

## Antibacterial Activities of *Cinnamomum verum* and *Curcuma longa* Extracts against some Multi-Drug Resistant (MDR) Bacterial Isolates

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**Abstract:** Spices are rich sources of bioactive molecules and play a crucial role in health maintenance and promotion due to their health-enhancing properties. The *in vitro* antimicrobial activity of *Cinnamomum verum* and *Curcuma longa* extracts was investigated against multidrug resistant bacteria using agar well diffusion method. The phytochemical analysis of both extracts showed the presence of saponins, tannins, glycoside, alkaloids, flavonoids and terpenoids which were confirmed by GC-MS data that identified the major constituents to be aromatic, phenolic, saturated, mono-unsaturated fatty acids and bioactive compounds with useful therapeutic properties. The findings revealed that *Curcuma longa* and *Cinnamomum verum* methanolic and aqueous extracts had significant ( $P < 0.05$ ) antibacterial activities against *Klebsiella pneumoniae*, *Bacillus* spp. and *Escherichia coli* ( $6.00 \pm 0.00 - 18.0 \pm 0.12$  mm zone of inhibitions). However, the antimicrobial activities of *Curcuma longa* methanolic extract against the multi drug resistant (MDR) pathogens at the various concentrations were found to be higher than the antimicrobial activity observed in other extracts used in the study. The combination of *Cinnamomum verum* and *Curcuma longa* extracts at various concentrations (i.e., 80:20; 60:40; 50:50; 40:60 and 20:80) had an antagonistic effect on the antibacterial activity of the extracts compared to the antibacterial activity of individual spices. The present study indicated that *Curcuma longa* and *Cinnamomum verum* with remarkable antimicrobial properties could be applied to improve existing drugs or to create new agents against MDR bacteria.

**Keywords:** Antimicrobial, Cinnamon, Pathogens, Phytochemical, Spices, and Turmeric

## INTRODUCTION

For centuries, antibacterial agents have been engaged to curb the mechanism and actions of pathogenic organisms. However, antimicrobial agents have also been associated with adverse effects on the host including hypersensitivity, immune suppression, and allergic reactions (Hegde *et al.*, 2012). Also, the continuous evolution of bacterial resistance to currently available antimicrobial agents has necessitated the search for novel and effective antimicrobial compounds from alternative and natural sources (Bakht *et al.*, 2011; Singh and Jain, 2011; Bakht *et al.*, 2013; Gul and Bakht, 2015). Natural sources like plants, spices and herbs are rich in bioactive molecules (e.g., polyphenols, carotenoids, and flavonoids) with therapeutic effects in the treatment of chronic as well as infectious diseases (Diallo *et al.*, 1999; Singh *et al.*, 2016).

A spice is dried seed, fruit, root, bark or flower of a plant or a herb used in small quantities for flavouring, coloring or as food preservative. Spices are distinguished from herbs, which are the leaves, flowers, or

stems from plants used for flavouring or as a garnish (Dubey, 2017; Sachan *et al.*, 2018). Spices such as clove, sage, turmeric, rosemary, and cinnamon are rich sources of bioactive molecules such as sulfur-containing compounds, tannins, alkaloids, phenolic diterpenes, glycosides and vitamins, especially flavonoids and polyphenols (Neveu *et al.*, 2010; Opara and Chohan, 2014; Yashin *et al.*, 2017). Spices are also used in traditional medicines for their health-enhancing properties as they are cheap and easily available (Sachan *et al.*, 2018). Previous studies have reported that bioactive constituents of spices possess tremendous importance in traditional medicines (Srinivasan, 2005; Tapsell *et al.*, 2006; Neveu *et al.*, 2010). Their constituents possess analgesic, antioxidative, antidotal, carminative, antispasmodic, diuretic, anti-inflammatory, antiperspirant, antiseptic, anticarcinogenic properties, which are actively used in preclinical, clinical, and therapeutic trials investigating new treatment of diseases (Duke *et al.*, 2003; Ravindran *et al.*, 2007; Jiang, 2019).

Turmeric (*Curcuma longa*) has been used for centuries as food preservative, colouring and flavouring agent, and traditional medicine (Naz *et al.*, 2010). *Curcuma longa* (Zingiberaceae family) has gained significant attention of researchers due to a wide array of biological activities it possesses (Gul and Bakht, 2015). It has been touted by Ancient Indians to possess antimicrobial, antioxidant, astringent, insect repellent and other useful therapeutic properties (Rudrappa and Bais, 2008; Chaturvedi *et al.*, 2009; Kim *et al.*, 2012; Khajehdehi, 2012; Sachan *et al.*, 2016).

Cinnamon (*Cinnamomum verum*), belonging to the family Laureceae grows in tropical Asia and Africa. The dried bark of the tree is usually used as a spice in food and dessert recipes. Cinnamon is rich in essential oils and tannins which inhibit microbial growth (Puangpronpitag and Sittiwet, 2009). This spice is regarded as antipyretic, antiseptic, astringent, balsamic, carminative, diaphoretic, fungicidal, stimulant, and stomachic. The powdered bark of this spice in water is applied to alleviate headaches and neuralgia (Dugoua *et al.*, 2007). It has been regarded as a folk remedy for indurations (of spleen, breast, uterus, liver, and stomach) and tumors (especially of the abdomen, liver and sinews) (Duke *et al.*, 2003; Ammon, 2008; Sachan *et al.*, 2018). Various studies have shown the antimicrobial effects of extracts of *Curcuma longa* and *Cinnamomum verum* on various microorganisms (Kim *et al.*, 2005; Park *et al.*, 2005; Lopez *et al.*, 2007; Senhajiet *et al.*, 2007; Niamsa and Sittiwet, 2009). However, the synergetic effects of these spices have never been reported. Hence, the need for the current study to evaluate the antimicrobial activities of *Curcuma longa* and *Cinnamomum verum* extracts on multidrug resistant bacteria.

