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Evaluation of Antimicrobial, Antioxidant and Cytotoxic Activity of *Lovoa trichiliodes* Extracts and Essential Oils

Opawale BO[1,*], Onifade AK[2] and Ogundare AO[2]

[1]*Department of Science Laboratory Technology, Rufus Giwa Polytechnic, Owo, Ondo State, Nigeria*
[2]*Department of Microbiology, Federal University of Technology, P.M.B. 704, Akure, Ondo State, Nigeria*

Abstract

Lovoa trichiliodes is a medicinal plant used in many African countries by traditional practitioners for the treatment of some infectious diseases. The extracts and essential oils from the leaves and stem bark of *Lovoa trichiliodes* were investigated for their antioxidant, antimicrobial and cytotoxic properties using standard techniques. The 2, 2-diphenyl-1-picryl-hydrazyl scavenging activity ranged from 03.33 ± 0.03% at 0.05 mg/ml of acetone stem bark extracts to 88.14 ± 0.03% at 2.0 mg/ml of stem bark essential oil. The IC_{50} ranged from 0.81 ± 0.15 to 1.65 ± 0.03 for the extracts and essential oils. However, the IC_{50} of the leaf extract (1.65 ± 0.03) and that of the leaf oil (1.52 ± 0.03) were significantly ($p \leq 0.05$) higher than that of ascorbic acid (0.40 ± 0.15) used as control. The antimicrobial assay of the samples revealed a high activity against both the typed and clinical isolates of test pathogens at 50 mg/ml for extracts and 50 µg/ml for oil respectively. The leaf extract showed lower level of activity than the stem bark extract against the test organisms compared to the controls. The essential oils from both the leaf and stem bark exhibited higher activities against bacteria than fungi. *Bacillus subtilis* showed the highest susceptibility to the extracts while *Pseudomonas aeruginosa* exhibited the least susceptibility to the plant materials. The minimum inhibitory concentration (MIC) and minimum bactericidal/fungicidal concentration (MBC/MFC) ranged from 2.5 to 200 mg/ml and 5 to 50 mg/ml for the extracts while that of oils ranged from 3 to 40 µg/ml and 5 to 75 µg/ml respectively. All the extracts and essential oils showed high level of lethality on brine shrimp larva with LC_{50} ranging from 0.71 to 52.65 ppm. These results confirm the basis for the use of this plant in traditional medicine as remedy against several diseases and prospect for antibiotic drug development for the treatment of ailments caused by the test pathogens.

Keywords: *Lovoa trichiliodes*; Essential oils; Antimicrobial assay; Traditional medicine; Infectious diseases

Introduction

The folkloric concepts of a wide range of medicinal plants have been proven scientifically and had led to the development of drugs to fight various infectious diseases [1]. Plant extracts and essential oils have been widely explored for their therapeutic activities against most microbial infections. It has been established that about 80% of the world's population relies on plant derived medicines for their healthcare needs and 3.5 billion people in the world depend on the exploitation medicinal plants and herbal products around them for their health needs [2]. These extracts and essential oils contain a variety of volatile molecules such as terpenes, terpenoids and phenols derived from aromatic and aliphatic compounds which might have antibacterial, antiviral and fungicidal consequences [3]. There has been an increasing interest in the radical scavenging activities of some natural antioxidants, especially those found in medicinal plants, which may play a role in preventing various chronic diseases [4]. There is also increasing need to search for new compounds with cytotoxic activity as the treatment of cancer with the available anticancer drugs is often unsatisfactory due to the problem of toxicity to the normal cells.

Lovoa trichiliodes is a species of plant in the Meliaceae family. It is common in West Africa and it is a large forest tree that could grow up to 40 m high with a dark heavy crown. It is used in traditional medicine for the treatment of many microbial infections. Based on the ethno medical information on the plant, the present study was aimed at demonstrating the antimicrobial activity of the extracts and essential oils from the plant materials against some human pathogens and to evaluate their potential antioxidant and cytotoxic activities.

Materials and Methods

Collection of plants and extraction procedure

Fresh leaves and stem bark of *Lovoa trichiliodes* Harm. were harvested from uncultivated farmlands located in Owo, Ondo State, South-Western Nigeria in May, 2011. The plant materials were then authenticated at the Herbarium of the Department of Botany, University of Lagos and voucher specimens (LVH3699) were deposited at the Department of Forestry and Wood Technology, Federal University of Technology, Akure. The authenticated plant materials were washed and cleaned thoroughly with tap water and then air-dried under shade. The dried samples were then ground into coarse powder with the aid of a mechanical grinder and were stored in clean air-tight containers, and kept in a cool, dry place until required for use.

