Asian Plant Research Journal

2(3): 1-11, 2019; Article no.APRJ.46504

Studies on the Antibacterial Activity and Chemical Composition of Methanol Extract of *Cochlospermum tinctorium* Root

R. Abdulaziz[1], M. H. Usman[2], U. B. Ibrahim[1], B. M. Tambari[3], A. Nafiu[2], I. F. Jumare[2], Mohammad A. Said[4] and A. D. Ibrahim[1*]

[1]Department of Microbiology, Usman Danfodiyo University, Sokoto, Nigeria.
[2]Department of Microbiology, Sokoto State University, Sokoto, Nigeria.
[3]School of Remedial Studies, Sokoto, Nigeria.
[4]Department of Biology, Alqalam University Katsina, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. Author RA designed the study. Author AID performed the statistical analysis. Author MHU and AN wrote the protocol and wrote the first draft of the manuscript. Author UBI and BMT managed the analyses of the study. Author FIJ and MAS managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/APRJ/2019/v2i330049
Editor(s):
(1) İrfan Ersin Akinci, Professor, Department of Horticulture, Kahramanmaras Sutcuimam University, Kahramanmaras, Turkey.
(2) Dr. Nesreen Houssien Abou- Baker, Associate Professor, Department of Soil and Water Use, Division of Agricultural and Biological Research, National Research Centre, Giza, Egypt.
Reviewers:
(1) E. S. Agoro, Federal Medical Centre, Yenagoa, Nigeria.
(2) Sara, Yahaya Galo, Umar Suleiman College of Education, Yobe State, Nigeria.
Complete Peer review History: http://www.sdiarticle3.com/review-history/46504

Original Research Article

Received 25 December 2018
Accepted 20 February 2019
Published 25 April 2019

ABSTRACT

The aim of study was to evaluate the antibacterial activity of *Cochlospermum tinctorium* against ten (10) strains of antibiotic resistant food-borne pathogens *Staphylococcus aureus* and *Listeria monocytogene*. Ten (10) strains of antibiotic resistant food-borne pathogens *Staphylococcus aureus* and *Listeria monocytogene* procured from Microbiology Research Laboratory Usman Danfodiyo University Sokoto. The roots of *Cochlospermum tinctorium* were collected from the rock side in Dambu Gomo, Zuru Local Government Area of Kebbi State, Nigeria. The roots were washed, air-dried and milled to powder using mortal and pestle and sieved to obtained fine powder. Maceration was used for extraction using methanol as solvent. The antibacterial activity of the plant

**Corresponding author: E-mail: Ibrahim.ibrahim@udusok.edu.ng, muhammadhiz04@gmail.com;*

was determined on Mueller Hinton agar using agar well diffusion method. Minimum concentration (MIC) and minimum inhibitory concentration (MBC) of plant extract was also determined. Thin layer chromatography and column chromatography was employed for separation and fraction of different compounds in the plant extract. The fractions were screened for antibacterial activity and active fractions having high antibacterial activity were subjected Gas Chromatography Mass Spectoscopy (GC-MS) analysis. The result of methanol extraction yield 5.17% extracts. The methanol extract of *Cochlospermum tinctorium* was effective in inhibiting the isolates at high concentration of 10 mg/mL. The results thin layer chromatography revealed four spots with Rf values 0.02, 0.37, 0.44 and 0.80 respectively. The GC-MS analysis of the active methanol extract of *Cochlospermum tinctorium* root powder revealed the existence of major peaks 1-(+)-Ascorbic acid 2,6-dihexadecanoate (R.T: 13.666), Diethyl phthalate (R.T: 10.440), Undecyl acetate (R.T: 10.007), 3-tetradecanone (R.T: 9.793), 3-hexadecanone (R.T: 12.427). It therefore concluded that the root of *Cochlospermum tinctorium* has immense potential to be used in the area of pharmacology as it possess antimicrobial activity against the antibiotic resistant food-borne pathogens, thus could be exploited as alternative antimicrobial drugs.

Keywords: Methanol; Cochlospermum tinctorium; antibiotic-resistant; pharmacology and pathogens.

