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## Phytochemical Screening and Antimicrobial Activity of Ethanol and Methanol Extracts of *Lantana camara* Leaf

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### ABSTRACT

This study investigated the phytochemical composition and antimicrobial activities of ethanol and methanol leaf extracts of *Lantana camara* Linn against some clinical pathogens. The ethanol and methanol extracts were obtained by soaking each of the powdered leaf in each solvent. The soaked powdered leaf was allowed to stand for four days at room temperature and later filtered using Whatman filter paper. The filtrate was further concentrated using rotary evaporator and then freeze-dried. The minimum inhibitory concentration (MIC) of the ethanol and methanol leaf extracts was carried out using agar well diffusion method. The phytochemical analysis was done using standard techniques. Data were analysed using Analysis of Variance (ANOVA) to test for significance. Means were separated using Duncan's Multiple Range Test (DMRT). The results of the antimicrobial activity revealed that *V. cholerae* was the most susceptible while *E. coli* was the most resistant to plant extracts. The phytochemicals present in the plant leaf had antimicrobial properties and may serve as a good substitute for resistant human pathogens.

**Keywords:** Phytochemical, Antimicrobial, Activity, Ethanol, Methanol, *Lantana camara*, leaf

## 1. INTRODUCTION

*Lantana camara* Linn, belongs to family Verbenaceae, commonly known as wild or red sage, is the most widespread species of this genus and regarded both as a notorious weed and a popular or ornamental garden plant (Ganjewala *et al.*, 2009).

*L. camara* leaves have been reported to make animals ill after ingestion and its berries are toxic before they become ripe (Wolfson and Solomon, 1964; Mc Lennan and Amos, 1989; Motion, 1994). *L. camara* oil and extracts are used in herbal medicine for the treatment of various human diseases such as skin itches, leprosy, cancers, chicken pox, measles, asthma, ulcers, tumors, high blood pressure, tatanus, rheumatism etc. (Begun *et al.*, 1995; Ghisberti, 2000; Ross, 1999).

Extracts from the leaves have been reported to have antimicrobial, fungicidal, insecticidal and nematocidal activity (Saksena and Tripathi, 1985; Begun *et al.*, 1995; Sharma *et al.*, 1999; Deena and Thoppil, 2000). The essential oil of *L. camara* from different regions of the world has been reported by many workers (Da Silva *et al.*, 1999; Sefidkon, 2002; Kasali *et al.*, 2004). The oils differ in their chemical compositions according to geographic origin of the plants. Da Silva *et al.* (1999) reported differences in essential oil composition of *L. camara* collected at different places in the Amazon region of North Brazil. The essential oil composition of *L. camara* from Nigeria has been previously reported (Kasali *et al.*, 2004). *L. camara* essential oil containing B-caryophyllene, geranyl acetate, terpinyl acetate, bornyl acetate and limonene remarkably inhibited the growth of many tested bacteria and fungi. *P. aeruginosa*, *A. niger*, *F. solani* and *C. albicans* appeared as the most sensitive ones (Deena and Thoppil, 2000).

A tea prepared from the leaves and flowers is taken against fever, influenza and stomach ache (Ghisberti, 2000). *L. camara* flowers extract from coconut oil provides protection against *Aedes* mosquitoes (Kumar and Maneemegalai, 2008). The aim of this study was to investigate the phytochemical composition and antimicrobial activities of ethanol and methanol extracts of *Lantana camara* leaf.

## 2. MATERIALS AND METHODS

### Collection of Sample

The plant leaves used in this study were collected from the wild at Nnamdi Azikiwe University, Awka, Anambra State. The analysis was carried out at Central Service Laboratory of National Root Crops Research Institute, Umudike, Abia State, Nigeria. The botanical identity of the plant was authenticated by the Horticulture Unit of the institute.

### Preparation of Sample for Analysis

The fresh plant leaves were washed with clean water and oven dried at a temperature of 60 °C for 72 hours. The sample was ground into fine powder using grinding machine and stored in an air-tight container for analysis.