The powdered sample (100 g) was soaked in 300 ml acetone for 72 hr with intermittent stirring using sterile spatula. The plant extracts were then filtered through Whatman No1. filter paper into bijou bottles and then dried using rotary evaporator at a temperature of 50°C to yield crude extracts [5]. Different concentrations of the extracts were prepared by diluting 0.10 g, 0.20 g, 0.30 g, 0.40 g and 0.50 g of the extracts in 100 ml of 0.01% Tween-20 to obtain concentrations of 10 mg/ml, 20 mg/ml, 30 mg/ml, 40 mg/ml and 50 mg/ml respectively [6].

A 500 g portion of the respective plant parts in 2000 ml of distilled water was hydro distilled using Clevenger type of apparatus for 5 hr to

***Corresponding author:** Opawale BO, Department of Science Laboratory Technology, Rufus Giwa Polytechnic, Owo, Ondo State, Nigeria, Tel: 2348035925955; E-mail: benjaminopawale@yahoo.com

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obtain the oil. The steam distillate was dried over anhydrous sodium sulphate and 10 mg of it was diluted with 100 ml of 0.01% Tween-20 to obtain a 100 µg/ml solution. Serial dilution of each 100 ml stock solution was made with 0.01% Tween-20 to give test solutions 50, 25, 12.5 and 6.3 µg/ml respectively [7,8].

Test microorganisms

The microorganisms employed in the study were fifteen clinical isolates (*Bacillus subtilis, Escherichia coli, Enterococcus faecalis, Klebsiella pneumonia, Pseudomonas aeruginosa, Salmonella typhi, Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus spp, Streptococcus pyogenes, Aspergillus flavus, Candida albicans, Candida glabrata, Cryptococcus neoformans* and *Trichophyton rubrum*) and five typed cultures (*Bacillus substilis* ATCC 6633*, Stapylococcus aureus* ATCC 25923*, Escherichia coli* ATCC 25922, *Salmonella typhi* ATCC 6539 and *Candida albicans* ATCC 10231) obtained from Federal Medical Center, Owo and Federal Institute of Industrial Research, Oshodi, Lagos State, Nigeria respectively.

In vitro antimicrobial susceptibility test

The extracts and essential oils obtained from the test plants were screened against the test organisms by agar well diffusion method [9]. A 25 ml aliquot of Mueller-Hinton agar (MHA, Lab Oratorios Britania, Argentinia) and Saboraud Dextrose agar (SDA) was poured into each Petri plate. When the agar solidified, test organisms were inoculated on the surface of the plates (1×10^6 *cfu*/ml and 1 x 10^6 *sfu*/ml for bacteria and fungi respectively) using a sterile glass spreader, allowed to set and punched with 6 mm cork borer. A portion of 50 µl of each of the extract concentrations was introduced into the wells. Control wells containing the same volume of 30% Dimethyl sulphoside (DMSO) served as negative control, while Chloramphenicol (100 µl) and Miconazole (100 µl) were used as positive controls for bacterial and fungal plates respectively. The tests were carried out in triplicates. Bacterial plates were incubated at 37°C while fungal plates were incubated at 25°C for 24 h and 72 h respectively. The diameters of the zones of inhibition were then measured in millimeters.

Minimum inhibitory concentration (MIC) and Minimum bactericidal/fungicidal concentrations (MBC/MFC)

Two-fold serial dilutions of the extracts were prepared in Mueller-Hilton broth and Saboraud broth for bacteria and fungi respectively to achieve a decreasing concentrations ranging from the least concentration that produced clear zone of inhibition (10 mg/ml to 0.156 mg/ml). All tubes including the controls were labeled accordingly. Each dilution was seeded with 1 ml of standardized inoculums (1.0×10^6 *cfu*/ml for bacteria and 1.0×10^6 *sfu*/ml for fungi) incubated at 37°C for 24 hr and 25°C for 72 hr for bacteria and fungi respectively. A tube containing only seeded broth (i.e. without plant extracts) was used as the positive control while the un-inoculated tube was used as negative control. The lowest concentration of each oil sample that showed a clear zone of inhibition was when compared with the controls was considered as the MIC. The minimum bactericidal/fungicidal concentration (MBC/MFC) was determined by inoculating 1 ml aliquot of the MIC tube culture on antibiotic free Muller Hinton Agar and Saboraud Dextrose Agar plates and incubated at 37°C for 24 hr for bacteria and 25°C for 72 hr for fungi. The lowest concentration of the extracts at which there was no observable growth was taken as the MBC/MFC.

