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Lantana camara: Phyto-constituents and Antimicrobial Activity Study

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Abstract

Background: The development of resistance by pathogenic organisms has caused renewed interest in medicinal plants as alternative antimicrobial agents in treating diseases and infections. *Lantana camara* is an evergreen shrub commonly found in the tropical and sub-tropical regions, different parts of the plant have been used traditionally in the treatment of fever, diarrheal, scabies, cold, skin infections etc. This study therefore, seeks to investigate the phytochemical composition and potential of *Lantana camara* extracts as an alternative antimicrobial agents.

Methods: According to standard methods in this study, qualitative and quantitative phytochemical screening of *Lantana camara* leaves extracts was done. *In vitro* antimicrobial activity of the extract were evaluated against gram-positive and negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella oxytoca*, *Proteus vulgaris*, *Staphylococcus aureus*, and *Salmonella typhi*) and fungal strains (*Aspergillus flavus*, *Aspergillus niger*, *Aspergillus fumigatus*, *Fusarium poae*, and *Fusarium solani*) using well-diffusion and mycelia inhibition method, respectively.

Results: Phytochemical screening of the extracts revealed saponins, phenols, tannins, flavonoids, steroids, cardiac glycosides, and alkaloids, with tannins (70.454 ± 0.002 mg/g) found to be abundant in ethanolic extract. The extracts displayed moderate to high inhibitory activities on the tested strains with *E.coli* and *A. flavus* highly inhibited by the extracts.

Conclusion: The present study clearly showed that the crude extracts are rich in diverse phytochemicals and are potential sources of antimicrobial principles that could be used in the management of diseases and infections.

Keywords: *Lantana camara*, alternative antimicrobial agents, antibacterial activity, antifungal activity.

1.0 INTRODUCTION

The use of plants in the treatment and management of diseases and infections dates back to history. The last few decades have witnessed a renewed interest in plants as alternative antimicrobial and therapeutic agents due to increasing microbial resistance to available antibiotics. These plant parts owe their therapeutic effects to different secondary metabolites with diverse mechanisms of action.

Lantana camara Linn commonly called wild or red sage, belongs to the family Verbenaceae. It is an evergreen notorious wild shrub that grows well in tropical and temperate regions [1]. It is a woody-smelling plant growing up to 1 – 3 meters; the flowers are often in clusters and diverse colors ranging from red, violet, white, yellow, and pink, often cultivated as an ornament plant but have now become an invasive weed [2]. The leaves are usually green, opposite, simple and oval-oblong in shape with a length between 2-10 cm and width 2 - 6 cm, the stems are woody and thorny while the roots are very strong [3].

Different parts of the plant have found application in folk medicine; the leaves are reportedly used to relieve toothache and stomachache, stimulate wound healing, and treat diarrheal, rheumatism, skin infection, ulcer, bronchitis, and biliary fever [4]. Essential oil from the plant is used to treat and manage tumors, leprosy, measles, tetanus, skin itches, chicken pox, cancer, asthma, ulcers, and high blood pressure [5]. Insecticidal, fumigant, and antiseptic activity of the plant have also been reported [6]. Antimicrobial, anti-inflammatory, antinociceptive, anti-mycobacterial, anticancer, antioxidant, anti-helminthic, and antiulcer activities of the plant have also been reported in the literature [7].

2.0 METHODOLOGY

2.1 Preparation of the Plant Material

L. camara leaves were collected in Ogbomoso Oyo state Nigeria in January 2020. The sample was identified by Professor A.T.J. Ogunkunle of the Department of Pure and Applied Biology, Ladoko Akintola University of Technology, Ogbomoso, Nigeria. The leaves were washed in distilled water (to remove dust, dirt, and other contaminants), air-dried, and pulverized using QBL-20L40 QASA blender. The pulverized plant material (1.40 kg) was successively extracted with n-hexane, ethyl

acetate, and ethanol for one (1) week each, filtered, and the filtrate concentrated *in vacuo*. The resulting crude extracts were kept in airtight container till further analysis, and the percentage yield was calculated as follows:

$$\% \text{ yield} = \frac{\text{mass of plant extract}}{\text{mass of dried plant sample}} \times 100$$