## **Extraction of Plant Materials**

### **Ethanol and Methanol Extractions**

The aqueous decoction of the plant was prepared by soaking 500g each of the powdered sample of leaves in 2.5L of ethanol and methanol respectively. The whole setup were left at room temperature for 72 hours. The extracts were then concentrated using a rotary evaporator and the solvents were allowed to evaporate. The concentrated extracts were stored in air-tight container in a cold store at a regulated temperature of 20 °C until they were required for analyses (Ezeabara and Chukwudi, 2019)

### **Qualitative Determination of Phytochemicals in *L. camara* Leaf**

Qualitative tests were conducted to evaluate the presence or absence of phytochemical of interest. It was conducted using standard methods described below. The extracts were screened for the following phytochemical compounds: alkaloids, saponins, flavonoids, tannins and terpenoids (Aborode, 2020; Chinyere, 2021; Paula-Peace, 2021).

#### **Alkaloid Determination**

The presence of alkaloid in the samples were investigated using Meyer's colourimetric method described by Harborne (1973). Ethanolic extract of the samples were obtained by shaking 2g of the samples in 20 ml of ethanol for 30 mins before filtrating over the funnel using Whatman filter paper in 100 ml beaker. 2.5 ml of each filtrate was taken and poured in the test tube labelled A, B, C and D, placed in a test tube rack. Few drops of Meyer's reagent were added to each of the test tube respectively. Formation of orange precipitate/colour shows the presence of alkaloid.

#### **Saponin Determination**

The froth test and emulsion test described by Harborne (1973) were used to determine the presence of saponin. 5 ml of distilled water was used to dissolve 1g of powdered samples in 250 ml conical flask. Each of them was shaken and placed in water bath for 5mins. They were filtered hot over the funnel using Whatman filter paper in 100 ml beaker. 2.5 ml of each cooled filtrate was poured into the test tube labelled A, B, C and D, and placed in a test tube rack. 10ml of distilled water was used to dilute each of the tube respectively.

#### **Froth Test**

Each of the flask was shaken vigorously for few minutes and observed. A stable froth (foam) upon standing indicates the presence of saponin.

#### **Emulsion Test**

Two drops of olive oil was added to the four test tubes respectively and shaken vigorously. The formation of emulsion indicates the presence of saponin.

#### **Tannin Determination**

The presence of tannins was determined using the Harborne (1973) method. 1g of powdered samples were boiled with 5ml of distilled water in a water bath for 5 minutes. They

were filtered hot with Whatman filter paper folded over a funnel in 100 ml beakers. Four test tubes labelled A, B, C and D, was positioned in a test tube rack. 1ml of the cooled filtrates was added to each test tube accordingly. 10 ml of ferric chloride was added to each of the test tube and observed. A greenish brown precipitate was observed which indicates the presence of tannin.

### **Flavonoid Determination**

The presence of flavonoid in the samples was determined using the Harborne (1973), Sofowora (1993) methods. 1g of powdered samples were dissolved with 10 ml of distilled water in 250 ml conical flask, shaken and placed in water bath for 5 mins. They were filtered hot using Whatman filter paper folded over the funnel in 100 ml beaker. The filtrates were allowed to cool. Two drops of 20% NaOH was added to 1ml of each of the filtrates in a test tubes labelled A, B, C and D. A yellow amber colour was observed in tubes A and D, light yellow in B and C. To each of the test tube was also added two drops of one normal sulphuric acid and observed. No colour change was observed in all the samples after addition of two drops of sulphuric acid. It implied that flavonoid is present in the plant due to formation of colourless solution.

### **Terpenoid Determination**

5 ml of aqueous extracts from each of the samples was poured into the test tube labelled A, B, C and D. 2 ml of chloroform was added to each of the test tube. 1ml of concentrated sulphuric acid was also added to each of the flask to form a layer. A reddish brown precipitate at the interface indicates the presence of terpenoid.

### **Quantitative Determination of Phytochemicals in *L. camara* Leaf**

#### **Determination of Alkaloid**

2g of each sample was analysed in accordance with the alkaline precipitation gravimetric method (Harborne, 1973). The weighed samples were soaked in 100 ml of 10% acetic acid solution in ethanol and allowed to stand for 4 hours at room temperature before filtering using Whatman filter paper. The filtrates were reduced to a quarter of their original volume by evaporation over a steam bath. Alkaloids in the extracts were precipitated by drop wise addition of concentrated NH<sub>4</sub>OH solution until full turbidity was obtained. The precipitate was recovered by filtration using weighed filter papers and then washed with 1% NH<sub>4</sub>OH solution, dried in the oven at 100 °C for an hour. They were cooled in desiccator and reweighed. By difference, the weight of alkaloids present in the samples were determined and expressed as percentage for the samples and analysed using the formula:

$$\% \text{ Alkaloid} = \frac{W_2 - W_1 \times 100}{\text{Weight of sample}}$$

where:

W1 = weight of empty filter paper

W2 = weight of paper + alkaloid precipitate

### **Determination of Saponin**

Saponin content of the samples were determined by double solvent extraction gravimetric method (Harborne, 1973). 2g of the powdered samples were mixed with 50 ml of 20% aqueous ethanol solution. The mixtures were heated with periodic agitation in water bath for 30mins at 55 °C. They were filtered, the residues were extracted with 50 ml of ethanol and both extracts were put together. The combined extracts were reduced to about 40 ml at 90 °C and transferred to a separating funnel where 40ml of diethyl ether was added and shaken vigorously. Separation was by partition during which the ether layer was discarded and the aqueous layer reserved. Reaction was carried out until the aqueous layer became clear. The saponins were extracted with 60 ml of normal butanol. The combined extracts were washed with 5% aqueous NaCl solution and evaporated to dryness in a pre-weighed evaporating dish. They were dried at 60 °C in the oven and reweighed. The saponin content was calculated as percentage of original sample as:

$$\% \text{ Saponin} = \frac{W2-W1 \times 100}{\text{Weight of sample}}$$

where:

W1 = weight of evaporating dish

W2 = weight of dish + sample

### **Determination of Tannin**

The Follins – Dennis spectrophotometric (Pearson, 1976) was used. 2g of the powdered samples were dispensed into 50 ml of distilled water in a conical flask and shaken for 30 mins in a shaker. The mixtures were filtered. 5 ml of the filtrates were measured into 50 ml volumetric flask and then diluted with 35 ml of distilled water. Also, 5 ml of standard tannic acid solution and 5ml of distilled water were measured with separate flasks to serve as standard and blank respectively. They were diluted with 35 ml of distilled water separately. 1ml of Follins – Dennis Reagent was added to each of the flask followed by 2.5 ml of saturated sodium carbonate solution. The content of each flask was filled to mark level with distilled water and incubated for 90 mins at room temperature. The absorbance of the developed colour was measured at 76 nm wavelength with the reagent blank at zero. The tannin content was calculated as shown below:

$$\% \text{ Tannin} = \frac{100}{w} \times \frac{AU}{As} \times \frac{VF}{VA} \times D$$

where:

W = weight of the sample analysed

AU = absorbance of test sample

AS = concentration of standard in mg/ml

C = total volume of extract

VF = volume of filtrate analysed

D = dilution factor (where applicable)

### Determination of Flavonoid

Flavonoids determination was done using Bohamand Kocipai method. 10g of the plant samples were extracted repeatedly with 100 ml of 80% aqueous methanol in conical flask at room temperature. The whole solution was filtered using a weighed Whatman filter paper. The filtrates were transferred into crucible and dryness in the oven, cooled in the desiccator and weighed. The percentage flavonoid was expressed as the weight of sample analysed using the formula:

$$\% \text{ Flavonoid} = \frac{W2 - W1 \times 100}{\text{Weight of sample}}$$

where:

W1 = weight of empty filter paper

W2 = weight of filter paper + flavonoid precipitate

### Microbial Analysis

Using a wire loop, colonies of the test organisms (*Escherichia coli*, *Salmonella typhi*, *Vibrio cholerae*, *Micrococcus luteus*, *Candida albicans*, *Microsporium gypseum* and *Aspergillus flavus*) were collected from the Department of Microbiology, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria.

### Antimicrobial Sensitivity Test of Crude Extracts

#### Preparation of Stock Solution

Stock solutions of the ethanol and methanol extracts of *L. camara* leaves were prepared by weighing 2.0g of each extract using electronic weighing machine. This was then dissolved completely in 2 ml of dimethyl sulphoxide (DMSO) in a sterile test tube, giving stock solution concentration of 1000 mg/ml per extract. The stock solution was then labelled and stored at room temperature until needed for use (Ezeabara and Chukwwudi, 2019).