DPPH free radicals scavenging assay

The DPPH free radical (2, 2-diphenyl-1-picrylhydrazyl) scavenging assay was determined using the method described by Koto-Nyiwa et al. [10]. A 4 mg portion of DPPH was dissolved in methanol to get 100 µM methanol solution of DPPH. A 3 ml portion of the extract concentrations (0.00 to 2.0 mg/ml) was added to 1 ml of 100 µM methanol solution of DPPH. The mixture was shaken vigorously and incubated in the dark at room temperature for 30 min. The absorbance at 517 nm was measured against the blank (methanol) and ascorbic acid as positive control using a spectrophotometer. The DPPH radical scavenging activity (%) was then determined by the following equation:

DPPH radical scavenging: Activity (%)=[(Ao −As) /Ao] × 100 where Ao=absorbance of DPPH without sample; As=absorbance of mixture of sample and DPPH. The radical scavenging activity of the samples (Median inhibitory concentration, IC_{50}) value was determined from an equation line obtained by plotting a graph of concentration against percentage inhibition.

Determination of cytotoxic effect of plant extracts

The brine shrimp (*Artemia salina*) lethality bioassay was carried out according to the method described by Hag et al. [11]. Brine shrimp eggs were hatched in artificial sea water prepared by dissolving 38 g of salt in 1 liter of distilled water, filtered and put in shallow rectangular dish. A plastic divider with several holes of 2 mm size was clamped in the dish to make two equal compartments. Brine shrimp eggs were placed in one side of the compartment while the other compartment was illuminated. After 48 h of illumination, phototrophic nauplii (Brine shrimp larvae) were collected by using pipette from the lightened side. Samples were then prepared by dissolving 20 mg each of the extracts and essential oils respectively in 2 mls of DMSO from where further diluted concentrations of 1000, 100, 10 and 1 ppm were prepared. A 4 ml portion of the artificial sea water was added into each test tube and 20 shrimps were transferred into it. This was followed by the addition of 1 ml of each of the test extracts and essential oils of previously prepared concentrations and maintained under illumination at room temperature. Survivors were counted with the aid of magnifying glass after 24 h. The percentage mortality was calculated using Abbot's formula and the LC_{50} was also determined [12,13].

Data analysis

Data were presented as mean ± standard error (SE). Significance difference between different groups was tested using two-way analysis of variance (ANOVA) and treatment means were compared with Duncan's New Multiple Range Test (DNMRT) using SSPS window 7 version17.0 software. The significance was determined at the level of $p \leq 0.05$.

Results and Discussion

Extracts and essential oils derived from medicinal plants are potential sources of novel antimicrobial compounds especially against pathogenic organisms. The results of the *in vitro* antimicrobial studies in this work showed that the acetone extracts (Tables 1 and 2) and essential oils (Tables 3 and 4) of *L. trichiliodes* leaf and stem bark significantly ($p \leq 0.05$) inhibited the growth of most of the test pathogens. The results revealed that the plant extracts and essential oils exhibited strong activity against the selected pathogens with varying magnitude. The acetone extracts showed the inhibition zones range of: leaf (12.00 ± 0.00-17.00 ± 0.58), stem bark (10.67 ± 0.58-22.33 ± 0.33) while the essential oils revealed: leaf (5.33 ± 0.33-18.00 ± 0.58) and stem bark (6.00 ± 0.58-20.33 ± 0.88) with the reference antibiotic standards ranging from: Chloramphenicol (10.00 ± 0.58-17.33 ± 0.33) and Myconazole (10.00 ± 0.58-14.33 ± 0.33) at 50 mg/ml and 50 µg/ml for the extracts and