2.2 Qualitative Phytochemical Evaluation

Secondary metabolites present in n-hexane, ethyl acetate, and ethanolic extracts of *Lantana camara* leaves were evaluated according to the study by Harbourne [8]. Phytochemicals tested for include, Alkaloids (Wagner's Test), Flavonoids (Lead acetate Test), Saponins (Froth's Test), Glycosides (Liebermann's test), Tannins (Braymer's Test), Cardiac Glycosides (Keller Killiani Test), carbohydrate (Molisch's test), Steroids (Salkowaski's Test), Phenols (Ferric chloride), Coumarins (Reaction with 10 % NaOH), Terpenoids (Salkowaski's test) and Anthraquinones (Borntrager's test). The presence or absence of the phytochemical was indicated by positive (+) and negative (-) signs, respectively.

2.2.1 Alkaloids

To 0.2 g of the plant extracts in a test tube was added 2 ml of Wagner's reagent (iodine dissolved in potassium iodide), the appearance of a yellow precipitate showed the presence of alkaloids.

2.2.2 Flavonoids

Exactly 1 ml of 10 % lead acetate was added to 0.2 g of *Lantana camara* leaf extracts, the development of a yellow precipitate is a preliminary test for the presence of flavonoids.

2.2.3 Cardiac Glycosides

Two drops of FeCl₃ (ferric chloride solution) was added to 1 ml of 5% glacial acetic acid, the resulting solution was added to 0.2 g of *L. camara* leaf extract, after which 3 drops of concentrated solution of tetroxosulphate (VI) acid (H₂SO₄) was added, appearance of a brown ring is a positive indication for cardiac glycosides.

2.2.4 Saponins

Exactly 0.2 g each of the extracts was dissolved in 10 ml of distilled water, shaken vigorously, and allowed to stand for 30 minutes. The appearance of a stable froth is a positive indication for saponins.

2.2.5 Glycosides

To 2 ml of chloroform was added 2 ml of acetic acid. The resulting solution was added to 0.2 g of *L. camara* leaf extract. A green ring at the interface is a positive test for glycosides

2.2.6 Tannins

In this test, 0.2 g each of the extracts were dissolved in 3 ml of distilled water, after which 2 drops of 5 % FeCl₃ solution was added, formation of a green precipitate suggests the presence of tannins.

2.2.7 Carbohydrates

0.2 g of *L. camara* extract was dissolved in 5 ml of distilled water and filtered. Three drops of 1% alcoholic alpha naphthol was added along the side of the test tube, after which 2 ml concentrated tetraoxosulphate (VI) acid was added. The appearance of a violet ring at the interface indicated the presence of carbohydrates.

2.2.8 Steroids

0.2 g of *L. camara* extract was added to 2 ml of chloroform, after which of 3 drops of concentrated H₂SO₄ was added along the side of the test tube. The appearance of a reddish-brown colour at the interface was indicative of steroids.

2.2.9 Phenols

Phenolic content was determined by adding 0.2 g of the crude plant extract to 2 ml of distilled water, followed by three (3) drops of FeCl₃ solution. The appearance of an intense green colour showed the presence of the phenolic group

2.2.10 Coumarin

Exactly 1 ml of 10 % sodium hydroxide was added to 0.2 g each of the plant extracts. The appearance of yellow colour is indicative of the presence of Coumarin .

2.2.11 Terpenoids

A combination of 2 ml of acetic anhydride and 3 ml of concentrated tetraoxosulphate (VI) acid was added to 0.2 g of the plant extract in a test tube. The appearance of a deep red colour is a positive test for terpenoids.