#### Determination of Inhibitory Activity

The inhibitory activity of the ethanol and methanol extracts of the leaves of *L. camara* was determined using the disc diffusion method in 5 mm discs. The antibiotics and dimethyl sulphoxide served as positive and negative controls respectively. The concentrations used were as follows: 1000 mg/ml of the ethanol and methanol extracts, 100 mg/ml of antibiotics (Streptomycin) and 1000 mg/ml of DMSO. After the discs had absorbed the leaf extracts, antibiotics and DMSO, the discs were removed, dried and placed on media on which the test microorganisms were freshly inoculated, then incubated at 37 °C for 24 hours for bacteria and 25 °C for 72 hours for fungi. Antimicrobial activity was determined after 24 hours of incubation for bacteria and 72 hours of incubation for fungi by measuring the zone of inhibition around each paper disc in millimetres (mm) (Reynolds, 2003).

#### Determination of Minimum Inhibitory Concentration

The minimum inhibitory concentrations (MIC) of the absolute (stock) concentrations were determined using the agar well diffusion method; five sterile plates were prepared and

nutrient broth was poured into each of the plates and then allowed to dry. Using standardized inocula ( $10^6$  cfu/ml), a loop full of the different test organisms was streaked onto each of the five plates when dry. Then, 10 holes were dug using an agar borer for the varying concentrations of ethanol and methanol extracts of leaves of *L. camara*. A ruler was used to separate the holes for each of the extracts. Varying concentrations of the ethanol and methanol extracts were made by serial dilution using DMSO as the diluent. The absolute/stock concentrations of the extracts used were 1000 mg/ml (equivalent to 100%). Five test tubes per extract were prepared in a test tube rack. The dilutions were as follows: 50% (500 mg/ml), 25% (250 mg/ml), 12.5% (125 mg/ml) and 6.25% (62.5 mg/ml) in each of the test tubes. A measured 50 UL volume of each dilution was added aseptically into the holes seeded with the test organisms in the nutrient agar plate using a syringe and placed in an incubator at 35 °C for 24 hours. Minimum inhibitory concentration was considered as the lowest concentration of ethanol and methanol extracts showing a clear zone of inhibition (Thongson *et al.*, 2004).

### Determination of Minimum Bactericidal/Fungicidal Concentration

The plates with the minimum inhibitory concentration were incubated for a further 24 hours at 35 °C to test which organism would grow on the zones of inhibition. Those plates on which organisms were completely killed after 24 hours, and clear zones remained, were referred to as bactericidal for bacteria and fungicidal for fungi (Espinel-Ingroff, 2002).

### Statistical Analysis

All data were analysed with Analysis of Variance (ANOVA) to test for significance. Means were separated using Duncan's New Multiple Range Test (DNMRT) at 5% level of probability using Statistical Analysis Software (SAS) package (SAS 1999).

## 3. RESULTS

### Qualitative Determination of Phytochemical Composition of Ethanol and Methanol Extracts in *L. camara* Leaf

The results of the qualitative phytochemical analysis of the ethanol and methanol leaf extracts of *L. camara* showed the presence of alkaloids, saponins, flavonoids, tannins and terpenoids. (Table 1).

**Table 1.** Qualitative phytochemical composition of ethanol and methanol leaf extracts of *L. camara*.

Phytochemicals	Methanol	Ethanol
Alkaloids	+	+
Flavonoids	+	+
Saponins	+	+

Tannins	+	+
Terpenoids	+	+

Key: + = Presence

### Quantitative Determination of Phytochemicals in *L. camara* Leaf Extracts

The results of the quantitative phytochemical analysis of the leaf extracts of *L. camara* revealed that ethanol extract had the highest composition of saponin ( $10.75 \pm 3.21$ ) while the lowest was alkaloid ( $0.08 \pm 0.12$ ). The methanol extract showed the highest composition of saponin ( $8.63 \pm 2.38$ ) while the lowest was tannin ( $0.10 \pm 0.01$ ) (Table 2).

**Table 2.** Quantitative phytochemical composition of ethanol and methanol leaf extracts of *L. camara*.

Plant Extracts	Alkaloids	Saponins	Flavonoids	Tannins	Terpenoids
Ethanol	$0.08 \pm 0.12^a$	$10.75 \pm 3.21^b$	$0.56 \pm 0.78^b$	$0.25 \pm 0.06^b$	$0.85 \pm 0.08^a$
Methanol	$0.21 \pm 0.15^b$	$8.63 \pm 2.38^a$	$0.35 \pm 0.44^a$	$0.10 \pm 0.01^a$	$0.85 \pm 0.06^b$

Results are in Mean  $\pm$  Standard Deviation. Means with the same letter in a column are not significantly different ( $p < 0.05$ )

