measured at 420 nm.

*Cardiac glycosides/Cardenolides*

1 g of the sample was added into 40 ml water and place in the oven at 100°C for 15 min. To 1 ml of the extract was added 5 ml water, 2 ml glacial acetic acid add one drop of $FeCl_3$ and 1 ml concentrated $H_2SO_4$. Measurement of the absorbance of the resulting solution was done at 410 nm.

*Phytates*

1 g of the sample was added into 10 ml 3% TCA. The phytate was precipitated as ferric phytate with 0.1% ammonium ferric sulphate. The ferric phytate was converted to ferric hydroxide and sodium phytate by adding 10 ml 0.5 M sodium hydroxide, followed by boiling and the precipitate dissolved by adding dilute acid. Measurement of absorbance of the solution was carried out at 519 nm.

**Qualitative phytochemical analysis of extracts of *C. longa***

Tests for the presence of the following plant secondary metabolites: alkaloids, saponins, tannins, flavonoids, steroids, cardiac glycosides, glycosides, phenols were carried out on the methanol, acetone, chloroform and ethanol extract of the plant. All tests were performed as described by Sofowora [12].

*Test for alkaloids*

To 1 ml of aqueous hydrochloric acid was added 2 ml of the extract, followed by addition of a few drops of saturated picric acid solution. A cream precipitate obtained indicated the presence of alkaloids.

*Test for tannins*

A 2 ml of the plant extract was dissolved in 10 ml of distilled water and filter followed by addition of 2 ml $FeCl_3$. A blue-black precipitate indicated the presence of tannins.

*Test for saponins (Frothing test)*

This was done by adding 0.5 ml of the extract to 5 ml distilled water. Frothing indicated the presence of saponins.

*Test for flavonoids*

To 2 ml of plant extract was added dilute NaOH and dilute HCl. A yellow solution indicates the presence of flavonoids.

*Test for steroids*

A 10 ml volume of the plant extract was dissolved in 10 ml chloroform and filter. To 2 ml of the filtrate was added 2 ml acetic anhydride and concentrated $H_2SO_4$. The presence of a blue-green ring indicated the presence of terpenoids.

*Test for cardiac glycosides (Keller-kiliani test)*

To 2 ml of extract was added 1 ml glacial acetic acid, 1 ml $FeCl_3$ and 1 ml concentrated $H_2SO_4$. The presence of a green-blue precipitate indicated that cardiac glycosides are present.

*GC-MS analysis of C. longa oil*

Essential oils of *C. longa* were extracted with petroleum ether. Identification of the chemical compounds present in the extracted oil was carried out by Gas Chromatography-Mass Spectrometry (GC-MS) according to methods described by Sohan and co-workers [13], using the GCMS-QP2010 Ultra High-End GC-MS (Shimadzu Europa GmbH®, Germany). Analysis was carried out following strictly the manufacturer's operational procedures.

**Antimicrobial activity of turmeric extracts using agar well diffusion method**

Susceptibility of the organisms to the acetone, ethanol, methanol and chloroform extracts of the *C. longa* was determined by agar well diffusion technique using Mueller-Hinton agar [11]. 7 mm diameter wells were prepared on agar containing a suspension of each isolated organism. The acetone, ethanol, methanol and chloroform extracts were diluted into two folds, using Dimethyl Sulfoxide (DMSO) as diluent and different concentrations were added to the wells. The plates were left at ambient temperature for 15 min and then incubated at 37°C for 24 h, after which the zones of inhibition were measured and recorded.

**Determination of Minimum Inhibitory Concentrations (MIC)**

MIC is defined as the lowest concentration that results in the maintenance or reduction of inoculum's viability, was determined by serial tube dilution technique for the bacterial isolates. Different concentrations 0.02-10.0 mg/ml of the extract and 0.03-250 µg/ml of tetracycline were differently prepared by serial dilution in Mueller Hinton broth medium [14]. Each tube was then inoculated with 100 µl of each of the adjusted bacterial strain. Two blank Mueller Hinton broth tubes, with and without bacterial inoculation, were used as the growth and sterility controls. The bacterial containing tubes were incubated aerobically at 37°C for 24 h after the incubation period, the tubes were observed for the MICs by checking concentration of the first tube I the series (ascending extract and antibiotic concentrations) that showed no visible trace of growth. The first tube in the series with no visible growth after the incubation period was taken as the MIC. The MIC values were used for time-kill kinetic study.

**Time-Kill kinetic analysis**

The rate of bacterial and fungal killing was determined using *Curcuma longa* extracts with least MIC value for each of the 3 bacteria namely: *Staphylococcus aureus* ATCC25923, *Escherichia coli* ATCC25922 and *Pseudomonas aeruginosa*; and 3 fungi namely, *Candida albicans* ATCC 10231, *Candida albicans* (clinical isolate) and *Malassezia furfur* ATCC 44349 by time-kill kinetic assay as described by Miyasaki et al. [15] with slight modifications. Fungal and bacterial cultures were diluted into 20 ml nutrient broth and Sabouraud's dextrose broth respectively and then supplemented with 1 × MIC and 2 × MIC of the methanol and ethanol *C. longa* extract separately for each test organism. The cultures were grown at 37°C and frequently shaken. The aliquots of cultures were collected at different time intervals (0 h, 6 h, 12 h, 24 h and 48 h) and plated onto Muller Hilton agar plates. After incubating the plates at 37°C for 16 h viable colonies were enumerated. The results were recorded in terms of CFU and plotted against time for each bacterial and fungal isolate.

**Statistical analysis of data**

One way ANOVA was used to determine significant difference between groups. SPSS V-16.0 was used for data analysis and the values from $P \leq 0.05$ to $P<0.001$ were considered significant at 2 tailed tests.

## RESULTS

Rhizomes of *Curcuma longa* purchased from farmers in Ado Ekiti, Nigeria, were analysed for their nutritional and phytochemical qualities, as well as the composition of its essential oil. The proximate analysis showed that the dried turmeric rhizomes were rich in energy (3002.00 ± 5.77 kcal/kg), protein (18.37 ± 0.15%), carbohydrate (45.8%) and fat (3.10 ± 0.10%), as well as vitamins and minerals. It contains high concentrations of $Ca^{2+}$, $Fe^{2+}$, $K^-$, $PO_4^{2-}$ and ascorbic acid; with low levels of $Zn^{2+}$, thiamine, niacin and riboflavin (Table 1).

Quantitative phytochemical analyses of the dried turmeric rhizomes revealed high concentrations of alkaloids (538 ± 0.25 mg/100 g), tannins (455 ± 13.23 mg/100 g) and saponnins (420 ± 8.66 mg/100 g); moderate level of oxalate (156.67 ± 10.41 mg/100 g); low level of phytates (45.0 ± 5.0 mg/100 g); with no detectable cyanogenic glycosides (Table 1).

Qualitative phytochemical analyses of the extracts of *C. longa* indicated the presence of alkaloids, tannins and phenols in the acetone, and ethanol extracts; saponin only in the ethanol extract; while none of the tested phytochemicals were detected in the chloroform extract (Table 2).