2.2.12 Anthraquinone

To 0.2 g of *L. camara* extract was added 2 ml of dilute H₂SO₄, the resulting solution was filtered, washed with 2 ml of chloroform, and shaken vigorously, after which 2 ml of ammonia solution was added. A pink colouration suggests the presence of anthraquinone

2.3 Quantitative Phytochemical Evaluation

2.3.1 Alkaloids

The quantity of alkaloids present in *L. camara* extracts was evaluated using a previously established method [8]. Exactly 0.5 g of the crude extract was extracted with a mixture of ethanol and ethanoic acid (9:1) for 4 h. The mixture was filtered with Whatman No. 42 filter paper, and the volume of the filtrate was reduced to 30 ml over a hot water bath. The concentrated filtrate was then precipitated with concentrated aqueous ammonia. The precipitate was filtered with Whatman No. 42 filter paper and dried till constant weight in an oven. Total alkaloid content was calculated as a percentage.

2.3.2 Flavonoids

$$\text{Total alkaloid content} = \frac{\text{weight of precipitate}}{\text{weight of extract}} \times 100$$

Aluminum chloride colorimetric assay was used for flavonoid quantification. Thus, 13 mg of each *L. camara* extract was dissolved in 26 ml of methanol and filtered, 4 ml of distilled water and 0.30 ml of 5 % sodium nitrite were added to the filtrate and shaken vigorously for 5 min. Then, 0.3 ml of 10 % aluminum chloride and 2 ml of 1 M sodium hydroxide were added, and the resulting flavonoid-aluminum complex was diluted with 3 ml of distilled water. Graded concentrations of Quercetin (20, 40, 60, 80, and 100 µg/ml) were used as standard, and absorbance taken at 510 nm. Total flavonoid concentration present in *L. camara* extract was expressed as milligram of Quercetin equivalent per gram of the extract [9].

2.3.3 Saponins

Total The total saponin content of *L. camara* extracts was evaluated accordingly [10]. Exactly 0.5 g each of the extracts were extracted with 100 cm³ of 90 % ethanol, the resulting extract was filtered, and volume of the filtrate was reduced to 40 ml over a hot water bath. The concentrated filtrate was further extracted with 10 ml of diethyl ether (to remove fats) in a separating funnel. The ether layer was discarded while the aqueous layer was hydrolyzed with 60 ml of n-butanol to liberate any aglycone present and washed with 10 ml of 5 % aqueous sodium chloride. The resulting solution was evaporated to constant weight in a beaker, and percentage saponins content was calculated.

$$\text{Total saponin content} = \frac{\text{weight of evaporated filtrate}}{\text{weight of extract used}} \times 100$$

2.3.4 Tannins

Total amount of tannins present in *L. camara* leave extracts was determined using the Folin - Ciocalteus method as described [11]. Exactly 13 mg each of the crude extracts were extracted with 26 ml of methanol and filtered through Whatman No. 42 filter paper. 0.1 ml of the filtrate was diluted with 7.5 ml of distilled water, after which 0.5 ml of Folin-Ciocalteus reagent (mixture of phosphomolybdate and phosphotungstate) and 1 ml of 35 % Na_2CO_3 were added. The resulting mixture was vigorously shaken and allowed to stand for 30 minutes to form bluish-green colour. Gallic acid solution (20, 40, 60, 80, and 100 $\mu\text{g}/\text{ml}$) was used as a standard for comparison while absorbance was read at 725 nm. The total amount of tannin present in the extract was calculated as a milligram of Gallic acid equivalent per gram of the extract.

2.3.5 Phenol

The folin-Ciocalteus method was used in the quantitative determination of phenol content of *L. camara* crude extract. Thus, 13 mg of the crude extracts were dissolved in methanol and filtered through Whatman No. 42 filter paper. 1 ml of Folin-Ciocalteus reagent (mixture of phosphomolybdate and phosphotungstate) and 9 ml of distilled water were added to 1 ml of the filtrate and the mixture was allowed to stand for 5 min. 10 ml of 7% sodium carbonate (Na_2CO_3) was further added to enhance the oxidation of phenol in the extract by Folin-Ciocalteus reagent. The graded concentration of Gallic acid (20, 40, 60, 80, and 100 $\mu\text{g}/\text{ml}$) were prepared and used as a standard while absorbance was read at 550 nm. Total phenol content was calculated and presented as milligram (mg) of Gallic acid equivalent per gram (GAE/gm) of the extract [11].