### Antimicrobial Sensitivity Test of Crude Extracts

The results of the inhibitory activity of ethanol and methanol leaf extracts of *L. camara* against the test organisms at 62.5 mg/ml concentration revealed that ethanol extract showed highest inhibition of *V. cholerae* ( $17.14 \pm 0.06$ ) while the lowest inhibition was observed on *E. coli* ( $12.08 \pm 0.22$ ). The methanol extract showed highest inhibition of *V. cholerae* ( $13.19 \pm 0.06$ ) and lowest inhibition of *M. luteus* ( $9.22 \pm 0.09$ ). In comparison, drug showed highest inhibition of the test organisms. There was a significant difference in the inhibitory activity of the plant extracts against all the test organisms ( $p < 0.05$ ) (Table 3).

**Table 3.** Zone of inhibition (mm) of test organisms by the ethanol and methanol leaf extracts of *L. camara* at 62.5 mg/ml concentration.

Plant Extracts	<i>E. coli</i>	<i>S. typhi</i>	<i>C. albicans</i>	<i>M. gypseum</i>	<i>M. luteus</i>	<i>V. cholerae</i>
Drug	$18.11 \pm 0.16^a$	$20.10 \pm 0.04^a$	$19.37 \pm 0.08^b$	$21.87 \pm 0.29^b$	$14.95 \pm 0.09^b$	$22.15 \pm 0.06^a$
Ethanol	$12.08 \pm 0.22^b$	$15.32 \pm 0.04^b$	$15.58 \pm 0.06^a$	$12.55 \pm 0.12^b$	$13.15 \pm 0.09^c$	$17.14 \pm 0.06^b$
Methanol	$11.01 \pm 0.14^c$	$12.24 \pm 0.05^c$	$13.18 \pm 0.14^c$	$10.12 \pm 0.18^a$	$9.22 \pm 0.09^a$	$13.19 \pm 0.06^b$

Results are in Mean  $\pm$  Standard Deviation. Means with the same letter in a column are not significantly different ( $p < 0.05$ )

The results of the inhibitory activity of ethanol and methanol leaf extracts of *L. camara* against the test organisms at 125 mg/ml concentration showed that ethanol extract had the highest inhibition of *V. cholerae* ( $14.32 \pm 0.29$ ) while the lowest inhibition was noticed on *E. coli* ( $7.00 \pm 0.25$ ). The methanol extract had the highest inhibition of *C. albicans* ( $10.57 \pm 0.32$ ) while the lowest inhibition was observed on *V. cholerae* ( $6.67 \pm 0.95$ ). In comparison, drug showed the highest inhibition of the test organisms. There was a significant difference in the inhibitory activity of the plant extracts against all the test organisms ( $p < 0.05$ ) (Table 4).

**Table 4.** Zone of inhibition of test organisms by the ethanol and methanol leaf extracts of *L. camara* at 125 mg/ml concentration.

Plant Extracts	<i>E. coli</i>	<i>S. typhi</i>	<i>C. albicans</i>	<i>M. gypseum</i>	<i>M. luteus</i>	<i>V. cholerae</i>
Drug	15.43 $\pm$ 0.59 <sup>a</sup>	15.02 $\pm$ 0.09 <sup>a</sup>	13.14 $\pm$ 0.90 <sup>b</sup>	10.89 $\pm$ 0.27 <sup>a</sup>	10.07 $\pm$ 0.20 <sup>b</sup>	14.06 $\pm$ 0.95 <sup>b</sup>
Ethanol	7.00 $\pm$ 0.25 <sup>b</sup>	13.75 $\pm$ 0.12 <sup>b</sup>	12.44 $\pm$ 0.16 <sup>b</sup>	10.55 $\pm$ 0.45 <sup>a</sup>	9.68 $\pm$ 0.39 <sup>b</sup>	14.32 $\pm$ 0.29 <sup>b</sup>
Methanol	10.20 $\pm$ 0.20 <sup>c</sup>	9.17 $\pm$ 0.25 <sup>c</sup>	10.24 $\pm$ 0.24 <sup>a</sup>	10.24 $\pm$ 0.24 <sup>a</sup>	7.89 $\pm$ 0.60 <sup>a</sup>	6.67 $\pm$ 0.95 <sup>a</sup>

Results are in Mean  $\pm$  Standard Deviation. Means with the same letter in a column are not significantly different ( $p < 0.05$ )

The results of the inhibitory activity of ethanol and methanol leaf extracts of *L. camara* against the test organisms at 250 mg/ml concentration revealed that ethanol extract showed the highest inhibition of *V. cholerae* ( $10.58 \pm 0.11$ ) while *E. coli* showed the lowest inhibition ( $8.68 \pm 0.23$ ). The methanol extract showed the highest inhibition of *V. cholerae* ( $9.10 \pm 0.60$ ) while the lowest inhibition was observed on *C. albicans* ( $7.04 \pm 0.13$ ). In comparison, drug showed the highest inhibition of the test organisms. There was a significant difference in the inhibitory activity of the plant extracts against all the test organisms ( $p < 0.05$ ) (Table 5).