Conc. (mg/ml) Organisms	10	20	30	40	50	DMSO	Chl(100 µg/ml)	Myz(100 µg/ml)
B. S	NI	7.67 ± 0.58^{a}	10.67 ± 0.58^{b}	13.67 ± 0.58^{c}	15.33 ± 0.58^{d}	NI	11.00 ± 0.00^{b}	N.A
B. S ATCC6633	NI	8.33 ± 0.33^{a}	11.33 ± 0.33^{b}	14.33 ± 0.33^{c}	15.67 ± 0.33^{d}	NI	13.33 ± 0.33^{c}	N.A
S. A	5.67 ± 0.58^{a}	9.00 ± 0.00^{b}	11.00 ± 0.00^{c}	12.67 ± 0.58^{d}	15.00 ± 0.00^{e}	NI	20.00 ± 0.00^{f}	N.A
S. A ATCC25923	6.33 ± 0.33^{a}	9.33 ± 0.33^{b}	11.33 ± 0.33^{c}	13.33 ± 0.88^{d}	15.33 ± 0.33^{e}	NI	22.33 ± 0.33^{f}	N.A
S. E	4.33 ± 0.58^{a}	7.33 ± 0.58^{b}	10.67 ± 0.58^{c}	13.33 ± 0.58^{d}	15.00 ± 0.00^{e}	NI	11.00 ± 0.00^{c}	N.A
E. C	NI	6.33 ± 0.58^{a}	9.33 ± 0.58^{b}	12.67 ± 0.58^{c}	14.33 ± 0.58^{d}	NI	14.00 ± 0.00^{d}	N.A
E. C ATCC25922	NI	7.33 ± 0.33^{a}	9.67 ± 0.67^{b}	13.67 ± 0.33^{c}	14.67 ± 0.33^{c}	NI	16.00 ± 0.58^{d}	N.A
Ps. A	NI	5.33 ± 0.58^{a}	8.33 ± 0.58^{b}	11.33 ± 0.58^{c}	13.67 ± 0.58^{d}	NI	12.00 ± 0.00^{c}	N.A
A. f	3.67 ± 0.58^{a}	6.33 ± 0.58^{b}	11.67 ± 0.58^{c}	12.00 ± 0.00^{c}	12.00 ± 0.00^{c}	NI	N.A	17.33 ± 0.58^{d}
C. A	7.67 ± 0.58^{a}	11.33 ± 0.58^{b}	13.67 ± 0.58^{c}	15.67 ± 0.58^{d}	16.00 ± 0.00^{d}	NI	N.A	12.00 ± 0.00^{bc}
C. A ATCC10231	8.33 ± 0.33^{a}	11.67 ± 0.33^{b}	13.67 ± 0.33^{c}	16.67 ± 0.33^{d}	17.00 ± 0.58^{d}	NI	N.A	14.00 ± 0.58^{c}
C. N	NI	NI	8.00 ± 0.00^{a}	11.33 ± 0.58^{b}	14.00 ± 0.00^{c}	NI	N.A	14.33 ± 0.58^{c}
T. R	6.67 ± 0.58^{a}	9.67 ± 0.58^{b}	12.67 ± 0.58^{c}	15.67 ± 0.58^{d}	16.00 ± 0.00^{d}	NI	N.A	10.00 ± 0.00^{b}

Values are Mean ± S.E.M (mm), Values followed by different alphabet along the rows are significantly different at p ≤ 0.05, NI: No Inhibition, N.A: Not Applicable, Chl: Chloramphenicol, Myz: Miconazole, B.S: *Bacillus subtilis,* S.A: *Staphylococcus aureus,* E.C: *Escherichia coli,* Ps.A: *Pseudomonas aeruginosa,* A.F: *Aspergillus flavus,* C.A: *Candida albicans, C.N*: *Cryptococcus neoformans,* T. R: *Trichophyton rubrum,* S.E: *Staphylococcus epidermidis.*

Table 1: Antimicrobial activity of acetone extract of *Lovoa trichiliodes* leaf on selected human pathogens.

Conc. (mg/ml) Organisms	10	20	30	40	50	DMSO	Chl	Myz
B. S	8.67 ± 0.58^{a}	12.33 ± 0.58^{b}	16.33 ± 0.58^{c}	19.33 ± 0.58^{d}	22.00 ± 1.00^{e}	NI	11.33 ± 0.58^{b}	N.A
B. S ATCC6633	8.33 ± 0.33^{a}	13.00 ± 0.58^{c}	16.67 ± 0.33^{d}	19.67 ± 0.33^{e}	22.33 ± 0.33^{f}	NI	14.67 ± 0.58^{c}	N.A
S. A	3.67 ± 0.58^{a}	6.33 ± 0.58^{b}	10.33 ± 0.58^{c}	14.67 ± 0.58^{e}	19.67 ± 0.58^{f}	NI	13.67 ± 1.00^{d}	N.A
S. A ATCC25923	3.33 ± 0.33^{a}	6.67 ± 0.33^{b}	10.67 ± 0.33^{c}	15.00 ± 0.58^{e}	20.00 ± 0.58^{f}	NI	15.33 ± 0.33^{d}	N.A
E. C	6.33 ± 0.58^{a}	10.33 ± 0.58^{b}	13.33 ± 0.58^{d}	15.67 ± 0.58^{e}	19.33 ± 0.58^{f}	NI	11.33 ± 0.58^{c}	N.A
E. C ATCC25922	6.33 ± 0.33^{a}	10.67 ± 0.33^{b}	13.00 ± 0.00^{c}	16.00 ± 0.58^{d}	19.67 ± 0.33^{e}	NI	14.33 ± 0.67^{d}	N.A
K. P	5.33 ± 0.58^{a}	9.67 ± 0.58^{b}	13.33 ± 0.58^{c}	16.33 ± 0.58^{d}	21.33 ± 0.58^{e}	NI	13.33 ± 0.58^{c}	N.A
S. T	NI	7.33 ± 1.15^{a}	11.33 ± 0.58^{b}	14.67 ± 0.58^{c}	18.67 ± 0.58^{d}	NI	11.67 ± 0.33^{b}	N.A
S. T ATCC6539	NI	7.67 ± 0.33^{a}	11.67 ± 0.33^{b}	15.33 ± 0.33^{c}	19.33 ± 0.33^{d}	NI	14.33 ± 0.67^{c}	N.A
Ps. A	9.00 ± 0.00^{a}	12.33 ± 0.58^{b}	16.33 ± 0.58^{c}	18.67 ± 0.58^{d}	20.33 ± 0.58^{e}	NI	11.67 ± 1.00^{b}	N.A
A. F	NI	NI	6.00 ± 0.00^{a}	8.33 ± 0.58^{b}	10.67 ± 0.58^{c}	NI	N.A	10.00 ± 1.00^{c}
C. A	7.67 ± 0.58^{a}	11.33 ± 0.58^{b}	13.67 ± 0.58^{c}	16.33 ± 0.58^{d}	18.33 ± 0.58^{e}	NI	N.A	11.00 ± 0.00^{b}
C. A ATCC10231	7.67 ± 0.33^{a}	12.33 ± 0.33^{b}	14.67 ± 0.67^{c}	16.67 ± 0.33^{d}	19.33 ± 0.33^{e}	NI	N.A	13.67 ± 0.33^{c}