2.4 Antimicrobial Susceptibility Test

2.4.1 Test Organism Collection

The organisms used in this study are clinical isolates obtained from Bowen University Teaching Hospital, Ogbomosho, Oyo State, Nigeria. The bacteria isolates are *Escherichia coli*, *Klebsiella oxytoca*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Staphylococcus aureus*, and *Salmonella typhi* while the fungi used are *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger*, *Fusarium solani* and *Fusarium poae*.

2.5 Antibacterial Susceptibility

Agar well diffusion method was used in evaluating the susceptibility of the bacteria to n-hexane, ethyl acetate, and ethanol extracts of *L. camara* [12]. Each test bacteria

(*Proteus vulgaris*, *Klebsiella oxytoca*, *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Salmonella typhi*) were sub-cultured in sterilized peptone water and incubated for 18 h at 37 °C. The inoculum was applied on Mueller–Hinton Agar (Lab M Ltd.) plates using a sterile swab stick, after which cork borer of diameter 7 mm was used to create five (5) wells to which 100 μl of different concentrations of the crude extracts were added. An additional well was also created at the center of the plate to which DMSO (serving as negative control) was added, while a standard antibiotic disk (Abtek Biologicals Ltd., UK) containing the following antibiotics were used for gram-positive bacteria; ceftriaxone 30 μg , tetracycline 30 μg , augmentin 30, gentamycin 10 μg , ofloxacin 5 μg , amoxicillin 25 μg , ciprofloxacin 5 μg , and pefloxacin 10 μg . For gram-negative bacteria, the following antibiotics were used; ciprofloxacin 5 μg , amoxicillin 25 μg , pefloxacin 10 μg , ofloxacin 5 μg , chloramphenicol 30 μg , streptomycin 30 μg , cephalothin 30 μg , gentamycin 10 μg , cotrimoxazole 25 μg , and erythromycin 5 μg . The plates were incubated at 37°C for 24 hours, after which the antibacterial activity of the extract and the standards were recorded as visible zones in diameter.

2.6 Antifungal activity

Mycelia inhibition method was used in the evaluation of the antifungal potential of *L. camara* extracts as previously described [13]. Exactly 2 ml of 50 mg/ml of the crude extract was mixed with Potato Dextrose Agar (PDA), poured into a sterile Petri dish, and allowed to cool. Then, 6 mm agar plugs of fungi (*Aspergillus niger*, *Aspergillus fumigatus*, *Aspergillus flavus*, *Fusarium solani* and *Fusarium poae*) were placed at the center of the plate and incubated at 28 °C for 72 h. The fungal plugs inoculated on PDA plates without incorporation of *L. camara* served as control. Radial growth of the fungi were measured in diameters (mm) and was used to calculate percentage growth inhibitions:

$$\% \text{ Growth Inhibition} = \frac{D_{\text{control}} - D_{\text{test}}}{D_{\text{control}}} \times 100$$

D: is the fungal growth in diameter (mm) on PDA plates.

3.0 RESULTS AND DISCUSSION

3.1 Extraction with Different Solvent

Solvent extraction of secondary metabolites from plant parts is one of the most widely used approach in

therapeutic and pharmacological investigation of plant materials; it involves diffusion/penetration of the solvent into the solid plant material and compounds with higher affinity for each solvent are solubilized. Solvent extraction yields are often influenced by polarities of solvents, extraction method, duration of extraction, temperature of extraction among others. n-hexane, ethyl acetate and ethanol were used to successively extract secondary metabolites from *L. camara* leaves, the percentage yield of the extract are 4.30 %, 4.67% and 4.14% for n-hexane, ethyl acetate and ethanol respectively. These results clearly showed that more of the secondary metabolites were solubilized in ethyl acetate.

3.2 Qualitative and Quantitative Screening

Medicinal and therapeutic potential of plants have been associated with presence of phytochemicals, which have been found to have wide range applications in the treatment and management of diseases and infections. Phytochemical components of plants are influenced by factors such as extraction solvent, climate, geographical location, time of harvest, physiological age of plant among others. Preliminary qualitative phytochemical screening results provide essential information on the presence or absence of secondary metabolites which in turn helps in drug discovery from plants.