**Table 5.** Zone of inhibition of test organisms by the ethanol and methanol leaf extracts of *L. camara* at 250 mg/ml concentration.

Plant Extracts	<i>E. coli</i>	<i>S. typhi</i>	<i>C. albicans</i>	<i>M. gypseum</i>	<i>M. luteus</i>	<i>V. cholerae</i>
Drug	14.52 $\pm$ 0.31 <sup>b</sup>	13.04 $\pm$ 0.18 <sup>c</sup>	11.43 $\pm$ 0.22 <sup>b</sup>	9.86 $\pm$ 0.66 <sup>b</sup>	9.20 $\pm$ 0.15 <sup>a</sup>	10.95 $\pm$ 0.36 <sup>a</sup>
Ethanol	8.68 $\pm$ 0.23 <sup>a</sup>	8.79 $\pm$ 0.62 <sup>b</sup>	10.49 $\pm$ 0.26 <sup>a</sup>	8.98 $\pm$ 0.54 <sup>b</sup>	9.05 $\pm$ 0.29 <sup>b</sup>	10.58 $\pm$ 0.11 <sup>b</sup>
Methanol	8.43 $\pm$ 0.76 <sup>a</sup>	7.14 $\pm$ 0.28 <sup>a</sup>	7.04 $\pm$ 0.13 <sup>b</sup>	8.50 $\pm$ 0.30 <sup>a</sup>	7.20 $\pm$ 0.60 <sup>a</sup>	9.10 $\pm$ 0.60 <sup>c</sup>

Results are in Mean  $\pm$  Standard Deviation. Means with the same letter in a column are not significantly different ( $p < 0.05$ )

The results of the inhibitory activity of ethanol and methanol leaf extracts of *L. camara* against the test organisms at 500 mg/ml concentration revealed that ethanol extract showed the highest inhibition of *V. cholerae* ( $8.83 \pm 0.40$ ) while *E. coli* showed the lowest inhibition ( $6.72 \pm 0.27$ ). The methanol extract showed the highest inhibition of *M. gypseum* ( $6.62 \pm 0.42$ ) while the lowest inhibition was observed on *V. cholerae* ( $4.28 \pm 0.39$ ). In comparison, drug showed the highest inhibition of the test organisms. There was a significant difference in the inhibitory activity of the plant extracts against all the test organisms ( $p < 0.05$ ) (Table 6).

**Table 6.** Zone of inhibition of test organisms by the ethanol and methanol leaf extracts of *L. camara* at 250 mg/ml concentration.

Plant Extracts	<i>E. coli</i>	<i>S. typhi</i>	<i>C. albicans</i>	<i>M. gypseum</i>	<i>M. luteus</i>	<i>V. cholerae</i>
Drug	12.24 $\pm$ 0.14 <sup>c</sup>	10.82 $\pm$ 0.05 <sup>c</sup>	9.66 $\pm$ 0.52 <sup>c</sup>	7.39 $\pm$ 0.23 <sup>a</sup>	7.65 $\pm$ 0.13 <sup>c</sup>	9.28 $\pm$ 0.20 <sup>b</sup>
Ethanol	6.72 $\pm$ 0.27 <sup>b</sup>	7.65 $\pm$ 0.18 <sup>b</sup>	7.58 $\pm$ 0.33 <sup>b</sup>	7.26 $\pm$ 0.45 <sup>a</sup>	6.89 $\pm$ 0.13 <sup>b</sup>	8.83 $\pm$ 0.40 <sup>b</sup>
Methanol	5.65 $\pm$ 0.23 <sup>a</sup>	6.25 $\pm$ 0.14 <sup>a</sup>	6.12 $\pm$ 0.25 <sup>c</sup>	6.62 $\pm$ 0.42 <sup>a</sup>	5.53 $\pm$ 0.36 <sup>a</sup>	4.28 $\pm$ 0.39 <sup>b</sup>