Identification of the bioactive constituent of the essential oil was carried out using GC-MS analysis. The GC-MS spectra of *C. longa* oil showed a total of 19 peaks (Figure 1). The identified compounds included Spiro[4,4]nona-1, 6-diene, (S)-, 2-Pentanone, 4-methyl-4-phenyl-, beta.-curcumene, Turmerone, Ar-tumerone, benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl- and benzene, (1,1,4,6,6-pentamethylheptyl among others (Table 3).

The clinical bacterial isolates used for assay of antibacterial activities of *C. longa* were all multidrug resistant bacteria (Table 4 and Figure 2). All the extracts showed high antimicrobial activities to the tested bacteria and fungi, with no significant difference, with exception of 4 (23.53%) organisms (*Propionibacterium acnes*, *Pediococcus acidilacti* concentration of the methanolic extract, 7 (41.18%) of 17 microbes tested were susceptible, while for the ethanol, acetone and chloroform extracts 7 (41.18%), 5 (29.41%) and 4 (23.53%) of the organisms respectively were susceptible. At 1.56 mg/ml methanolic extracts, 12(70.59%) tested organisms were susceptible, while the ethanolic, acetone and chloroform extracts gave 8 (47.66%), 8 (47.06%) and 9 (52.94%) susceptibility respectively (Table 5). *Bacillus cereus* and *Malassezia furfur*). The methanolic extracts, however, showed high superiority in antimicrobial activities, followed by the ethanolic extract at 0.78 mg/ml.

**Table 1: Proximate, vitamins, minerals and phytochemical composition of dried powder of *Curcuma longa* rhizome**

| | Minimum | Maximum | Mean ± Std. Deviation |
|---|---|---|---|
| **Proximate** | | | |
| Moisture % | 8.40 | 8.70 | 8.53 ± 0.15 |
| Protein % | 18.20 | 18.50 | 18.37 ± 0.15 |
| Fat % | 3.00 | 3.20 | 3.10 ± 0.10 |
| Ash % | 8.60 | 8.60 | 8.60 ±0.00 |
| Crude fibre % | 15.30 | 15.70 | 15.5333 ± 0.21 |
| Carbohydrate % | 45.80 | 45.80 | 45.80 ± 0.00 |
| Metabolizable energy Kcal/Kg | 2996.50 | 3008.00 | 3002.00 ± 5.77 |
| **Minerals** | | | |
| $Fe^{++}$ (mg/100 g) | 12.50 | 12.90 | 12.67 ± 0.21 |
| $Zn^{++}$ (mg/100 g) | 0.30 | 0.30 | 0.30 ± 0.00 |
| $Mg^{++}$ (mg/100 g) | 30.00 | 35.00 | 31.67 ± 2.88 |
| $Ca^{++}$ ( mg/100 g) | 360.00 | 365.00 | 363.33 ± 2.88 |
| $K^{+}$ ( mg/100 g) | 40.00 | 45.00 | 41.67 ± 2.88 |
| $PO4^{-}$ ( mg/100 g) | 265.00 | 285.00 | 276.67 ± 10.41 |
| **Vitamins** | | | |
| Ascorbic acid (mg/100 g) | 21.00 | 21.50 | 21.23 ± 0.25 |
| Thiamin (mg/100 g) | 0.40 | 0.50 | 0.43 ± 0.06 |
| Niacin (mg/100 g) | 0.30 | 0.30 | 0.30 ± 0.00 |
| Riboflavin (mg/100 g) | 0.80 | 0.90 | 0.87 ± 0.06 |
| **Phytochemicals** | | | |
| Alkaloids (mg/100 g) | 530.00 | 550.00 | 538.33 ± 10.41 |
| Phytates (mg/100 g) | 40.00 | 50.00 | 45.00 ± 5.00 |
| Tannins (mg/100 g) | 445.00 | 470.00 | 455.00 ± 13.23 |
| Saponins (mg/100 g) | 410.00 | 425.00 | 420.00 ± 8.66 |
| Oxalates(mg/100 g) | 145.00 | 165.00 | 156.67 ± 10.41 |
| Cyanogenic glycosides | 0.00 | 0.00 | |

**Table 2: Qualitative phytochemical composition of acetone, ethanol, methanol and chloroform extracts of dried powder of *Curcuma longa* rhizome**

| Phytochemicals | Acetone | Ethanol | Methanol | Chloroform |
|---|---|---|---|---|
| Alkaloids | ++ | ++ | ++ | - |
| Tannins and phenols | ++ | ++ | + | - |
| Saponins | + | - | + | - |
| Flavonoids | - | - | - | - |
| Terpenoids | - | - | - | - |
| Steroids | - | - | - | - |
| Cardiac glycosides | - | - | - | - |
| Glycosides | - | - | - | - |