The results obtained from the preliminary phytochemical investigation of *L. camara* leaves are summarized in Table 1, data from the table showed that alkaloids, flavonoids, tannins, phenols, steroids and cardiac glycosides were present in the three extracts (n-hexane, ethyl acetate and ethanol), terpenoids, carbohydrates and coumarins were found in both ethyl acetate and ethanol extracts while glycosides was identified only in ethanol extract. This investigation however, did not detect the presence of anthraquinones in the three extracts. The presence of alkaloids, saponins, phytosterols, glycosides, tannins, terpenoids, steroids and flavonoids had previously been reported in *L. camara* [14,15].

Similarly, Table 2 provides information on the quantitative phytochemical composition of *L. camara* leaf extracts, which clearly showed that flavonoids, tannins and phenols contents of the leaf increased with increasing polarity of the solvents. It is also important to note that tannin content of *L. camara* leaf are far higher than those of other phytochemicals detected in this study, with the

Table 1. Qualitative phytochemical screening of *L. camara* extracts

Parameters	Hexane	Ethyl acetate	Ethanol
1 Saponin (Froth's Test)	+	+	+
2 Alkaloid (Wagner's Test)	+	+	+
3 Flavonoid (Lead acetate Test)	+	+	+
4 Tannin (Braymer's Test)	+	+	+
5 Phenol (Ferric chloride)	+	+	+
6 Steroid (Salkowaski's Test)	+	+	+
7 Cardiac Glycosides (Keller Killiani test)	+	+	+
8 Terpenoid (Salkowaski's test)	-	+	+
9 Carbohydrate (Molisch's test)	-	+	+
10 Coumarin (Reaction with 10 % NaOH)	-	+	+
11 Glycosides (Liebermann's test)	-	-	+
12 Anthraquinone (Borntrager's test)	-	-	-

highest amount of tannin found in ethanol extract (70.454±0.002 mg/g). A closer inspection of the table revealed that the leaves are especially rich in phenolic compounds (flavonoids ((24.500±0.012 mg/g)), tannins (70.454±0.002 mg/g) and phenol (10.733±0.014 mg/g).

Phenolics are natural bioactive compound having one or more aromatic groups to which one or more hydroxyl groups are attached, it comprises of the simple phenolic acid to the more complex polyphenols (e.g tannins and flavonoids)[16].

Table 2. Quantitative Screening of *L. camara* Extracts

Parameters	Hexane	Ethyl acetate	Ethanol
Saponins (%)	1.500±0.006	5.500±0.019	3.500±0.033
Alkaloids (%)	0.500±0.002	6.600±0.003	0.700±0.003
Flavonoids (mg/g)	11.325±0.051	17.806±0.045	24.500±0.012
Tannins (mg/g)	39.860±0.006	45.687±0.002	70.454±0.002
Phenols (mg/g)	1.225±0.003	1.767±0.009	10.733±0.014

There is a large volume of published studies describing antioxidant potential of phenolics, and in fact, the relationship between consumption of fruits/vegetables and reduced risk of cardiovascular diseases has been linked to their phenolic content [17].

This activity have been attributed to their ability to donate hydrogen atoms, number and position of hydroxyl groups on the aromatic ring, and nature of substitution on the aromatic ring [18]. These secondary plant metabolites serve as free radical scavengers, singlet oxygen quenchers, and transition metal chelators, enhancing their antioxidant properties [16]. Anti-inflammatory, antiviral, antibacterial, anti-diabetic, anti-carcinogenic, antitumor, and hepato-protective abilities of tannins have been reported in the literature [19]. Similarly, flavonoids have been found to possess anti-mutagenic, anti-inflammatory, antiviral anticancer, hepato-protective, anti-cholinesterase, antioxidant and cardio-protective properties [20].