## MATERIALS AND METHODS

### Study area

The study area was conducted in Kano state from different area each vegetable was collected from each of the six markets, the samples were collected from Bayero University, Kano, Kabuga, Janbulo, Rijiyar Zaki, Danbari and Rimi and transported to the Laboratory for analysis.

### Sample Collection

Total of 30 vegetable samples of 5 *Brassica oleracea* (cabbage), 5 *Cucumis sativus* (cucumber), 5 *Daucus carota* (carrot), 5 *Spinacia oleracea* (spinach) and 5 *Solanum lycopersicum* (tomatoes) were collected in sterile polythene bags from six different retail markets in Kano city.

### Preparation of Plant Materials

Dried cinnamon bark and turmeric rhizomes were purchased from RijiyarZaki market, Kano. The cinnamon bark and turmeric rhizomes spices were identified in the Herbarium of Plant Biology Bayero University, Kano with accession number of BUKHAN 119 and BUKHAN 188 as *Cinnamomum verum* and *Curcuma longa* respectively. The cinnamon bark and turmeric were grinded into powder using laboratory blender and then sieved with 5mm mesh size to obtain very fine powder then stored in a dry and sterile container to avoid contaminations by environmental pathogens (John *et al.*, 2003).

### Extraction of Spice Materials

Percolation and maceration extraction were obtained according to the method of Fatope *et al.* (2001), about 100g of cinnamon bark and turmeric powder were separately weighed using weighing balance and soaked in 1000ml of methanol and distilled water in conical flasks for one week at 37°C with regular shaking. The solution was filtered, and the solvent evaporated using a rotary evaporator and kept at 4°C prior to sensitivity test. The spices were allowed to air dry in order to obtain dried extracts.

### Phytochemical analysis of the plant extracts

The phytochemical analysis of *Cinnamomum verum* and *Curcuma longa* methanolic and aqueous extracts were carried out to find the presence or absence of bioactive secondary metabolites, saponins, tannins, glycoside, alkaloids, flavonoids and terpenoids by adopting standard protocols according to the method of Trease *et al.* (1983) with modification by Hegde *et al.* (2010).

### Multidrug-Resistant (MDR) Bacterial Strains

The MDR bacterial strains used in this study were the following: Gram-positive MDR bacteria, *Bacillus* spp.; Gram-negative MDR bacteria, namely, *Klebsiella pneumonia* and *Escherichia coli*. These MDR bacterial strains were isolated by Muazu, (2020) from five different fresh vegetables purchased from six different retail markets in Kano city, using standard cultural, morphological and biochemical procedures. The susceptibility patterns were obtained by disc diffusion method of Bauer-Kirby (1966) using six antibiotics (CLSI, 2015).

### Antibacterial Activities of Plant Extracts

The antibacterial assay was carried out using agar well diffusion method as described by Bauer-Kirby (1966). Mueller Hinton agar was prepared as specified by the manufacturer; 20ml of the media was autoclaved at 121°C for 15 minutes and poured aseptically into sterile petri-dish and allowed to solidify. Pure culture of isolates were inoculated in nutrient broth and incubated at 37°C for 24 hrs. Stock solution was prepared by separately dissolving 8000µg of each spice extract in 2ml dimethyl sulphoxide (DMSO) to obtain 4000µg/ml solution for methanolic and aqueous extracts of cinnamon bark and turmeric rhizomes. The double dilution procedure was prepared to obtain lower concentration of the extracts of 4000µg/ml, 2000µg/ml, 1000µg µg/ml, 500µg/ml, 250µg/ml, 125µg/ml and 62.5µg/ml respectively as described by Vashka (2009).

However, stock solution of the combined spice extracts was prepared by mixing the crude aqueous and methanolic extracts of cinnamon bark and turmeric rhizomes in different proportions (80:20; 60:40, 50:50, 40:60 and 20:80). For example, cinnamon bark crude aqueous extract of 80 ratio (0.8ml) + 20 ratio (0.2ml) turmeric rhizomes. These proportions were prepared for both 4000µg/ml and 500µg/ml extract concentrations as described by Thakur *et al.* (2012). The growth was standardized by diluting the culture with 4ml of sterile normal saline (0.8w/v) to match 0.5 McFarland turbidity standards (Cheesebrough, 2006). A loopful of standardized isolate suspension was streaked evenly on each agar plate under aseptic conditions. Agar plate was punched with a sterile Cork borer of 6mm size and 0.1ml of each spice extracts at different concentrations of 4000µg/ml, 2000µg/ml, 1000µg µg/ml, 500µg/ml, 250µg/ml, 125µg/ml and 62.5µg/ml were prepared using cropipette in each bored hole. Gentamicin served as positive control. The plates were then allowed to stand for 30mins for pre-diffusion of the extract and were incubated at 37°C for 48hrs. The antibacterial activity of the extract was determined after incubation by measuring the mean diameter zones of inhibition produced by each of the extracts against the bacterial species and the results were recorded in millimeter (mm) (CLSI, 2011).

### Determination of Minimum Inhibitory Concentration (MIC)

The spice extracts that showed significant antibacterial activity by agar well diffusion method were subjected to MIC assay by preparing a stock solution of 30,000 µg in 10ml of dimethylsulfoxide (DMSO) the serial doubling dilution using dimethyl sulphoxide to arrive at different concentration of 250 µg/ml, 500 µg/ml, 750 µg/ml, 1000 µg/ml, 1250 µg/ml and 1500 µg/ml. Equal volume of 1ml of Muller Hinton broth was prepared according to manufacturer's instruction, autoclaved and then dispensed into test tubes.