KEYS: + = Positive; - = Negative

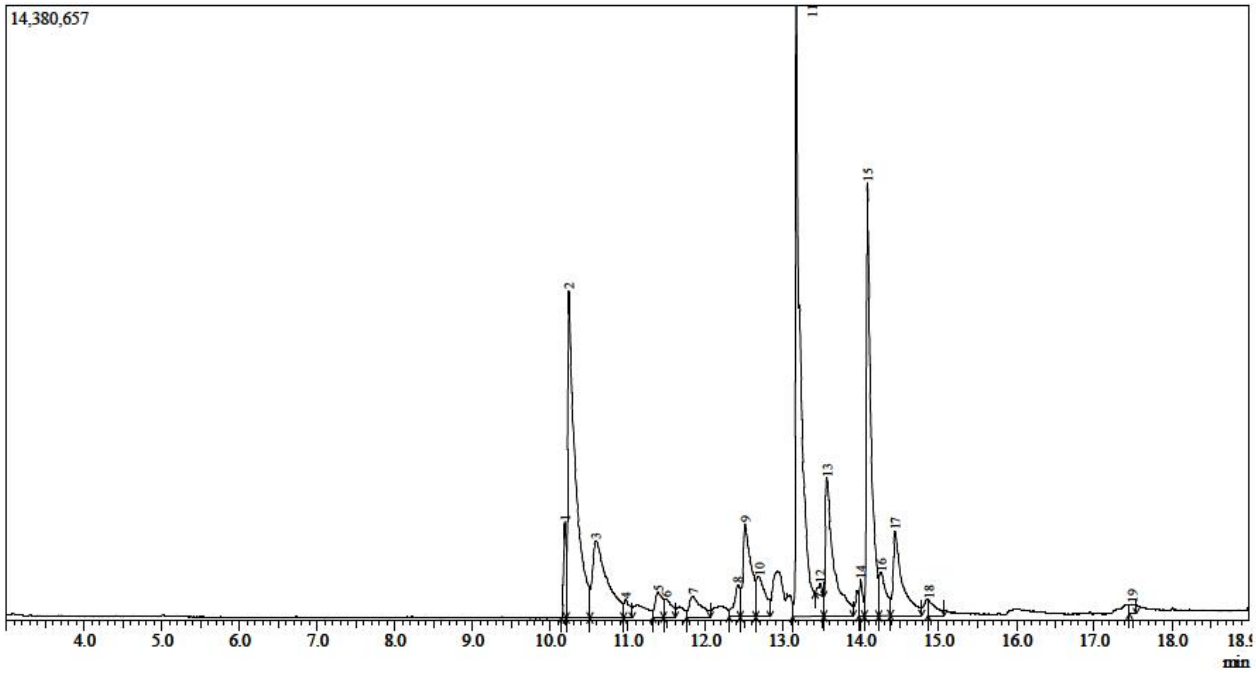


**Figure 1: Spectra of GC-MS analysis of *Curcuma longa* essential oil**

**Table 3: Bioactive constituents of the essential oil**

| Peak# | Retention time | Area | Area% | Height | Height (%) | A/H | Name |
|---|---|---|---|---|---|---|---|
| 1 | 10.189 | 4818424 | 1.72 | 2245909 | 4.51 | 2.15 | Spiro[4.4]nona-1,6-diene, (S)- |
| 2 | 10.242 | 51877169 | 18.48 | 7650812 | 15.37 | 6.78 | 2-Pentanone, 4-methyl-4-phenyl- |
| 3 | 10.585 | 24079584 | 8.58 | 1803945 | 3.62 | 13.35 | N-Methyl-N-benzyl-4-oxo-4-phenyl-butyramide |
| 4 | 10.072 | 2205834 | 0.79 | 435139 | 0.87 | 5.07 | Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl- |
| 5 | 11.387 | 3680352 | 1.31 | 584430 | 1.17 | 6.30 | Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl- |
| 6 | 11.487 | 3012896 | 1.07 | 436303 | 0.88 | 6.91 | beta.-curcumene |
| 7 | 11.833 | 5268581 | 1.88 | 491848 | 0.99 | 10.71 | cis-sesquisabinene hydrate |
| 8 | 12.415 | 3659669 | 1.30 | 745902 | 1.50 | 4.91 | Tridecane, 2-methyl-2-phenyl- |
| 9 | 12.511 | 15777873 | 5.62 | 2170290 | 4.36 | 7.27 | Benzene, (1,1,4,6,6-pentamethylheptyl)- |
| 10 | 12.685 | 7595277 | 2.71 | 933040 | 1.87 | 8.14 | 6-(p-Tolyl)-2-methyl-2-heptenol, trans- |
| 11 | 13.170 | 64257803 | 22.89 | 14278665 | 28.68 | 4.50 | Ar-tumerone |
| 12 | 13.476 | 886616 | 0.32 | 259998 | 0.52 | 3.41 | Tumerone |
| 13 | 13.561 | 23498999 | 8.37 | 3238964 | 6.51 | 7.26 | Curlone |
| 14 | 13.996 | 2575800 | 0.92 | 841821 | 1.69 | 3.06 | 7-Oxabicyclo[4.1.0]heptane, 1-(1,3-dimethyl-1,3-butadienyl)-2,2,6-trimethyl-, (E)- |
| 15 | 14.084 | 41515204 | 14.79 | 10112648 | 20.31 | 4.11 | 1,3-Dioxolane, 2-(2-phenyl-2-propyl)- |
| 16 | 14.263 | 6313965 | 2.25 | 1002693 | 2.01 | 6.30 | Bicyclo[3.1.1]hept-2-ene, 2,6-dimethyl-6-(4-methyl-3-pentenyl)- |
| 17 | 14.437 | 16163691 | 5.76 | 1975410 | 3.97 | 8.18 | 1,3,7-Octatriene, 2-methyl-3-trimethylsilyl- |
| 18 | 14.871 | 2517400 | 0.90 | 362862 | 0.73 | 6.94 | Methoprene |
| 19 | 17.491 | 1053202 | 0.38 | 209246 | 0.42 | 5.033 | Oleic Acid |
| | | 280758339 | 100.00 | 49779925 | 100.00 | | |