An appreciable amount of saponins and alkaloid (5.500 ± 0.019 and 6.600 ± 0.003 respectively) were also found in the ethyl-acetate extract of *L. camara*. Saponins are phytochemicals of great nutritional and therapeutic value as they possess the ability to lower blood pressure and cholesterol, protect liver cells, and are important adjuvant in vaccine preparations [21]. They are also good anti-inflammatory, antioxidant, antimicrobial, anti-mutagenic anticancer, immune-modulatory, neuro-protective, and anticoagulant agents [22]. Alkaloids, on the other hand, are active antimicrobial, analgesic, anti-cholinesterase, and anti-inflammatory agents. They have also been proven to be potent anticancer and antitumor agents. These result findings suggest that phytochemicals present in *L. camara* in varying amounts and working synergistically with a different and novel mechanism of actions may be responsible for the reported use in folk medicine as well as pharmacological activities. Consumption of these phyto-rich plants is of great benefit in human health and is probable sources of bioactive compounds in managing infectious diseases.

3.3 Antibacterial Susceptibility

Table 3 represents the results obtained for investigating the susceptibility of the tested bacterial strains to *L. camara* leaf extracts. The extracts displayed moderate to high inhibitory activities on all the tested bacterial, and susceptibility increases with increasing concentration of the extracts. The study also found that the bacterial were more

susceptible to ethanolic extract compared to other extracts used in the study, with the highest antimicrobial activity recorded against the growth of *E. coli* at a concentration of 150 mg/ml (25.50 ± 0.55 mm). The observed high antibacterial effect of the ethanolic extract could be attributed to the presence of different classes of phytochemicals in the extract, which works synergistically as revealed in Table 1. Also, the high flavonoid, tannins, and phenol contents of the ethanolic extract, as shown in Table 2, could be responsible for this observation. It is important to note that n-hexane (21.83 ± 0.73 mm at 150 mg/ml) and ethanolic extract (22.50 ± 0.41 mm at 150 mg/ml) were shown to be better antibacterial agents against *S. aureus* compared to ciprofloxacin (19.50 mm) and ofloxacin (15.50 mm) used as standard antibiotic in this study. Although the antibacterial activity of *L. camara* extracts was lower compared to those of standards antibiotics in the current study (except for *S. aureus*), the extracts could be said to have an appreciable inhibitory effect on all the tested organisms as shown in Plate 1. Hence, they are promising alternative antibacterial agents which could be sourced in the drug discovery process. This result compares favourably with the results of Dubey and Padhy which showed that *L. camara* extract had high inhibitory activity on *S. aureus*, *P. vulgaris* and *P. aeruginosa* [15], and that of Barretto and co-workers, which also established the inhibitory activity of *L. camara* extract on *E. coli*, *P. vulgaris*, *S. aureus* and *P. aeruginosa* [23].

S. aureus is an opportunist commensal bacteria found in humans. It is a leading cause of community-associated skin and soft tissue bacteremia [24]. *K. oxytoca* is also an opportunist healthcare-associated bacteria primarily found in intensive care and immune-compromised patients as well as surfaces in hospital environments [25]. They are usually found in the intestinal and respiratory tracts and are the leading cause of urinary tract infections, bronchopneumonia, wound infection [26]. *P. aeruginosa*, on the other hand, are also opportunistic bacteria in immune-compromised individuals. They are causative organisms of chronic and acute lung infections, unitary tract, and gastrointestinal tract infections. The organism has also been found to pose intrinsic resistance to antibiotics; hence, its infections are often difficult to treat [27]. *S. typhi* is found in the bloodstream, liver, spleens, and small intestine of an infected individual. It causes typhoid fever common in developing countries where poor sanitation and poor hygiene practices prevail [28]. *E. coli* is a com-