Equal volume (1ml) of serial dilution of the different extracts were added to 1ml Muller Hinton broth and 0.1ml standardized inocula of the test organism adjusted to McFarland turbidity standard was added to each test tube and incubated at 37°C for 24 hours for bacteria. Tubes containing broth and cinnamon/turmeric extract without inocula serves as negative control while the tube containing broth and inocula serves as organism control. The lowest concentration of the extract that inhibited the growth of each organism was considered as minimum inhibitory concentration (MIC) (Akinyemi *et al.*, 2005 and Khurram *et al.*, 2009).

#### **Minimum Bactericidal Concentration (MBC)**

Sterile Mueller Hinton agar plates were inoculated with samples from the MIC tubes that showed no visible bacterial growth and incubated at 37°C for 24hrs. The medium where no growth observed was taken as the MBC. It is defined as the concentration of the antimicrobial that results in a 99.9% reduction in CFU/ml compared with the organism concentration in the original inoculums (CLSI, 2015).

#### **GC/MS Analysis**

A gas chromatography from Agilent USA hyperated to a mass spectrophotometer (7890A GC system, 5675C inter MSD with triple axis detector equipped with an auto injector (10µl string) was used while Helium gas was used as a carrier gas. All chromatographic separation was performed on capillary column having specification: length: 30m, internal diameter: 0.2 µm, thickness: 250 µm, treated with phenyl methyl silox. Other GC/MS condition are ion sources (EI), 250°C, interface temperature, 300°C, pressure, 6.2 psia, out time, 1.8mm, 1µl injector in split mode with a split ratio 1:50, the injection temperature of 300°C the column temperature started at 35°C for 5mins and change to 150°C at the rate of 4°C/min, the temperature was raised to 250°C at the rate of 20°C/min and held for 5mins. The total elution was 47.5 minute. Mass Spectral Solution software provided by supplier was used to control the

system and to acquire the data; identification of the compounds was based on the comparison of their mass spectra and their retention time with standard mass spectra from National Institute of Standards and Technology (NIST) Mass Spectral Search Library Software version 2.0.

## **RESULTS**

### **Physical Properties of Plant Extracts**

Table 1 showed the physical properties of *Curcuma longa* and *Cinnamomum verum*, both methanolic and aqueous extracts revealed that methanolic *Curcuma longa* (MCL), methanolic *Cinnamomum verum* (MCV), aqueous *Curcuma longa* (ACL) and aqueous *Cinnamomum verum* (ACV) extracts had the recovery of 15.6%, 12.7%, 5.8% and 4.7% respectively. The results also showed that the textures of MCL and MCV were sticky semi-solid and sticky crisp-solid respectively while, the textures of ACL and ACV were sticky semi-solid and dry solid respectively. The current study further showed that the extracts existed between acidic and neutral pH.

### **Phytochemical Composition of the Extracts**

The result of the phytochemical constituents of the plant extracts revealed that saponins, tannins, glycoside, alkaloids, flavonoids and terpenoids were observed in the methanolic *Curcuma longa* (MCL) and aqueous *Cinnamomum verum* (ACV) extracts. From the result, flavonoid and tannins were observed to be absent in methanolic *Cinnamomum verum* (MCV) and aqueous *Curcuma longa* (ACL) extracts respectively (Table 2).

### **Antibacterial Activity of Plant Extracts against Bacterial Isolates**

The plant extracts against the bacterial isolates showed that *Curcuma longa* methanolic extract had the highest antibacterial activity at 4000µg/ml (zone of inhibition of 18.00±1.41mm) against *Klebsiella pneumoniae* followed by the antibacterial activity of *Curcuma longa* methanolic extract against *E. coli* at

4000µg/ml (zone of inhibition of 17.3±0.15mm).

The least antibacterial activities of the plant extracts against the bacterial isolates were observed at 62.5µg/ml of the extract concentrations. The result also revealed that the antibacterial activities of *Curcuma longa* methanolic and aqueous extracts against *Klebsiella pneumoniae* ranged from 6.73±0.37 to 18.0±0.12mm while, *Cinnamomum verum* methanolic and aqueous extracts' zone of inhibitions ranged between 6.0±0.00 and 14.53±0.48mm. From the result, *Curcuma longa* methanolic and aqueous extracts exerted an inhibition that ranged between 6.0±0.00 and 15.2±0.08 against *Bacillus* spp. while, *Cinnamomum verum* methanolic and aqueous extracts had a zone of inhibition that ranged from 6.0±0.00 and 13.1±0.10mm against *Bacillus* spp. However, there was a significant difference between the different concentrations at probability level of  $p < 0.05$  (Table 3).

#### **Antibacterial Activity of *Cinnamomum verum* and *Curcuma longa* extracts in combination**

Results on zones of inhibition (mm) of *Cinnamomum verum* and *Curcuma longa* methanolic and aqueous extracts against multidrug resistant *Bacillus* spp., *Klebsiella pneumoniae* and *E. coli* at various concentrations (500µg/ml and 4000µg/ml) at ratio of 80:20, 60:40, 50:50, 40:60 and 20:80. At 4000 µg/ml, methanolic extracts of *Cinnamomum verum* and *Curcuma longa* had 10mm – 13mm zone of inhibition against *Klebsiella pneumoniae* while the zone of inhibition for its aqueous counterpart ranged between 8.0mm – 13mm. From the study, the zone of inhibition of *Cinnamomum verum* and *Curcuma longa* methanolic extracts against *Bacillus* spp. ranged between 11mm – 14mm while the aqueous extracts of *Cinnamomum verum* and *Curcuma longa* had 10mm – 14mm zone of inhibition against *Bacillus* spp. were presented in Table 4. The present study revealed that *Curcuma longa* methanolic (MT) extract had the highest zone of

inhibition against the test organisms at 4000µg/ml compared to the zone of inhibitions of other extracts at the various concentrations.