Values are Mean ± S.E.M (mm), Values followed by different alphabet along the rows are significantly different at p ≤ 0.05, NI: No Inhibition, N.A: Not Applicable, Chl: Chloramphenicol, Myz: Miconazole, B.S: *Bacillus subtilis,* S.A: *Staphylococcus aureus,* E.C: *Escherichia coli, K.P*: *Klebsiella pneumoniae,* Ps.A: *Pseudomonas aeruginosa, S.T*: *Salmonella typhi,* A.F: *Aspergillus flavus,* C.A: *Candida albicans.*

Table 2: Antimicrobial activity of acetone extract of *Lovoa trichiliodes* stem bark on selected human pathogens.

Conc. (µg/ml)/ Organisms	6.3	12.5	25	50	DMSO	Chl	Myz
B. S	NI	7.33 ± 0.33^{a}	10.00 ± 0.58^{b}	12.67 ± 0.33^{c}	NI	11.00 ± 0.58^{b}	N.A
B. S ATCC6633	5.33 ± 0.33^{a}	8.00 ± 0.58^{b}	10.00 ± 1.00^{c}	13.00 ± 0.58^{d}	NI	13.33 ± 0.33^{d}	N.A
S. A	6.33 ± 0.33^{a}	7.33 ± 0.33^{a}	11.33 ± 0.33^{b}	12.33 ± 0.33^{b}	NI	11.33 ± 0.33^{b}	N.A
S. A ATCC25923	6.00 ± 0.00^{a}	8.33 ± 0.33^{b}	11.33 ± 0.33^{c}	14.33 ± 0.33^{e}	NI	13.00 ± 0.58^{d}	N.A
S. P	NI	NI	NI	5.33 ± 0.33^{a}	NI	11.67 ± 0.33^{b}	N.A
S. E	NI	NI	4.33 ± 0.33^{a}	8.00 ± 0.58^{b}	NI	15.67 ± 0.33^{c}	N.A
SSP	NI	NI	NI	3.33 ± 0.88^{a}	NI	13.00 ± 0.58^{b}	N.A
E. C	6.00 ± 0.00^{a}	8.33 ± 0.33^{b}	11.33 ± 0.33^{c}	14.67 ± 0.33^{e}	NI	13.67 ± 0.33^{d}	N.A
E. C ATCC25922	6.33 ± 0.33^{a}	9.67 ± 0.67^{b}	11.33 ± 0.33^{b}	16.00 ± 0.58^{c}	NI	15.67 ± 0.67^{c}	N.A
E. F	7.00 ± 0.00^{a}	10.00 ± 0.58^{b}	14.33 ± 0.33^{d}	18.00 ± 0.58^{e}	NI	11.33 ± 0.33^{c}	N.A
K. P	NI	NI	6.33 ± 0.33^{a}	8.33 ± 0.33^{b}	NI	10.00 ± 0.58^{c}	N.A
A.F	NI	NI	NI	7.33 ± 0.33^{a}	NI	N.A	10.00 ± 0.58^{b}
C. A	NI	NI	NI	9.33 ± 0.88^{a}	NI	N.A	11.00 ± 0.58^{b}
C. A ATCC10231	NI	NI	NI	8.00 ± 0.58^{a}	NI	N.A	14.33 ± 0.33^{b}
C. G	NI	4.00 ± 0.58^{a}	6.33 ± 0.33^{b}	9.33 ± 0.33^{c}	NI	N.A	11.67 ± 0.33^{d}