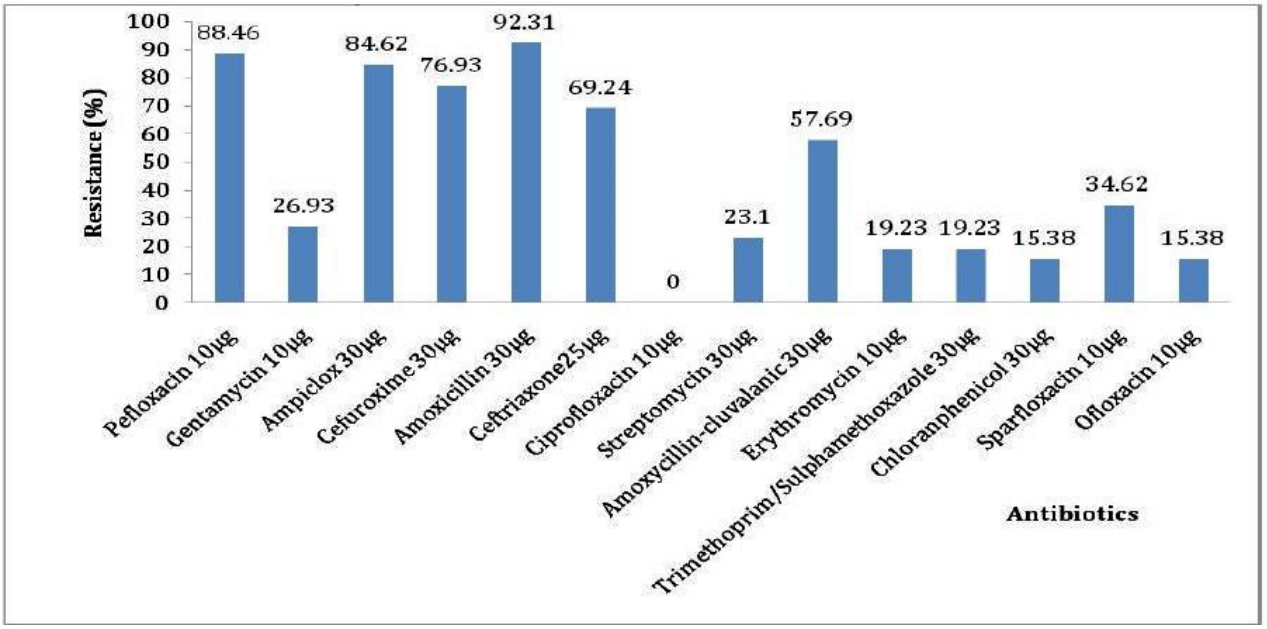


**Figure 2: Susceptibility bacteria used to the antibiotics**

**Table 4: Cluster of antibiotic resistance among the clinical bacteria used**

| Organisms | Antibiotics Cluster |
|---|---|
| *Propionibacterium acnes* | PEF/APX/CXM/CXM/AMX/S/AMC/E/SPX |
| *Corynebacterium accolens* | PEF/CN/APX/CXM/AMX/CRO/AMC/E/SPX |
| *Pediococcus acidilacti* | PEF/CN/APX/CXM/AMX/CRO/AMC/SXT/C |
| *Bacillus cereus* | PEF/APX/CXM/AMX/CRO/S/AMC |
| *Bacillus megaterium* | PEF/APX/CXM/AMX/CRO/S |
| *Brachibius cervices* | PEF/AMX/CRO |
| *Arthrobacter mysorens* | PEF/APX/CXM/AMX/CRO/S/AMC/E/SXT |
| *Staphylococcus aureus* | PEF/APX/CXM/AMX/CRO/AMC/C/SPX |
| *Escherichia coli* | PEF/APX/CXM/AMX/CRO/S/AMC/SXT/C/SPX/OFX |
| *Proteus mirabilis* | APX/CXM/AMX/CRO/S/AMC/E |
| *Pseudomonas aeruginosa* | PEF/CN/APX/AMX/AMC/SXT/C/OFX |
| *Bordetella trematum* | PEF/CN/APXCXM/AMX/AMC/SPX |
| *Cetobacterium somarae* | PEF/AMX |

**Table 5: Antimicrobial activities of turmeric extract on the tested bacteria and fungi (average zone of inhibition in mm)**

| Extracts | | *Propionibacterium acnes* | *Escherichia coli* | *Proteus mirabilis* | *Pediococcus acidilacti* | *Corynebacterium accolens* | *Arthrobacter mysorens* | *Bordetella trematum* | *Cetobacterium somarae* | *Pseudomonas aeruginosa* | *Staphylococcus aureus ATCC 25923* | *Brachibius cervices* | *Bacillus megaterium* | *Bacillus cereus* | *Escherichia coli ATCC 25922* | *Malassezia furfur ATCC 44349* | *Candida albicans ATCC 10231* | *Candida albicans (Clinical isolate)* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Acetone | 100 mg/ml | 10.5 | 13 | 8 | 13 | 6.5 | 10.5 | 17 | 14 | 8 | 12 | 11 | 12 | 10 | 15 | 22 | 19 | 24 |
| | 50 mg/ml | 7 | 10.5 | 8 | 10.5 | 4.5 | 9.5 | 15 | 13 | 7 | 10.5 | 10 | 10.5 | 9.5 | 10 | 21 | 17 | 22 |
| | 25 mg/ml | 6.5 | 10.5 | 7 | 9 | 3.5 | 8 | 14.5 | 11 | 5 | 9 | 9.5 | 10 | 9 | 9 | 13 | 13.5 | 20.5 |
| | 12.5 mg/ml | 6 | 6.5 | 6.5 | 7.5 | 3.5 | 7.5 | 12 | 10.5 | 3 | 8 | 9 | 9 | 8 | 7 | 10 | 8.5 | 15.5 |
| | 6.25 mg/ml | 6 | 4.5 | 6 | 7.5 | 3 | 7 | 10 | 10.5 | 2 | 4 | 8 | 8 | 6 | 4 | 5.5 | 8 | 15 |
| | 3.13 mg/ml | 5 | 4 | 5.5 | 5 | 3 | 5 | 10 | 5 | 0 | 0 | 6 | 4 | 3 | 3.5 | 5 | 6 | 13 |
| | 1.56 mg/ml | 5 | 4 | 0 | 0 | 0 | 0 | 5 | 3.5 | 0 | 0 | 0 | 0 | 0 | 3 | 5 | 6 | 12.5 |
| | 0.78 mg/ml | 3 | 3.5 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5.5 | 6 |
| Ethanol | 100 mg/ml | 11 | 7.5 | 8.5 | 7.5 | 11 | 9 | 12 | 16 | 9 | 13 | 13 | 7.5 | 21 | 9 | 18 | 15 | 20 |

| | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 50 mg/ml | 10 | 7 | 8 | 5.5 | 11 | 8.5 | 10.5 | 12 | 7.5 | 8.5 | 11 | 7 | 19 | 6 | 16 | 13 | 17 |
| | 25 mg/ml | 8.5 | 6.5 | 7 | 5.5 | 10.5 | 8 | 10 | 10.5 | 7.5 | 6 | 10 | 7 | 13 | 6 | 15.5 | 10 | 16.5 |
| | 12.5 mg/ml | 8 | 6 | 6.5 | 3 | 7.5 | 7 | 9 | 10.5 | 6 | 5 | 9.5 | 7 | 13 | 5 | 11 | 9.5 | 13 |
| | 6.25 mg/ml | 8 | 6 | 6.5 | 0 | 6 | 6.5 | 8 | 10 | 4 | 3 | 8 | 7 | 9 | 3 | 9.5 | 6 | 11 |
| | 3.13 mg/ml | 7 | 5 | 6 | 0 | 4.5 | 6 | 7 | 9 | 0 | 1 | 0 | 3 | 5.5 | 1 | 8.5 | 4 | 11 |
| | 1.56 mg/ml | 5 | 0 | 5 | 0 | 4 | 0 | 5.5 | 8 | 0 | 0 | 0 | 0 | 0 | 0 | 8 | 4 | 10 |
| | 0.78 mg/ml | 4.5 | 0 | 0 | 0 | 1 | 0 | 3 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 4.5 | 3 | 4 |
| Methanol | 100 mg/ml | 8 | 5.5 | 9 | 9 | 13 | 14.5 | 13 | 17 | 17 | 13 | 12 | 14.5 | 11 | 10 | 23 | 9.5 | 16.5 |
| | 50 mg/ml | 8 | 5 | 9 | 8.5 | 11.5 | 11 | 7.5 | 15 | 13 | 7 | 10 | 12 | 10 | 7 | 21 | 9 | 16 |
| | 25 mg/ml | 6 | 5 | 6 | 8 | 10 | 6 | 7.5 | 13 | 13 | 7 | 9 | 12 | 9 | 6.5 | 18.5 | 9 | 16 |
| | 12.5 mg/ml | 5.5 | 4 | 5 | 8 | 9.5 | 4.5 | 4.5 | 11 | 6 | 5 | 9 | 11 | 8 | 5.5 | 17 | 8 | 15 |
| | 6.25 mg/ml | 5 | 3.5 | 4.5 | 7.5 | 7 | 4.5 | 3.5 | 10.5 | 0 | 2 | 5 | 9 | 7.5 | 5 | 15 | 6.5 | 13.5 |
| | 3.13 mg/ml | 4.5 | 3.5 | 4.5 | 7 | 7 | 4 | 4 | 10.5 | 0 | 0 | 4 | 8.5 | 7 | 5 | 9 | 6.5 | 13.5 |
| | 1.56 mg/ml | 4 | 3 | 0 | 5.5 | 4.5 | 4 | 3 | 5.5 | 0 | 0 | 0 | 0 | 5 | 4 | 6 | 5 | 12 |
| | 0.78 mg/ml | 0 | 0 | 0 | 2.5 | 3 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 3 | 2 | 5 | 5 |
| Chloform | 100 mg/ml | 13 | 19 | 9 | 6 | 11 | 11.5 | 11 | 13.5 | 12 | 12.5 | 11.5 | 13 | 9 | 17 | 15 | 13 | 17 |
| | 50 mg/ml | 10 | 10.5 | 7.5 | 5.5 | 7 | 11.5 | 9.5 | 12 | 8.5 | 12 | 11 | 12 | 7 | 13 | 12 | 10 | 17 |
| | 25 mg/ml | 9.5 | 10 | 7 | 5 | 7 | 11 | 7.5 | 11 | 7 | 7 | 8.5 | 12 | 5 | 11 | 8 | 9 | 16 |
| | 12.5 mg/ml | 9 | 10 | 6 | 5 | 6 | 11 | 6 | 10 | 5 | 6 | 8 | 11.5 | 3 | 10 | 6.5 | 7.5 | 14.5 |
| | 6.25 mg/ml | 8.5 | 7.5 | 5.5 | 3 | 4.5 | 9.5 | 5.5 | 8 | 0 | 4 | 7 | 11 | 1 | 6 | 6 | 6 | 13 |
| | 3.13 mg/ml | 8.5 | 4.5 | 5.5 | 2 | 4 | 6 | 5.5 | 5.5 | 0 | 0 | 5 | 0 | 0 | 3 | 0 | 6 | 12 |
| | 1.56 mg/ml | 8 | 2 | 3 | 0 | 0 | 4.5 | 4 | 3 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 5 | 8 |
| | 0.78 mg/ml | 5 | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 5 |
| | F-value | 4.363 | 0.327 | 0.412 | 5.012 | 2.595 | 0.789 | 2.321 | 1.549 | 0.494 | 0.020 | 0.038 | 0.435 | 3.877 | 0.932 | 4.434 | 0.154 | 0.218 |
| | P-value | 0.012* | 0.725 | 0.668 | 0.017* | 0.100 | 0.468 | 0.124 | 0.237 | 0.618 | 0.890 | 0.963 | 0.653 | 0.038* | 0.410 | 0.025* | 0.858 | 0.806 |