#### **Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of *Cinnamomum verum* and *Curcuma longa* plant extracts**

Table 5 showed the MIC and MBC of *Cinnamomum verum* and *curcuma longa* extract against the test organisms. The MIC range of 250 to 750µg/ml of *Cinnamomum verum* and *curcuma longa* plant extracts were observed to inhibit the activity of *Klebsiella pneumoniae* at an MBC range of 1000 and 1250µg/ml. The results also showed that the MICs of aqueous *Curcuma longa* (ACL) and aqueous *Cinnamomum verum* (ACV) extracts against *Klebsiella pneumoniae* were the highest (750µg/ml) while MIC of 250µg/ml on *Klebsiella pneumoniae* was observed to be the least. Similarly, aqueous *Curcuma longa* (ACL) and aqueous *Cinnamomum verum* (ACV) plant extracts had the highest MICs at 750µg/ml respectively against *Bacillus* spp. and *Escherichia coli*.

#### **Gas Chromatography - Mass Spectrophotometry (GC-MS) Analysis of Plant Extracts**

The current study revealed the chemical characteristics of the compounds identified during GC-MS analysis of the plants methanolic and aqueous extracts (Table 6 and 7). From the results, octadecanoic acid (stearic acid), n-hexadecanoic acid (palmitic acid), hexadecanoic acid, methyl ester (methyl palmitate), octadecanedioic acid, 4-phosphorinanone, 1-methyl-, octadecanoic acid, 2,3-dihydroxypropyl ester and Cis-9-hexadecenoic acid (palmitoleic acid) were chemical compounds observed in the methanolic extract of *Cinnamomum verum* after GC-MS analysis. Similarly, *Cinnamomum verum* aqueous extract yielded cycloheptane (C<sub>7</sub>H<sub>14</sub>), 1-octadecene (C<sub>18</sub>H<sub>36</sub>), Phenol, 3, 5 bis (1,1-dimethylethyl) (C<sub>14</sub>H<sub>22</sub>O), pentane, 3-methylene (C<sub>6</sub>H<sub>12</sub>), n-hexadecanoic acid

(C<sub>16</sub>H<sub>32</sub>O<sub>2</sub>), hexadecanoic acid, methyl ester (C<sub>17</sub>H<sub>34</sub>O<sub>2</sub>) and spiroxamine-1 (C<sub>18</sub>H<sub>35</sub>NO<sub>2</sub>) (Table 6). The current result also revealed the chemical constituents, their molecular weights and formula observed in *Curcuma longa* methanolic and aqueous extracts. The chemical constituents of *Curcuma longa* methanolic extract were, curlone (218g, C<sub>15</sub>H<sub>22</sub>O), tumerone (218g, C<sub>15</sub>H<sub>22</sub>O), oleic acid (282g, C<sub>18</sub>H<sub>34</sub>O<sub>2</sub>), phenol, 2-methoxy- (124g, C<sub>7</sub>H<sub>8</sub>O<sub>2</sub>), 2-methoxy-4-vinylphenol (150g, C<sub>9</sub>H<sub>10</sub>O<sub>2</sub>) and benzene, 1-(1,5-dimethylhexyl)-4-methyl- (204g, C<sub>15</sub>H<sub>24</sub>). However, benzene, 1-fluoro-4-methoxy- (126g, C<sub>7</sub>H<sub>7</sub>FO), isoborneol (154g, C<sub>10</sub>H<sub>18</sub>O), cycloheptasiloxane, tetradecanethyl- (519g, C<sub>14</sub>H<sub>42</sub>O<sub>7</sub>Si<sub>7</sub>), Borneol, trifluoroacetate (ester) (250g, C<sub>12</sub>H<sub>17</sub>F<sub>3</sub>O<sub>2</sub>), and cyclohexene, 4-methylene-1-(1-methylethyl)- (136g, C<sub>10</sub>H<sub>16</sub>) were observed in the GC-MS data of *Curcuma longa* aqueous extract (Table 7).

## DISCUSSION

Phytochemical analyses of *Cinnamomum verum* and *Curcuma longa* extracts showing antimicrobial activity revealed the presence of different active constituents in different extracts. Both *Cinnamomum verum* and *Curcuma longa* extracts contained saponins, tannins, glycoside, alkaloids, flavonoids and terpenoids. Similar observations have been reported for *Curcuma longa* from the study of Gupta *et al.* (2015). In addition, the current findings corroborated with the work of Rajesh *et al.* (2013), Dhanalaxmi *et al.* (2014) and Ofentse *et al.* (2015) who reported the presence of same phytochemical constituents and attributed the antimicrobial activity of *Cinnamomum verum* and *Curcuma longa* to the presence of these compounds. There are reports showing that alkaloids, flavonoids, tannins, and phenolic compounds are responsible for the antibacterial activities in plants (Cordell *et al.*, 2001 and Shreya *et al.*, 2015). The extraction of phytochemical compounds from plant materials is largely dependent on the type of solvent used in the extraction

procedure, however, traditional practitioners used water as the primary solvent (Hegde *et al.*, 2012).