Values are Mean ± S.E.M (mm), Values followed by different alphabet along the rows are significantly different at p ≤ 0.05, NI: No Inhibition, N.A: Not Applicable, Chl: Chloramphenicol, Myz: Miconazole, *B.S*: *Bacillus subtilis,* S.A: *Staphylococcus aureus,* E.C: *Escherichia coli, E.F*: *Enterococcus faecalis, K. P*: *Klebsiella pneumoniae,* A.F: *Aspergillus flavus,* C.A: *Candida albicans, C.G*: *Candida glabrata, S.P*: *Streptococcus pyogenes, SSP*: *Streptococcus species, S.E*: *Staphylococcus epidermidis.*

Table 3: Antimicrobial activity of essential oil extracts of *Lovoa trichiliodes* leaf on selected human pathogens

Conc. (µg/ml)/ Organisms	6.3	12.5	25	50	DMSO	Chl	Myz
B. S	6.33 ± 0.33^{a}	7.67 ± 0.67^{b}	13.33 ± 0.33^{d}	17.33 ± 0.33^{e}	NI	11.00 ± 0.00^{c}	N.A
B. S ATCC6633	6.33 ± 0.33^{a}	10.33 ± 0.33^{b}	12.00 ± 0.00^{c}	18.00 ± 0.58^{e}	NI	14.67 ± 0.67^{d}	N.A
S. A	7.33 ± 0.33^{a}	11.33 ± 0.33^{b}	14.67 ± 0.67^{c}	19.33 ± 0.33^{d}	NI	13.67 ± 0.00^{c}	N.A
S. A ATCC25923	8.00 ± 0.00^{a}	11.67 ± 0.33^{b}	15.33 ± 0.33^{c}	19.67 ± 0.33^{d}	NI	15.33 ± 0.33^{c}	N.A
E. C	7.33 ± 0.33^{a}	11.67 ± 0.33^{b}	14.33 ± 0.33^{c}	18.33 ± 0.33^{d}	NI	11.33 ± 0.00^{b}	N.A
E. C ATCC25922	8.33 ± 0.33^{a}	13.33 ± 0.33^{b}	17.00 ± 0.58^{c}	20.33 ± 0.88^{d}	NI	14.33 ± 0.67^{b}	N.A
E. F	5.33 ± 0.33^{a}	7.00 ± 0.00^{b}	10.00 ± 0.58^{c}	16.33 ± 0.33^{d}	NI	17.33 ± 0.33^{d}	N.A
K. P	NI	8.33 ± 0.33^{a}	10.67 ± 0.33^{b}	12.33 ± 0.33^{c}	NI	13.33 ± 0.00^{d}	N.A
S. T	NI	NI	NI	6.00 ± 0.58^{a}	NI	11.67 ± 0.00^{b}	N.A
S. T ATCC6539	NI	NI	5.33 ± 0.33^{a}	11.33 ± 0.33^{b}	NI	14.33 ± 0.67^{c}	N.A
Ps. A	NI	6.33 ± 0.33^{a}	9.33 ± 0.33^{b}	13.33 ± 0.33^{d}	NI	11.67 ± 0.00^{c}	N.A
A. F	NI	NI	6.33 ± 0.33^{a}	7.33 ± 0.33^{b}	NI	N.A	10.00 ± 0.00^{c}
C. A	NI	3.00 ± 0.58^{a}	8.33 ± 0.33^{b}	10.67 ± 0.33^{c}	NI	N.A	11.00 ± 0.00^{c}
C. A ATCC10231	NI	4.33 ± 0.33^{a}	8.33 ± 0.33^{b}	11.33 ± 0.33^{c}	NI	N.A	13.67 ± 0.33^{d}

Values are Mean ± S.E.M (mm), Values followed by different alphabet along the rows are significantly different at p ≤ 0.05, NI: No Inhibition, N.A: Not Applicable, Chl: Chloramphenicol, Myz: Miconazole, *B.S*: *Bacillus subtilis*, S.A: *Staphylococcus aureus*, E.C: *Escherichia coli*, *E.F*: *Enterococcus faecalis*, *K. P*: *Klebsiella pneumoniae*, A.F: *Aspergillus flavus*, C.A: *Candida albicans*, *C.G*: *Candida glabrata*, *S.P*: *Streptococcus pyogenes*, *SSP*: *Streptococcus species*, *S.E*: *Staphylococcus epidermidis*.