Results are in Mean  $\pm$  Standard Deviation. Means with the same letter in a column are not significantly different ( $p < 0.05$ )

The results of the inhibitory activity of ethanol and methanol leaf extracts of *L. camara* against the test organisms at 1000 mg/ml concentration revealed that ethanol extract showed the highest inhibition of *V. cholerae* ( $9.06 \pm 0.04$ ) while *E. coli* showed the lowest inhibition ( $7.03 \pm 0.09$ ). The methanol extract showed the highest inhibition of *V. cholerae* ( $8.40 \pm 0.02$ ) while the lowest inhibition was observed on *E. coli* ( $6.00 \pm 0.08$ ). In comparison, drug showed the highest inhibition of the test organisms. There was a significant difference in the inhibitory activity of the plant extracts against all the test organisms ( $p < 0.05$ ) (Table 7).

**Table 7.** Zone of inhibition of test organisms by the ethanol and methanol leaf extracts of *L. camara* at 1000 mg/ml concentration.

Plant Extracts	<i>E. coli</i>	<i>S. typhi</i>	<i>C. albicans</i>	<i>M. gypseum</i>	<i>M. luteus</i>	<i>V. cholerae</i>
Drug	15.79 $\pm$ 0.03 <sup>a</sup>	13.18 $\pm$ 0.05 <sup>a</sup>	9.23 $\pm$ 0.58 <sup>c</sup>	9.84 $\pm$ 0.01 <sup>b</sup>	10.67 $\pm$ 0.13 <sup>b</sup>	14.98 $\pm$ 0.05 <sup>a</sup>
Ethanol	7.03 $\pm$ 0.09 <sup>c</sup>	9.04 $\pm$ 0.02 <sup>b</sup>	8.54 $\pm$ 0.12 <sup>c</sup>	8.01 $\pm$ 0.55 <sup>a</sup>	8.05 $\pm$ 0.74 <sup>a</sup>	9.06 $\pm$ 0.04 <sup>b</sup>
Methanol	6.00 $\pm$ 0.08 <sup>a</sup>	7.01 $\pm$ 0.18 <sup>c</sup>	6.29 $\pm$ 0.59 <sup>b</sup>	8.00 $\pm$ 0.01 <sup>c</sup>	6.02 $\pm$ 0.09 <sup>a</sup>	8.40 $\pm$ 0.02 <sup>b</sup>

Results are in Mean  $\pm$  Standard Deviation. Means with the same letter in a column are not significantly different ( $p < 0.05$ )

#### 4. DISCUSSION

This study showed that both ethanol and methanol leaf extracts of *L. camara* were able to inhibit the growth of human pathogens tested. This is probably as a result of the synergistic actions of the active components present in the plant leaf.

The ethanol extract of *L. camara* was found to have higher inhibitory activity against all the test organisms than the methanol extract. This agrees with the report of Ekwenye and Elegalam (2005) on garlic who attributed this to the fact that ethanol is an organic solvent and will dissolve organic compounds better, hence liberate the active compounds needed for antifungal activity. The inhibitory activity of the ethanol and methanol leaf extracts increased with concentration. This agrees with the report that more active ingredients in plants are dissolved in solution at higher concentrations (Amadioha, 2000; Onifade, 2002; Okigbo and Ogbonnaya, 2006). Comparison of the inhibitory activity of the antibiotic (Streptomycin) and plant extracts indicated that the antibiotic showed the higher inhibition than the plant extracts against all the organisms tested.

The result of the susceptibility profile of the test organisms indicated that *V. cholerae* was the most susceptible while *E. coli* was the most resistant to the plant extracts. The higher resistant of *E. coli* is a source of concern since it has been associated with various human disease conditions such as gastroenteritis, typhoid fever, dysentery, cholera, and urinary tract infections (Orji *et al.*, 2006).

#### 5. CONCLUSION

This study has confirmed that the ethanol and methanol leaf extracts of *L. camara* were able to inhibit the growth of test organisms such as *E. coli*, *S. typhi*, *C. albicans*, *M. gypseum*, *M. luteus* and *V. cholerae*. It is recommended that the leaf extract of *L. camara* should be used as natural antibiotic in place of the chemically combined ones. This will reduce the cost and allergy often associated with synthetic drugs.

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