Various studies have shown the antimicrobial activity of *Curcuma longa* and *Cinnamomum verum* extracts against an array of pathogens (Kim *et al.*, 2005; Park *et al.*, 2005; Lopez *et al.*, 2007; Senhaji *et al.*, 2007 and Niamsa and Sittiwet, 2009). In the present study, the findings revealed that the antibacterial activities of *Curcuma longa* and *Cinnamomum verum* methanolic and aqueous extracts against *Klebsiella pneumoniae*, *Bacillus* spp. and *Escherichia coli* ranged from 6.00±0.00 to 18.0±0.12mm. However, the antimicrobial properties of *Curcuma longa* methanolic extract against the test pathogens at the various concentrations were found to be better than other extracts used in the study. Gul and Bakht (2015), in their study observed that methanolic extract of turmeric exhibited higher antibacterial against the bacterial isolates than aqueous extract. However, the zone of inhibition exhibited by the different turmeric extracts against the test organisms in their studies were slightly different (6.0 mm – 13.5 mm) from the zone of inhibition observed in the current study (6.0±0.00 mm – 18.0±0.12 mm). Chandrana *et al.* (2005) and Kim *et al.* (2005) in their separate studies reported that *Curcuma longa* (turmeric) extract inhibited the growth of *Escherichia coli*, *Bacillus subtilis* and *Staphylococcus aureus* which might be due to the presence of curcuminoid, a phenolic compound. Similarly, Negi *et al.* (1999) reported that turmerone and curlone components of turmeric possessed better antibacterial activities against a wide range of microbes including *Bacillus cereus*, *Bacillus coagulans*, *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*. The antimicrobial activities of turmeric is reported to be due to the presence of essential oil, curcumin, curcuminoids, turmeric oil, turmerol and veleric acid (Cikricki *et al.*, 2008 and Basniwal *et al.*, 2011).

Also, between the two spices turmeric had the highest activity in correspondence to the work of Rawat and Rawat (2015). The antibacterial activity of cinnamon is decreased when added to food materials containing high fat content. In addition, the spice is required in high quantity in foods to allow the inhibition of food pathogen. This is mostly unacceptable by some food consumers as high cinnamon content increases the intensity of the food flavor (Domadia *et al.*, 2007 and Vangalapati *et al.*, 2012).

The interactions (synergistic, additive, or antagonistic) that exists when constituents of various herbal medicines are combined revealed their uniqueness (Heinrich *et al.*, 2012). Synergy is an effect of a combination of substances which is greater than would be expected by adding together their separate individual contributions (Williamson, 2001). In the present study, the combination of *Cinnamomum verum* and *Curcuma longa* at various ratios (i.e., 80:20; 60:40, 50:50, 40:60 and 20:80) had an antagonistic effect on the antibacterial activity of the extracts compared to the activities observed when the spices acted individually. These findings concurred with the study of Mohadeseh *et al.* (2012) who observed an antagonistic effect when spices were combined at 1:1. This might be due to some components in cinnamon bark and turmeric rhizomes which are antagonist in nature and might neutralize each other and weaken their anti-microbial activity. Also, Ebi and Ofoefule (1997) reported that crude plant extracts may contain inactive substances which may antagonize the antimicrobial actions of one another.

Fourteen volatile and semivolatile compounds in the *Cinnamomum verum* methanolic and aqueous extracts were identified based on the comparison of their GC relative retention time and mass spectra with those of National Institute of Standards and Technology (NIST) Mass Spectral Search Library Software version 2.0. The antimicrobial compounds identified include

octadecanoic acid, n-Hexadecanoic acid, 2,3-dihydroxypropyl ester, cycloheptane (Table 6). In a previous study on *Cinnamomum verum*, twenty-six compounds were identified as constituents of the plant bark (Vazirian *et al.*, 2015). Similarly, eleven chemical compounds were identified in the *Curcuma longa* extracts and these include curlone, tumerone, benzene, 1-fluoro-4-methoxy-, Isoborneol, cycloheptasiloxane, and borneol. The GC-MS data revealed that *Cinnamomum verum* extracts constituted more of phenols, cycloalkane, saturated and mono-unsaturated fatty acids. Phenol containing plants have been known to possess excellent antioxidants properties (Jiang, 2019). Stearic acid has wide applications in ethnomedicine, cosmetics, food, beverages, preservation, fragrance, and pharmaceutical industries (Beare-Rogers *et al.*, 2001). Similarly, palmitic acids have been utilized in the prevention of stroke and anti-inflammatory diseases; management of obesity and serves as natural additives in food (Seidell, 1952 and Abraham *et al.*, 1989). From the current findings, *Curcuma longa* extracts were observed to be dominated by fatty acid, aromatic and phenolic compounds. The bioactive compound curlone, indicated several positive biological properties, such as antioxidant, antiviral, antibacterial, antifungal, insecticidal, and anticancer activities (Naz *et al.*, 2010; Khajehdehi, 2012; and Sachan *et al.*, 2016). Isoborneol is commonly used as soothing stomachache, skin healing, food additive, flavouring agent, and a natural insect repellent (Ammon and Wahl, 1991).

According to Singh *et al.* (2016), plants that are rich in bioactive molecules (e.g., polyphenols, carotenoids, and flavonoids) possess therapeutic purposes in delaying the onset of some diseases like cardiovascular disorders, diabetes, and cancer. Therefore, the presence of high concentration of these chemical compounds in the extracts is a possible phytochemical characteristic feature among *Curcuma longa* and *Cinnamomum verum*, pointing to the potential of these

plant species as promising sources of these antimicrobial metabolites against human pathogens. The variation in the chemical composition of the *Curcuma longa* and

*Cinnamomum verum* extracts provided evidence that ecological conditions for growth greatly affect the bioactive properties and functions of the medicinal plants.