Table 4: Antimicrobial activity of essential oil from *Lovoa trichiliodes* stem bark on selected human pathogens.

essential oils respectively. Minimum inhibitory concentration (MIC) obtained in the double broth dilution of the extracts and essential oils of the plant materials against all the test pathogens are shown in Tables 5 and 6 respectively. The results showed that all the extracts and oils have significant antimicrobial activity against most of the tested microbial strains in the ranges 2.5-200 mg/ml and 3.0-60 µg/ml for the extracts and essential oils respectively. This significant activity may be due to the presence of monoterpenes and sesquiterpenes as reported in the work of Soroglou et al. [14]. The pathogens: *Escherichia coli, Enterococcus faecalis, Klebsiella pneumoniae, Bacillus subtilis* and *Staphylococcus aureus* were the most sensitive strains while *Salmonella typhi, Streptococcus pyogenes, Staphylococcus epidermidis, Streptococcus species, Cryptococcus neoformans* and *Trichophyton rubrum* were insensitive to the extracts and essential oils. The extracts and essential oils had more significant (p ≤ 0.05) antibacterial activity than those of fungi. The susceptible pathogens were even more sensitive to the plant materials than the reference antibiotics. The results corroborate

Test Organisms	MIC		MBC/MFC	
	leaf	Bark	Leaf	Bark
Bacillus subtilis	25	2.5	50	7.5
Bacillus subtilis ATCC6633	25	2.5	40	5
Staphylococcus aureus	2.5	5	7.5	10
Staphylococcus aureus ATCC25923	2.5	5	5	5
Staphylococcus epidermidis	200	100	ND	ND
Escherichia coli	10	5	40	15
Escherichia coli ATCC25922	10	5	40	12.5
Klebsiella pneumoniae	15	5	30	20
Salmonella typhi	200	15	ND	30
Salmonella typhi ATCC6539	200	15	ND	25
Pseudomonas aeruginosa	2.5	2.5	5	7.5
Aspergilus flavus	25	25	50	50
Candida albicans	15	5	40	20
Candida albicans ATCC10231	15	5	40	20
Cryptococcus neoformans	ND	100	ND	ND
Trichophyton rubrum	ND	200	ND	ND

ND: Not Detected.
Table 5: The MIC and MBC/MFC of acetone extracts of *L. trichiliodes* Harm. on the selected organisms.

Test Organisms	MIC		MBC/MFC	
	Leaf	Bark	Leaf	Bark
B. subtilis	10	5	20	7.5
B. subtilis ATCC6633	10	5	20	10
S. aureus	6.3	5	10	7.5
S. aureus ATCC25923	5	5	10	10
S. pyogenes	30	ND	50	ND
S. epidermidis	25	ND	40	ND
Streptococcus spp	40	ND	60	ND
E. coli	3	3	5	5
E. coli ATCC25922	3	3	5	5
E. faecalis	3	5	7.5	10
K. pneumonia	10	7.5	15	15
S. typhi	ND	20	ND	40
S. typhi ATCC6539	ND	15	ND	25
Ps. aeruginosa	ND	7.5	ND	15
A. flavus	40	20	75	40
C. albicans	40	10	60	15
C. *albicans* ATCC10231	40	10	50	20

ND: Not Detected.
Table 6: The MIC and MBC/MFC of Essential oils from *L. trichiliodes* on the selected organisms (µg/ml).

the reported antimicrobial activity of some members of the Meliaceae and the plant may serve as good source of cheap and highly effective antimicrobial agents for bacterial infections caused by multi-resistant organisms.

The radical scavenging activity of the extracts and essential oils of *L. trichiliodes* was measured by the *in vitro* DPPH assay. The percentage (%) scavenging of DPPH free radical was found to be concentration dependent at 517 nm. The more rapidly the absorbance decreases, the more potent the antioxidant activity of the sample in term of its hydrogen atom donating capacity [15]. The results of antioxidant and cytotoxic activities of the acetone extracts and essential oils of the plant materials as presented in Tables 7 and 8 respectively showed that all extracts and essential oils exhibited good DPPH radical inhibition activity compared with ascorbic acid and high cytotoxic property.