*Significant

The rate of bacterial and fungal killing after exposure to 1 × MIC (1.58 mg/ml) and 2 × MIC (3.13 mg/ml) of respective extracts of *C. longa* for each isolate is summarized in Figures 2-7. In the time-kill kinetics of antimicrobial study against *C. albicans* (clinical isolate), *C. albicans* ATCC 10231, *M. furfur* ATCC44349, *P. aeruginosa*, *S. aureus* ATCC 25923 and *E. coli* ATCC 25922 showed significant reduction in viable cell count from 0 to 48 h. However 2 × MIC (3.13 mg/ml) of ethanolic extract showed the highest reduction in *C. albicans* from 3.993 log10 to zero at 48 h (Figure 2). While the rest average reduction at 48 h (Figures 3-7).

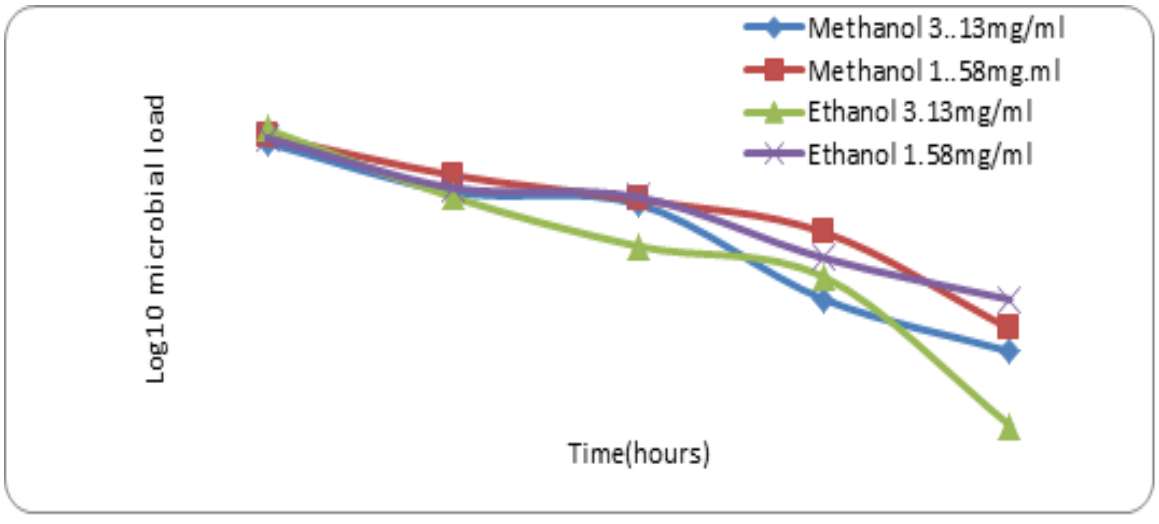


**Figure 3: Rate of killing of *Candida albicans* (clinical isolate)**

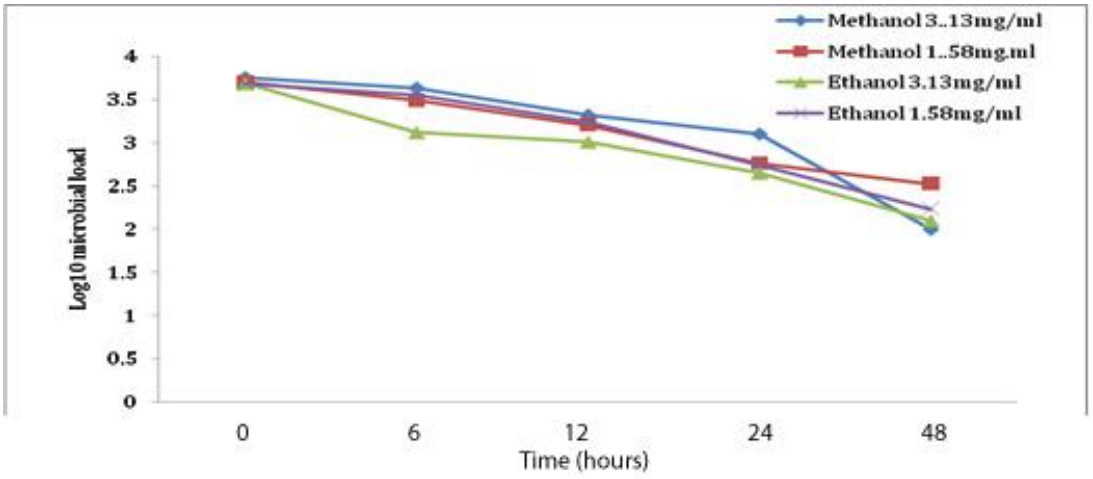


**Figure 4: Rate of killing of *Candida albicans* ATCC 10231**

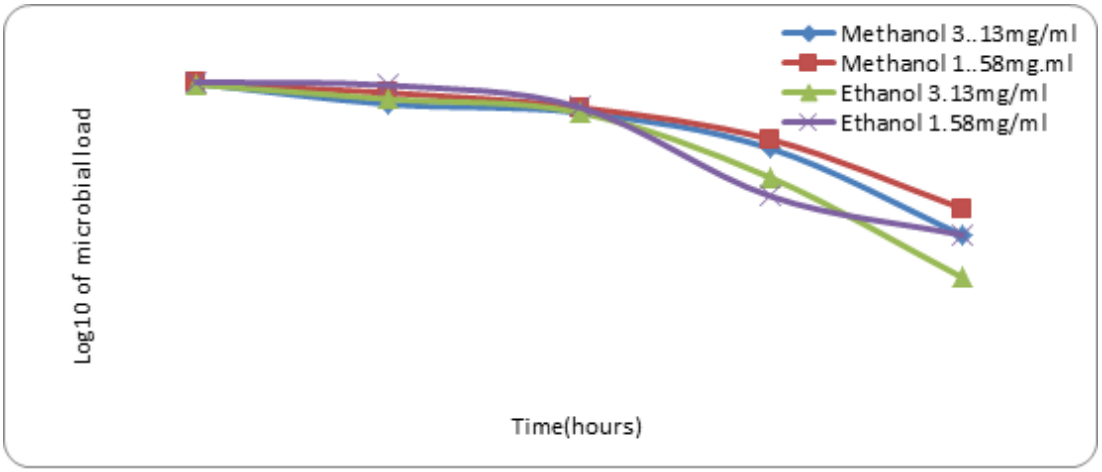