Table 3. Antibacterial susceptibility test for *L. camara* extracts

Organisms	Conc. (mg/ml)	n-Hexane (mm)	Ethyl acetate (mm)	Ethanol (mm)	DMSO	Standard (mm)
<i>S. aureus</i>	50	15.83 ± 0.62	15.00 ± 0.81	18.17 ± 0.65	-	CPX: 19.50
	75	17.50 ± 0.08	16.17 ± 0.10	19.00 ± 0.41	-	OFL: 15.50
	100	17.50 ± 0.82	17.33 ± 0.78	19.50 ± 0.69	-	
	125	18.17 ± 0.47	17.53 ± 0.13	19.67 ± 0.62	-	
	150	21.83 ± 0.73	18.00 ± 0.87	22.50 ± 0.41	-	
<i>P. vulgaris</i>	50	15.33 ± 0.01	13.33 ± 0.94	14.17 ± 0.36	-	TET: 26.50
	75	17.00 ± 0.41	14.00 ± 0.47	14.83 ± 0.85	-	
	100	17.17 ± 0.62	13.50 ± 0.27	15.17 ± 0.72	-	
	125	17.17 ± 0.31	18.17 ± 0.06	16.33 ± 0.65	-	
	150	17.43 ± 0.03	18.24 ± 0.49	18.33 ± 0.47	-	
<i>K. oxytoca</i>	50	12.33 ± 0.24	12.33 ± 0.10	16.50 ± 0.63	-	OFL:30.50
	75	14.33 ± 0.18	13.33 ± 0.50	17.33 ± 0.31	-	PFX:30.00
	100	14.67 ± 0.11	13.67 ± 0.78	17.33 ± 0.24	-	CPX:28.50
	125	17.50 ± 0.41	15.33 ± 0.47	18.00 ± 0.47	-	TET:27.50
	150	18.33 ± 0.85	17.50 ± 0.78	22.00 ± 0.08	-	
<i>E. coli</i>	50	7.83 ± 0.47	7.83 ± 0.47	18.33 ± 0.03	-	TET:31.00
	75	8.00 ± 0.82	11.50 ± 0.48	19.86 ± 0.24	-	CPX:28.00
	100	8.83 ± 0.85	12.50 ± 0.86	20.67 ± 0.03	-	PFX:28.00
	125	8.83 ± 0.24	14.50 ± 0.41	22.33 ± 0.89	-	
	150	9.50 ± 0.71	15.17 ± 0.65	25.50 ± 0.55	-	
<i>S. typhii</i>	50	11.50 ± 0.47	10.50 ± 0.23	16.17 ± 0.18	-	CRO:30.00
	75	11.83 ± 0.65	11.50 ± 0.78	16.67 ± 0.85	-	
	100	11.83 ± 0.85	11.67 ± 0.03	17.33 ± 0.24	-	
	125	13.33 ± 0.31	13.67 ± 0.65	18.33 ± 0.65	-	
	150	14.00 ± 0.82	16.67 ± 0.03	18.67 ± 0.47	-	
<i>P. aeruginosa</i>	50	11.23 ± 0.24	8.83 ± 0.62	17.00 ± 0.78	-	CRO:25.50
	75	11.33 ± 0.24	8.83 ± 0.24	17.33 ± 0.25	-	AUG:25.50
	100	13.83 ± 0.03	10.33 ± 0.84	19.83 ± 0.23	-	GEN:22.50
	125	13.67 ± 0.62	11.50 ± 0.68	20.00 ± 0.68	-	
	150	14.33 ± 0.85	13.33 ± 0.25	21.00 ± 0.09	-	

AUG= augmentin, OFL= ofloxacin, CRO= ceftriaxone, GEN= gentamycin, CPX=ciprofloxacin, PEF= pefloxacin and TET= tetracycline. * Each of the values are means of 3 determinations

mon pathogen of the gastrointestinal tracts of humans and animals. It is responsible for a broad spectrum of diseases, including unitary tract infections (UTIs), diarrheal, and food poisoning [29]. *Proteus vulgaris* is also opportunist bacteria, it cause UTIs, infections of the bloodstream and digestive tracts, respiratory tract infections, skin infection, and resistance to antibiotics. Hence, infections caused by them are difficult to treat [30]. Evidence from the susceptibility of the tested bacteria strains to *L. camara* extracts suggests its potential in the treatment of diseases and infections caused by them.