**Table 1: Physical properties and percentage yield of the extracts**

| Plant extract | Physical Appearance |                    | pH  | weight of plant material (g) | Weight yield (g) | (%) Yield (w/w) |
|---------------|---------------------|--------------------|-----|------------------------------|------------------|-----------------|
|               | Colour              | Texture            |     |                              |                  |                 |
| MCL           | Dark-orange, brown  | Sticky semi-solid  | 7   | 100                          | 15.6             | 15.6            |
| MCV           | Dark-coffee brown   | Sticky crisp-solid | 5.1 | 100                          | 12.7             | 12.7            |
| ACL           | Dark brown          | Sticky semi-solid  | 7   | 100                          | 5.8              | 5.8             |
| ACV           | Coffee brown        | Dry solid          | 5.1 | 100                          | 4.7              | 4.7             |

Key: MCL = Methanolic *Curcuma longa*, MCV = Methanolic *Cinnamomum verum*  
 ACL = Aqueous *Curcuma longa*, ACV = Aqueous *Cinnamomum verum*

**Table 2: Phytochemical Constituents of the Extracts**

| Extracts | Saponins | Tannins | Glycosides | Alkaloids | Flavonoid | Terpenoids |
|----------|----------|---------|------------|-----------|-----------|------------|
| MCL      | +        | +       | +          | +         | +         | +          |
| MCV      | +        | +       | +          | +         | —         | +          |
| ACL      | +        | —       | +          | +         | +         | +          |
| ACV      | +        | +       | +          | +         | +         | +          |

**Keys:** — = Negative, + = Positive,

MCL = Methanolic *Curcuma longa*, MCV = Methanolic *Cinnamomum verum*

ACL = Aqueous *Curcuma longa*, ACV = Aqueous *Cinnamomum verum*

**Table 3: Antibacterial activities of *Cinnamomum veruma* and *Curcuma longa* extracts (µg/ml) against Bacterial Isolates**

| MDR<br>Organism/extract            | 4000                    | 2000                    | 1000                    | 500                     | 250                     | 125                    | 62.5                   | Control              |
|------------------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|------------------------|------------------------|----------------------|
| <b><i>Klebsiella pneumonia</i></b> |                         |                         |                         |                         |                         |                        |                        |                      |
| MCL                                | 18.00±0.12 <sup>a</sup> | 15.70±0.56 <sup>a</sup> | 13.16±0.73 <sup>a</sup> | 12.26±0.15 <sup>a</sup> | 10.43±0.26 <sup>a</sup> | 9.23±0.28 <sup>a</sup> | 6.73±0.37 <sup>a</sup> | 27±0.00 <sup>a</sup> |
| MCV                                | 10.87±0.59 <sup>a</sup> | 9.87±0.59 <sup>a</sup>  | 8.97±0.59 <sup>a</sup>  | 8.30±0.33 <sup>a</sup>  | 7.63±0.32 <sup>a</sup>  | 7.00±0.00 <sup>a</sup> | 6.00±0.00 <sup>a</sup> | 27±0.00 <sup>a</sup> |
| ACL                                | 14.93±0.35 <sup>a</sup> | 13.20±0.50 <sup>a</sup> | 11.37±0.58 <sup>a</sup> | 10.20±0.70 <sup>a</sup> | 8.93±0.64 <sup>a</sup>  | 8.37±0.54 <sup>a</sup> | 7.47±0.32 <sup>a</sup> | 27±0.00 <sup>a</sup> |
| ACV                                | 14.53±0.48 <sup>a</sup> | 12.70±0.62 <sup>a</sup> | 12.30±0.65 <sup>a</sup> | 10.70±0.33 <sup>a</sup> | 9.70±0.35 <sup>a</sup>  | 8.70±0.37 <sup>a</sup> | 7.70±0.39 <sup>a</sup> | 27±0.00 <sup>a</sup> |
| <b><i>Bacillus spp.</i></b>        |                         |                         |                         |                         |                         |                        |                        |                      |
| MCL                                | 15.20±0.08 <sup>a</sup> | 13.10±0.06 <sup>a</sup> | 11.80±0.44 <sup>a</sup> | 9.90±0.03 <sup>a</sup>  | 7.70±0.88 <sup>a</sup>  | 6.00±0.00 <sup>a</sup> | 6.00±0.00 <sup>a</sup> | 25±0.00 <sup>a</sup> |
| MCV                                | 13.10±0.10 <sup>a</sup> | 12.10±0.06 <sup>a</sup> | 11.27±0.13 <sup>a</sup> | 9.80±0.12 <sup>a</sup>  | 8.80±0.15 <sup>a</sup>  | 6.00±0.00 <sup>a</sup> | 6.00±0.00 <sup>a</sup> | 25±0.00 <sup>a</sup> |
| ACL                                | 10.10±0.07 <sup>a</sup> | 9.10±0.13 <sup>a</sup>  | 8.20±0.17 <sup>a</sup>  | 7.20±0.20 <sup>a</sup>  | 6.00±0.00 <sup>a</sup>  | 6.00±0.00 <sup>a</sup> | 6.00±0.00 <sup>a</sup> | 25±0.00 <sup>a</sup> |
| ACV                                | 14.30±0.33 <sup>a</sup> | 13.00±0.00 <sup>a</sup> | 11.70±0.33 <sup>a</sup> | 10.70±0.33 <sup>a</sup> | 9.70±0.33 <sup>a</sup>  | 6.00±0.00 <sup>a</sup> | 6.00±0.00 <sup>a</sup> | 25±0.00 <sup>a</sup> |
| <b><i>Escherichia coli</i></b>     |                         |                         |                         |                         |                         |                        |                        |                      |
| MCL                                | 17.30±0.15 <sup>a</sup> | 15.00±0.29 <sup>a</sup> | 12.80±0.17 <sup>a</sup> | 9.00±0.00 <sup>a</sup>  | 7.00±0.58 <sup>a</sup>  | 6.00±0.00 <sup>a</sup> | 6.00±0.00 <sup>a</sup> | 21±0.00 <sup>a</sup> |
| MCV                                | 14.80±0.17 <sup>a</sup> | 13.20±0.15 <sup>a</sup> | 11.10±0.10 <sup>a</sup> | 9.00±0.00 <sup>a</sup>  | 6.70±0.33 <sup>a</sup>  | 6.00±0.00 <sup>a</sup> | 6.00±0.00 <sup>a</sup> | 21±0.00 <sup>a</sup> |
| ACL                                | 15.00±0.00 <sup>a</sup> | 13.30±0.15 <sup>a</sup> | 11.90±0.26 <sup>a</sup> | 9.00±0.00 <sup>a</sup>  | 7.70±0.33 <sup>a</sup>  | 7.00±0.00 <sup>a</sup> | 6.00±0.00 <sup>a</sup> | 21±0.00 <sup>a</sup> |
| ACV                                | 11.80±0.17 <sup>a</sup> | 10.80±0.17 <sup>a</sup> | 10.00±0.00 <sup>a</sup> | 9.00±0.00 <sup>a</sup>  | 7.70±0.33 <sup>a</sup>  | 7.00±0.00 <sup>a</sup> | 6.00±0.00 <sup>a</sup> | 21±0.00 <sup>a</sup> |