**Figure 5: Rate of kill of *Malassezia furfur* ATCC44349**

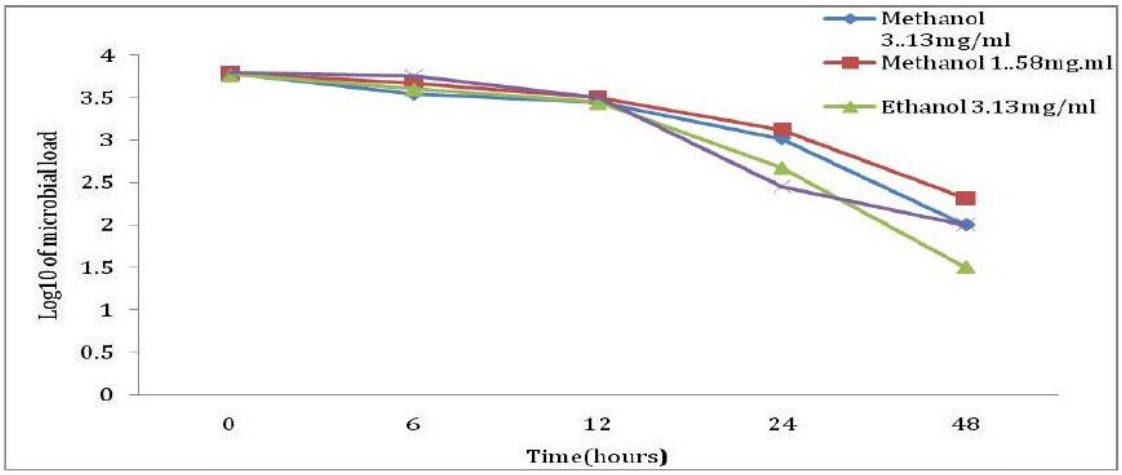


**Figure 6: Rate of kill of *Pseudomonas aeruginosa***

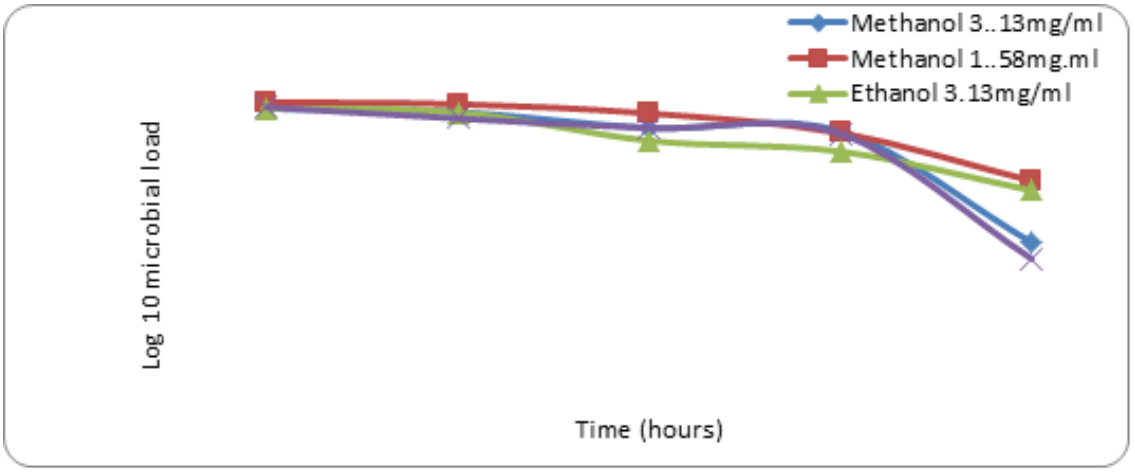


**Figure 7: Rate of kill of *Staphylococcus aureus* ATCC 25923**

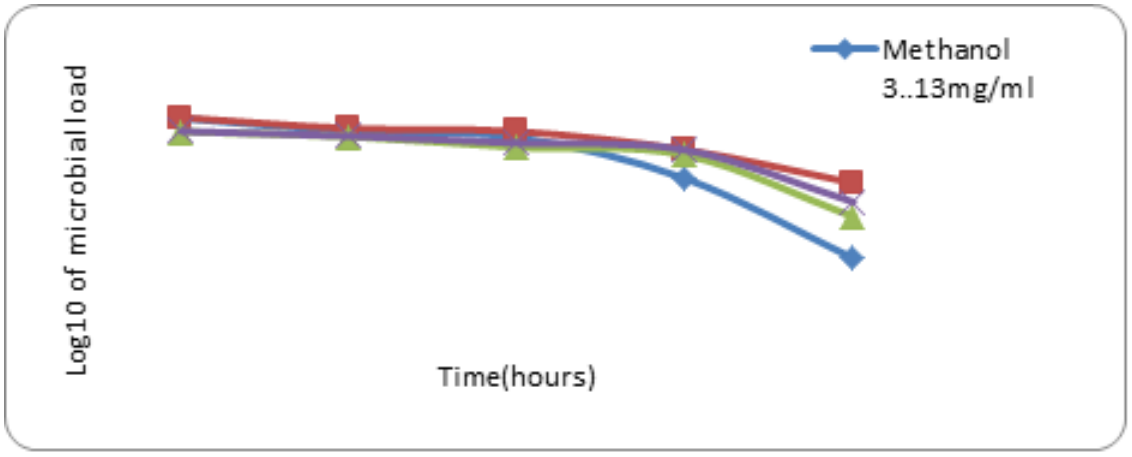


**Figure 8: Rate of kill of *Escherichia coli* ATCC 25922**

## DISCUSSION

Medicinal plants are an important source for new pharmacological agents and could be natural composite sources of new anti-infectious agents [16]. *C. longa* in previous studies have been seen to possess high nutritional and medicinal properties [17,18]. In this study the nutritional and medicinal potentials of *C. longa* were assessed through its proximate, minerals, vitamin and phytochemical analyses of dried rhizome of *C. longa*, as well as antimicrobial activities of its extracts.