3.4 Antifungal Activity Result of *L. camara* Extracts

The susceptibility of the tested fungi strains are presented in Table 4. The extracts displayed moderate to high antifungal activity on all the tested fungal strains. An increase in antifungal activity of the extracts as the polarity of solvents increases, was observed against *A. flavus* and *F. poae*, which might probably be due to the

presence of diverse phytochemical classes, coupled with increased quantities of tannins, flavonoids and phenols in the ethanolic extract as earlier shown in Table 2. Notably, the highest antifungal activity study was recorded against the growth of *A. flavus* (88.20 %). Similarly, *A. niger* (83.78 %) and *F. solanis* (84.47 %) were more susceptible to ethyl acetate extract than other extraction solvents. These findings could be attributed to the ethyl acetate extract's high saponins and alkaloids contents as shown in Table 2. The extracts also displayed good inhibitory activities on the growth of *A. fumigatus*, it is however not clear why *F. solani* was resistant to the ethanolic extract.

Aspergillus fumigatus, *A. flavus*, and *A. niger* are members of the *Aspergillus* genus. The genus is known to reproduce asexually and consists of filamentous fungi known to be agents of opportunistic infections in immune-compromised individuals [31]. Grains such as maize, millet, guinea corn, onions, melon, and other

Table 4. Antifungal activity of *L. camara*

Organism	n-Hexane		Ethyl acetate		Ethanol		Control
	FG (mm)	%GI	FG (mm)	%GI	FG (mm)	%GI	
<i>A. flavus</i>	9.50	86.81	9.00	87.50	8.50	88.20	72.00
<i>A. niger</i>	15.00	72.97	9.00	83.78	11.00	80.18	55.50
<i>A. fumigatus</i>	8.00	86.67	10.00	83.33	7.50	87.50	60.00
<i>F. solani</i>	9.00	82.52	8.00	84.47	26.50	48.54	51.50
<i>F. poae</i>	9.50	79.79	9.00	80.85	8.50	81.92	47.00

*FG = fungal growth, % GI = % growth inhibition

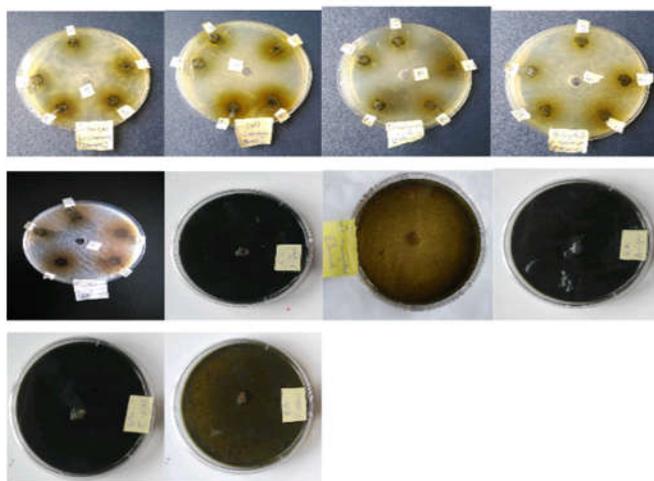


Plate 1. Plate Showing Antimicrobial Activities of *L. camara* extracts.

foodstuff have been prone to *Aspergillus* and *Fusarium* proliferation, leading to their deterioration. *Aspergillus spp* and *Fusarium spp* growth on such food items results in the production of mycotoxins. These mycotoxins are stable to heat processing and treatment with chemicals. Hence they are often difficult to remove once contaminated with food [32]. Serious health problems including stunted growth, immune system suppression, cancer, and loss of lives have been associated with mycotoxins ingestion. It is apparent from this study that *L. camara* displayed good antifungal properties and could be employed as an alternative antibacterial agent in the treatment of infection caused by the pathogenic strains.

Phytochemical screening of the leaf extracts revealed that the plant contains diverse phytochemicals of which tannins, phenols, and flavonoids are abundant. The plant

extract also displayed good antibacterial and antifungal properties on the tested pathogenic strains. These combinations of findings provide some support for the conceptual premises that phytochemicals present in plants are responsible for their various pharmacological activities and that *L. camara* is a potential source of alternative antimicrobial agents, especially in the treatment of diseases and infections caused by the tested pathogenic strains.

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Conflicts of Interest

The Authors declares that there is no conflict of interest.

Authors’ Contribution

SOO conceived and designed the study, contributed to data collection and data analysis tools. VAF, AJA and GJI contributed to data analysis tools, data analysis and manuscript writing. All authors approved the final copy of the manuscript.

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