**Keys:** MCL = Methanolic *Curcuma longa* extract, MCV = Methanolic *Cinnamomum verum* extract, ACL = Aqueous *Curcuma longa* extract, ACV = Aqueous *Cinnamomum verum* extract.

Mean values with the same superscript in the same column and row are significantly different at (p<0.05) level of significance.

**Table 4: Actibacterial Activity of *Cinnamomum verum* and *Curcuma longa* extracts against multidrug resistant bacterial isolates**

| Extracts<br>Concentrations | <i>Klebsiella pneumoniae</i> |          | <i>Bacillus spp</i> |          | <i>Escherichia coli</i> |          |
|----------------------------|------------------------------|----------|---------------------|----------|-------------------------|----------|
|                            | 4000µg/ml                    | 500µg/ml | 4000µg/ml           | 500µg/ml | 4000µg/ml               | 500µg/ml |
| MC                         | 11.0                         | 12.0     | 13.0                | 10.0     | 15.0                    | 09.0     |
| MT                         | 18.0                         | 12.0     | 15.0                | 10.0     | 17.0                    | 09.0     |
| MC:MT (80:20)              | 10.0                         | 12.0     | 11.0                | 12.0     | 14.0                    | 12.0     |
| MC:MT (60:40)              | 12.0                         | 11.0     | 12.0                | 11.0     | 12.0                    | 11.0     |
| MC:MT (50:50)              | 13.0                         | 10.0     | 12.5                | 14.0     | 15.0                    | 13.0     |
| MT:MC (80:20)              | 11.0                         | 11.0     | 14.0                | 12.0     | 13.0                    | 11.0     |
| MT:MC (60:40)              | 10.0                         | 09.0     | 13.0                | 10.0     | 11.0                    | 12.0     |
| AC                         | 14.0                         | 11.0     | 14.0                | 10.0     | 12.0                    | 09.0     |
| AT                         | 15.0                         | 10.0     | 10.0                | 07.0     | 15.0                    | 09.0     |
| AC:AT (80:20)              | 10.0                         | 12.0     | 14.0                | 12.0     | 10.0                    | 11.0     |
| AC:AT (60:40)              | 11.0                         | 11.0     | 15.0                | 10.0     | 11.0                    | 09.0     |
| AC:AT (50:50)              | 13.0                         | 09.0     | 16.0                | 14.0     | 13.0                    | 12.0     |
| AT:AC (80:20)              | 08.0                         | 11.0     | 12.0                | 10.0     | 08.0                    | 11.0     |
| AT:AC (60:40)              | 10.0                         | 13.0     | 15.0                | 11.0     | 10.0                    | 13.0     |

**Keys:** MC= *Cinnamomum verum* methanolic extract; MT= *Curcuma longa* methanolic extract; AC= *Cinnamomum verum* aqueous extract and AT= *Curcuma longa* aqueous extract.

**Table 5: MIC and MBC of *Cinnamomum verum* and *curcuma longa* extracts on bacterial isolates**

| Extract/organism             | MIC(µg/ml) | MBC (µg/ml) |
|------------------------------|------------|-------------|
| <i>Klebsiella pneumoniae</i> |            |             |
| MCL                          | 250        | 1000        |
| MCV                          | 500        | 1000        |
| ACL                          | 750        | 1000        |
| ACV                          | 750        | 1250        |
| <i>Bacillus spp.</i>         |            |             |
| MCL                          | 500        | 1000        |
| MCV                          | 250        | 1000        |
| ACL                          | 750        | 1000        |
| ACV                          | 750        | 1000        |
| <i>Escherichia coli</i>      |            |             |
| MCL                          | 500        | 1000        |
| MCV                          | 250        | 1000        |
| ACL                          | 750        | 1250        |
| ACV                          | 750        | 1250        |

**Keys:** MCL = Methanolic *Curcuma longa* extract, MCV = Methanolic *Cinnamomum verum* extract, ACL = Aqueous *Curcuma longa* extract, ACV = Aqueous *Cinnamomum verum* extract.