In Table 1, proximate analysis showed that the dried rhizome contains high amounts of carbohydrate (45.8%), fat (3.10 ± 0.10%) and protein (18.37 ± 0.10%) thus making it a rich source of energy (3002.00 ± 5.77 kcal/kg). Carbohydrate performs numerous roles in living organisms; they provide energy to cells such as brain, muscle and blood. They contribute to fat metabolism and spare proteins as energy source. They also act as mild laxatives for human beings [19]. Fat supplies calories to the body and is essential for the proper functioning of the body. Fats provide essential fatty acids such as linoleic and linolenic acids which are important for controlling inflammation, blood clotting and brain development [20]. Proteins being among the essential nutrients for human beings act as one of the building blocks of body tissue and can also serve as fuel for the body. They also contain amino acids which are used as precursors to nucleic acid, enzymes, hormones, immune response, cellular repair, and other molecules essential to life [21].

The mineral and vitamin contents of the dried rhizome of *C. longa* showed that it contains high concentrations of $Ca^{2+}$, $Fe^{2+}$, $K^{-}$, $PO_4^{2-}$, $Mg^{++}$ and ascorbic acid; with low levels of $Zn^{2+}$, thiamine, niacin and riboflavin (Table 1). Calcium, according to the National Osteoporosis Foundation plays an important role in building stronger, denser bones early in life as well as the prevention of colon cancer and the reduction of obesity [22]. The high levels of magnesium, potassium, phosphate and iron may contribute to its anti-inflammatory, neuroprotective, gastrointestinal, and hepatoprotective activity [23], due to these minerals role in homeostasis, regulating blood sugar levels, renal filtration, reabsorption and excretion, fluid and electrolyte balancing and gastrointestinal motility [24]. Vitamins such as niacin, thiamine, riboflavin and the mineral zinc, though in low concentrations, are important for body metabolism. Thiamine is a vitamin of the B complex group and its phosphate derivatives are involved in many cellular processes. Its deficiency can lead to metabolic coma and death [25]. Niacin and riboflavin act as co-enzymes, functioning in numerous oxidation and reduction reactions [26]. Ascorbic acid has antioxidant properties, reacting with oxidants of the reactive oxygen species, such as the hydroxyl radical which are damaging to animals and plants at the molecular level due to possible interaction with nucleic acids [27]. High concentrations of alkaloids, tannins, and saponins, moderate levels of oxalate and phytates, with no detectable cyanogenic glycosides, were reported in this study (Table 1). The use of *C. longa* as cardio-protective, anti-cancer, anti-microbial, antidiabetic, antiageing, neuroprotective and antioxidant medicinal plant has been attributed to its phytochemical constituents [28-30]. Qualitative

phytochemical analysis of the extracts of *C. longa* indicated that acetone, methanolic and ethanolic extracts contain alkaloids, tannin and phenols; only ethanolic extracts contained saponin, while none of tested phytochemicals were detected in the chloroform extract. This result differed from that obtained by Ankur et al. [31], where tannin and phenol, flavonoid and glycosides were detected in the chloroform extract and, flavonoid and glycosides in the methanolic extracts.

The GC-MS analysis of oil of *C. longa* revealed 19 peaks (Figure 1), and the bioactive constituents among which included Beta-curcumene, Ar-tumerone, curlone, oleic acid and methoprene, which are known for their anti-inflammatory properties, stem cell proliferation, antiproliferative effect against breast carcinoma, antipulmonary, beneficial effect on cancer, autoimmune and inflammatory disease [32-35].

The clinical bacterial isolates used for assay of antibacterial activity of *C. longa* were all multidrug resistant bacteria, with cluster of resistance to 2-10 antibiotics. Antimicrobial susceptibility tests of different extracts of *C. longa* rhizomes showed that all fractions of *C. longa* rhizome are highly active against typed and clinical bacteria isolates as well as the fungi tested, showing zones of inhibition ranging between 1 mm and 24 mm. This result was consistent with the finding of Mary et al. [36], who reported the inhibitory effects of methanol, acetone and hexane extract of turmeric against bacterial and fungal pathogens. Further observations revealed that the methanol extracts showed high superiority in antimicrobial activities, followed by ethanol extracts. At the lowest concentration (0.78 mg/ml) 7 (41.18%) of 17 microbes tested were susceptible to the methanol extract, while for the ethanol, acetone and chloroform extracts 7 (41.18%), 5 (29.41%) and 4 (23.53%) organisms respectively were susceptible. While at the highest (1.56 mg/ml) 12 (70.59%) tested organisms were susceptible to the methanol extracts, while for the ethanol, acetone and chloroform extracts 8 (47.66%), 8 (47.06%) and 9 (52.94%) organisms respectively were susceptible. All the extracts showed better antifungal activity than antibacterial activity which correlates with the study of Mithra et al. [37]. The chloroform extracts showed least susceptibility to the other three solvent extracts which is in agreement with the study of Praveen and Sharmishtha [38] on some medicinal plants. They attributed the minimal antimicrobial activity in chloroform extracts to low concentration of antimicrobial compounds in these extracts.

The time kill antimicrobial assay of the extracts of *C. longa* gave variable kinetics against susceptible bacteria and fungi as seen in Figures 2-8. The extract demonstrated both bacteriostatic and bactericidal effects as it shows a concentration and time dependent killing. A complete elimination of *C. albicans* (clinical isolate) was achieved at 48 h of exposure. While gradual reduction of microbial concentration occurred with time with 1.58 mg/ml and 3.13 mg/ml of methanolic and ethanolic extract. This study revealed that complete elimination of *C. albicans* (clinical isolate) and significant reduction in the other test organisms at 48 hours and after 12 h exposure respectively. This result obtained correlates with the study of Oladosu et al. [39].

## CONCLUSION AND RECOMMENDATION

The development of resistance to available antibiotics has led researchers to explore natural plant compounds which have been shown to poses antimicrobial properties being tested as alternatives to synthetic chemical agents. The result from this study shows that extracts from *C. longa* rhizome possess pharmacologically active compounds that may have worked in synergy against organisms associated with skin infection. Also *C. longa* extracts is nutritionally rich as it contains some essential vitamins and minerals. The oil of *C. longa* contains chemical components which are known to have anti-inflammatory and anticancer properties with beneficial effects to autoimmune diseases. Therefore, *C. longa* can not only be used in pharmaceutical industries to produce drugs against pathogenic microorganisms, but can also be used in the treatment and prevention of various diseases and as a source of nutrients for body's growth and development.