It was observed that the stem bark extract and essential oil

Conc. (mg/ml)	Stem bark extract	Leaf extract	Stem bark oil	Leaf oil	Ascorbic acid
0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
0.05	03.33 ± 0.03^{a}	04.17 ± 0.02^{a}	06.25 ± 0.03^{b}	05.33 ± 0.88^{b}	09.42 ± 0.04^{c}
0.10	11.42 ± 0.03^{a}	15.64 ± 0.57^{b}	56.86 ± 0.03^{d}	10.64 ± 0.03^{a}	46.27 ± 0.03^{c}
0.20	25.15 ± 0.01^{b}	24.27 ± 0.15^{b}	64.46 ± 0.07^{c}	15.33 ± 0.88^{a}	56.25 ± 0.03^{c}
0.40	27.46 ± 0.01^{b}	29.18 ± 0.00^{b}	71.88 ± 0.06^{d}	19.38 ± 0.01^{a}	64.38 ± 0.01^{c}
0.60	32.51 ± 0.00^{b}	32.62 ± 0.01^{b}	76.26 ± 0.02^{c}	26.25 ± 0.03^{a}	77.50 ± 0.06^{c}
0.80	38.17 ± 0.13^{b}	37.93 ± 0.01^{b}	80.64 ± 0.03^{c}	29.36 ± 0.03^{a}	81.86 ± 0.03^{c}
1.00	41.14 ± 1.15^{a}	38.16 ± 0.02^{a}	83.14 ± 0.03^{b}	38.76 ± 0.06^{a}	83.77 ± 0.02^{b}
1.20	52.21 ± 0.10^{b}	42.41 ± 0.15^{a}	86.88 ± 0.01^{c}	46.81 ± 0.12^{a}	86.40 ± 0.31^{c}
1.40	59.14 ± 0.01^{b}	48.05 ± 0.25^{a}	89.36 ± 0.03^{c}	51.85 ± 0.04^{a}	89.39 ± 0.06^{c}
1.60	63.75 ± 0.14^{b}	56.21 ± 0.00^{a}	88.14 ± 0.03^{c}	60.64 ± 0.03^{ab}	90.64 ± 0.03^{c}
1.80	62.33 ± 0.15^{a}	59.75 ± 0.01^{a}	90.33 ± 0.88^{b}	67.53 ± 0.07^{a}	91.81 ± 0.14^{b}
2.00	66.18 ± 0.02^{ab}	61.42 ± 0.01^{a}	88.14 ± 0.03^{c}	70.66 ± 0.02^{b}	93.81 ± 0.05^{c}
IC_{50}	**1.23 ± 0.15^{c}**	**1.65 ± 0.03^{d}**	**0.81 ± 0.15^{b}**	**1.52 ± 0.03^{d}**	**0.40 ± 0.15^{a}**

Table 7: Antioxidant activity of acetone extracts and essential oil of *Lovoa trichiliodes* (%DPPH scavenging inhibition).

Dosage(ppm) Leaf	Initial larvae	Acetone			Essential oil		
		No. of survivors	No. of deaths	% mortality	No. of survivors	No. of deaths	% mortality
1000	20	0	20.00 ± 0.00^{b}	100	2	18.00 ± 0.00^{a}	90
100	20	3	17.00 ± 1.00^{b}	85	6	14.33 ± 0.00^{a}	70
10	20	4	16.00 ± 0.02^{b}	80	16	4.00 ± 0.00^{a}	20
1	20	8	12.33 ± 0.05^{b}	60			
LC_{50}				0.71			52.65
Stem bark							
1000	20	0	20.00 ± 0.00^{b}	100	1	19.00 ± 0.00^{a}	95
100	20	6	14.00 ± 0.00^{a}	70	7	13.00 ± 0.00^{b}	65
10	20	7	13.00 ± 1.01^{a}	65	5	5.00 ± 0.00^{b}	25
1	20	10	10.33 ± 0.02^{b}	50			
LC_{50}				0.92			49.65

Values followed by different superscripts across each row are significantly different at $p \leq 0.05$

Table 8: Percentage mortality of brine shrimps at different concentrations of acetone extract and Essential oils of *Lovoa trichiliodes*.

showed higher DPPH free radical scavenging activity than the leaf extract and essential oil with IC_{50} values of 1.23 ± 0.15, 0.81 ± 0.15 and 1.65 ± 0.03, 1.52 ± 0.03 when compared with the ascorbic acid used as reference (0.40 ± 0.15) respectively. These results can be a strong scientific evidence of the use of this plant as a source of antioxidants.

Brine shrimps cytotoxic assay is a simple, quick and economical bioassay which has been designed to evaluate the cytotoxic potential of active natural products [16]. The result of cytotoxic activities of the *L. trichiliodes* extracts and essential oils had marked cytotoxic effect on brine shrimp larvae which was concentration dependent with percentage mortality of 50-100% and 25-95% and LC_{50} of 0.71 and 0.92; and 52.65 and 49.65 respectively for the leaf and stem bark acetone extracts and essential oils of the plant. It indicates the presence of anticancer activity of both the extracts and essential oils.

Conclusion

The results obtained in the study have shown that acetone extracts and essential oils of *L. trichiliodes* plant materials demonstrated significantly, good antimicrobial, antioxidant and anticancer activities against the test microorganisms, and may be exploited for discovery and development of new therapeutic agents. Further investigations should be conducted to isolate pure compounds in this plant for the development of new antimicrobial agents.

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