**Table 6: GC-MS data of *Cinnamomum verum* extracts**

| S/ No                                            | Molecular weight (g) | Molecular formula                               | IUPAC Name                                         | RT (MINS) | AREA (%) |
|--------------------------------------------------|----------------------|-------------------------------------------------|----------------------------------------------------|-----------|----------|
| <b><i>Cinnamomumverum</i> methanolic extract</b> |                      |                                                 |                                                    |           |          |
| 1.                                               | 284                  | C <sub>18</sub> H <sub>36</sub> O <sub>2</sub>  | Octadecanoic acid (stearic acid)                   | 38.709    | 1.471    |
| 2.                                               | 256                  | C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>  | n-Hexadecanoic acid (Palmitic acid)                | 37.408    | 25.518   |
| 3.                                               | 270                  | C <sub>17</sub> H <sub>34</sub> O <sub>2</sub>  | Hexadecanoic acid, methyl ester (methyl palmitate) | 37.001    | 1.094    |
| 4.                                               | 314                  | C <sub>18</sub> H <sub>34</sub> O <sub>4</sub>  | Octadecanedioic acid                               | 39.141    | 1.417    |
| 5.                                               | 130                  | C <sub>6</sub> H <sub>11</sub> OP               | 4-Phosphorinanone,1-methyl-                        | 40.285    | 4.073    |
| 6.                                               | 254                  | C <sub>16</sub> H <sub>30</sub> O <sub>2</sub>  | Cis-9- hexadecenoic acid (Palmitoleic acid)        | 38.578    | 7.348    |
| 7.                                               | 358                  | C <sub>21</sub> H <sub>42</sub> O <sub>4</sub>  | Octadecanoic acid,2,3dihydroxypropyl ester         | 42.106    | 12.802   |
| <b><i>Cinnamomumverum</i> aqueous extract</b>    |                      |                                                 |                                                    |           |          |
| 8.                                               | 98                   | C <sub>7</sub> H <sub>14</sub>                  | Cycloheptane                                       | 26.255    | 0.680    |
| 9.                                               | 252                  | C <sub>18</sub> H <sub>36</sub>                 | 1-Octadecene                                       | 26.705    | 0.452    |
| 10.                                              | 206                  | C <sub>14</sub> H <sub>22</sub> O               | Phenol,3,5bis(1,1 dimethylethyl)                   | 27.450    | 0.639    |
| 11.                                              | 84                   | C <sub>6</sub> H <sub>12</sub>                  | Pentane,3-methylene                                | 26.073    | 0.500    |
| 12.                                              | 256                  | C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>  | n-Hexadecanoic acid (Palmitic acid)                | 37.402    | 13.736   |
| 13.                                              | 270                  | C <sub>17</sub> H <sub>34</sub> O <sub>2</sub>  | Hexadecanoic acid, methyl ester (methyl palmitate) | 37.001    | 5.709    |
| 14.                                              | 297                  | C <sub>18</sub> H <sub>35</sub> NO <sub>2</sub> | SPIROXAMINE-1                                      | 39.047    | 1.667    |

**Table 7: GC-MS data of *Curcuma longa* plant extracts**

| S/ No                                          | Molecular weight (g) | Molecular formula                                              | IUPAC Name                                                  | RT (MINS) | AREA (%) |
|------------------------------------------------|----------------------|----------------------------------------------------------------|-------------------------------------------------------------|-----------|----------|
| <b><i>Curcuma longa</i> methanolic extract</b> |                      |                                                                |                                                             |           |          |
| 1.                                             | 218                  | C <sub>15</sub> H <sub>22</sub> O                              | Curlone                                                     | 18.636    | 22.873   |
| 2.                                             | 218                  | C <sub>15</sub> H <sub>22</sub> O                              | Tumerone                                                    | 18.067    | 55.275   |
| 3.                                             | 282                  | C <sub>18</sub> H <sub>34</sub> O <sub>2</sub>                 | Oleic acid                                                  | 25.948    | 2.212    |
| 4.                                             | 124                  | C <sub>7</sub> H <sub>8</sub> O <sub>2</sub>                   | Phenol,2-methoxy- (Guaiacol)                                | 6.019     | 3.196    |
| 5.                                             | 150                  | C <sub>9</sub> H <sub>10</sub> O <sub>2</sub>                  | 2-Methoxy-4-vinylphenol                                     | 11.055    | 3.023    |
| 6.                                             | 204                  | C <sub>15</sub> H <sub>24</sub>                                | Benzene,1-(1,5-dimethylhexyl)-4-methyl-                     | 16.828    | 2.730    |
| <b><i>Curcuma longa</i> aqueous extract</b>    |                      |                                                                |                                                             |           |          |
| 7.                                             | 126                  | C <sub>7</sub> H <sub>7</sub> FO                               | Benzene, 1-fluoro-4-methoxy-                                | 11.192    | 2.436    |
| 8.                                             | 154                  | C <sub>10</sub> H <sub>18</sub> O                              | Isoborneol                                                  | 14.764    | 6.608    |
| 9.                                             | 519                  | C <sub>14</sub> H <sub>42</sub> O <sub>7</sub> Si <sub>7</sub> | Cycloheptasiloxane, tetradecanethyl-                        | 17.310    | 3.061    |
| 10.                                            | 250                  | C <sub>12</sub> H <sub>17</sub> F <sub>3</sub> O <sub>2</sub>  | Borneol, trifluoroacetate(ester)                            | 17.873    | 23.513   |
| 11.                                            | 136                  | C <sub>10</sub> H <sub>16</sub>                                | Cyclohexene,4-methylene-1-(1-methylethyl)- (beta=terpinene) | 14.557    | 2.459    |

**CONCLUSION**

The phytochemical analyses of both extracts showed the presence of saponins, tannins, glycoside, alkaloids, flavonoids and terpenoids which were confirmed by GC-MS data. The extracts of *Curcuma longa* and *Cinnamomum verum* showed antimicrobial

activity against the MDR organisms. The *Curcuma longa* methanolic extract showed higher antibacterial activity against *Klebsiella pneumoniae*, *Bacillus* spp. and *Escherichia coli* in comparison to other extracts.

Thus, from the results obtained in the present study, it can be concluded that *Curcuma longa* and *Cinnamomum verum* extracts can prove to be effective antimicrobial agents against multidrug resistant pathogens. Hence, future studies

are directed towards the development of purified bioactive compounds and quantitative determination of safe concentrations that can be used to improve existing drugs or to create new agents against MDR bacteria.

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