## REFERENCES

[1] K. Iller, B.A. Selkin, G.J. Murakawa, *J. Investigative Dermatol. Symposium Proc*., **2001**, 6, 170-174.
[2] N.Y. Qin, F.Q. Yang, Y.T. Wang, S.P. Li, *J. Pharm. Biomed. Anal*., **2007**, 43, 486-492.
[3] M.C. Ramirez-Tortosa, M.D. Mesa, M.C. Aguilera, J.L. Quiles, L. Baro, C.L. Ramirez-Tortosa, E. Martinez-Victoria, A. Gil, *Atherosclerosis.*, **1999**, 147, 371-378.
[4] P.M. Luthra, R. Singh, R. Chandra, *Indian J. Clin. Biochem*., **2001**, 16, 153-160.
[5] S. Maho, K. Yoshiyuki, *Phytomedicine.*, **2009**, 16, 1137-1143.
[6] A.K. Dhiman, Common Drug Plants and Ayurvedic Remedies, 1st Edi., Press, New Delhi, India, **2004**.
[7] P. Paranjpe, Herbs for Beauty, 1st Edi., Chaukhambha Sanskrit Pratishthan, Delhi, India, **2001**.
[8] Clinical and Laboratory Standards Institutes, Performance Standard of Antimicrobial Susceptibility Testing, 23rd Information Supplement, CLSI, Wanye, USA.
[9] Association of Official Analytical Chemists, Offical Methods of Analysis 19th Edi., AOAC, Washington D.C., **2012**.
[10] J.B. Herboone, Phytochemical Methods, Chapman and Hall Ltd., London, **1998**.
[11] P.N. Mayuri, *J. Current Pharm. Res*., **2012**, 10(1), 19-219.
[12] A. Sofowora, Medicinal plants and Traditional Medicines in Africa, 3rd Edi., Spectrum Books Ltd. Ibadan, Nigeria, **2008**.
[13] S. Sohan, P. Arnab, G. Abhrajyoti, B. Maitree, *BMC Microbiol*., **2015**, 15, 170.
[14] A. Khan, M. Rhaman, S. Islam, *Turk J. Biol*., **2007**, 31, 167-172.
[15] Y. Miyasaki, J.D. Rabenstein, J. Rhea, M.L. Crouch, U.M. Mocek, P.E. Kittel, M.A. Morgan, W.S. Nicholas, M.M.V. Benschoten, W.D. Hardy, G.Y. Liu, *PLoS One*., **2003**, 8, E61594.
[16] P.I. Ushimaru, T.N. Mariama, C. Luiz, B. Di Luciano, F.J. Ary, *Brazilian J. Microbiol*., **2007**, 38, 717-719.
[17] P.S. Rana, D.A. Jain, *Int. J. Pharm. Life Sci*., **2012**, 3(1), 1368-1376.
[18] A. Ikpeama, G.I. Onwuka, C. Nwankwo, *Int. J. Sci. Eng. Res*., **2014**, 5(10), 2229-5518.
[19] S.O. Eze, O. Ernest, *Comm. Appl. Sci*., **2014**, 2(1), 8-24,
[20] A.H. Lichtenstein, L.J. Appeel, M. Brands, M. Carnethon, S. Danials, *Circulation.*, **2006**, 114, 82-96.
[21] S. Bilsbrough, M. Neil, *Int. J. Sport Nutr. Exercise Metab*., **2006**, 16, 129-152.
[22] L. Downing, M.A. Islam, *American J. Health-System Pharm*., **2013**, 70(13), 1132-1139.
[23] K. Vasavda, P.L. Hedge, A. Harini, *J. Homeopathy Ayurvedic Med*., **2013**, 2(4), 133.
[24] W.M. Haynes, CRC Handbook of Chemistry and Physics, 92nd Ed., Boca Raton, FL: CRC Press 4:122, **2011**.
[25] R.F. Butterworth, In: Modern Nutrition in Health and Disease, 10th Ed., Shils, M.E., Shike, M., Ross, A.C., Caballero, B. and Cousins, R.J. Editors). Baltimore: Lippincott Williams & Wilkin, **2006**.
[26] D.B. McCormick, In: Encyclopedia of Molecular Biology and Molecular Medicine, Meyers, R.A., Editors, Weinheim: VCH, **1997**, 396-

405.
[27] P.M. Dewick, Medicinal Natural Products: A Biosynthetic Approach, 3rd Edi.,), John Wiley and Sons, **2009**.
[28] B.P. Kanti, I.R. Syed, *Oxidative Med. Cellular Longevity*., **2009**, 2(5), 270-278.
[29] O.J. Akinjogunla, C.S. Yah, N.O. Eghafona, F.O. Ogbemudia, *Annals Biol. Res*., **2010**, 1920, 174-184.
[30] V. Habauzit, C. Morand, *Therapeutic Adv. Chronic Dis*., **2011**, 3(2), 87-106.
[31] G. Ankur, M. Surabhi, S. Rajendra, *Biotech. Reports*., **2015**, 6, 51-55.
[32] B.B. Aggarwal, A.B. Kunnumakkara, K.B. Harikumar, S.T. Tharakan, B. Sung, P. Anand, *Planta Medica*., **2008**, 74(13), 1560-1590.
[33] H.L. Li, C. Liu, G. Couto, M. Ouzounian, M. Sun, A.B. Wang, Y. Huang, C.W. He, Y. Shi, X. Chen, M.P. Nghien, Y. Liu, M. Chen, F. Dawood, M. Fukoka, Y. Maekawa, L. Zhang, A. Leask, A.K. Ghosh, L.A. Kirshenbaum, P.P. Liu, *The J. Clin. Invest*., **2008**, 118, 879-893.
[34] Y.Y. Chen, S.Y. Chiang, J.G. Lin, *Inter. J. Oncol*., **2010**, 36, 1113-1120.
[35] C.H. Sales, P.R. Souza, B.C. Peghini, J.S. de Silva, C.R. Cardoso, *Mini-Reviews Med. Chem*., **2013**, 13(2), 201-210.
[36] H.P.A. Mary, J.S.S. Prinitha, A.S.M. Madoen, J. Anoop, *Res. J. Pharm. Biol. Chem. Sci*., **2012**, 3(3), 49-55.
[37] N.H. Mithra, S. Shishir, Y. Mahalaxmi, P. Amit, *J. Pharm*., **2012**, 2(2), 192-198.
[38] D. Praveen, P. Sharmishtha, *Indian J. Pharm. Sci*., **2012**, 74(5), 443-450.
[39] P. Oladosu, N.R. Isu, K. Ibrahim, P. Okolo, D.K. Oladepo, *African J. Microbiol. Res*., **2013**, 7(46), 5248-5252.