1. INTRODUCTION

Cochlospermum tinctorium is a shrub that can grow up to 10 meters high. The slash is iodine-like in colour. Leaves are alternate, palmately lobed with stipules. Inflorescence consists of brightly colored yellow flowers that are regular and borne in racemes or panicles. Fruits are elongated, 3-5 valve, capsules containing seeds that are embedded in cotton foam. The seeds are bean-shaped with brown to black colour. It contains oily endosperm with broad cotyledon, it is a savannah plant found on fallow farm lands [1]. The bark, roots and seeds are used in the treatment of various ailments in different areas around the world. In Nigeria, a decoction of the root is used for treating gonorrhoea. It is used in the treatment of diabetes by the Igede people of Benue State [2]. The leaves are used in the treatment of malaria fever in some parts of Kogi State. In Mali the plant is variously used against jaundice, abdominal pains, haemorrhoids, intestinal worms, helminth, bilhazia and hepatitis. It was also reported to have been used against gastrointestinal diseases like ulcer, stomach ache, flatulence and constipation [3].

Staphylococcus aureus is capable of reproducing in wide range of physical conditions of temperature, pH and salt concerntration [4]. *Staphylococcus aureus* can be found in a variety of foods because of its ability to reside broad array of spaces in close proximity of human beings [5,6]. Moreover, *Staphyloccoccus aureus* is a leading cause of foodborne illness worldwide causing 2.41 million illnesses per year in the United State alone [7]. The basic cause of all these reported illness is by consuming food contaminated with *Staphyloccoccus aureus* derived toxins. About 1000 patients are hospitalized based on the severity of infection; 6 deaths may happen each year [7]. Severity of the symptoms depends on the amount of toxin consumed [8]. Disease condition is caused when the concerntration of toxin in the body is increased from 10^5 CFU/ml. Disease symptoms generally appear in 1-6 hours after eating the contaminated food.

Listeria monocytogenes, a member of the genus Listeria, naturally occurs in agricultural environments such as soil, manure and water [9]. Scientific literature frequently discusses the ability of this microorganism to survive in the food-processing, produce-packing environment and equipment, diverse habitat like soil, silage, marine and freshwater, sewage, vegetation, domestic and wild animal as well as humans [10,11,12]. Adzitey and Huda [13] pointed out that studies on *L. Monocytogene* and its association with foods is important to create more awareness in order to reduce its colonisation, transmission, cross contaminations and infections. Even though the reasons for the increasing number of pathogens causing food and water diseases in North America are found in Nigeria, occurence of food-borne *Listerial* infectionis not well reported. The reasons for the increasing number of pathogens include improved ability to isolate and identify organisms, import of a variety of products from abroad, large animal feeding stations and an increase in the number of immune compromised persons [14]. Hoelzer et al. [15] have reported that one major determinant of the listeriosis risk is the ability of a food to support the growth of *L. monocytogenes* during storage but data regarding the ability to support growth of the organisms are scarce or non-existent for many produce commodities.

Nigeria is bestowed with rich and diverse resources of plant wealth including an enormously large number of medicinal plants which are used extensively as anti-tumor, immune-modulators, anti-diabetics, purgatives, anti-inflammatory, anti-oxidants and antidotes. Most of these medicinal plants are undocumented in regards to their phytochemical characteristics, pharmacognostic characters, extractive value and also antibacterial activities. Since plants producea diverse range of bioactive molecules making them a rich source of different types of medicines, researches in bioactive substances might result to the discovery of new compounds that could be used to formulate new and more potent antibacterial drugs to overcome the problem of resistance to the currently available antibiotics. Also the importance of proper identification of these medicinal plants and their individual peculiar traits cannot be overstressed, it is vital that proper taxonomy is recorded in order not to confuse the plant in question with closely related species. The aim of this research is to study the antibacterial activity and chemical composition of methanol extract of *Cochlospermum tinctorium* root powder and to determine the chemical composition of the most active methanol extract of *Cochclospermum tinctorium* root powder using GC-MS (Gas chromatography- Mass spectrometry).

1.1 Sample Collection, Processing and Preparation

The roots of *Cochlospermum tinctorium* were collected from the rock side in Dambu Gomo, Rafin Zuru District, Zuru Local Government Area of Kebbi State, Nigeria. The samples were packaged in sterile polythene bags and it was transported to the Department of Microbiology Laboratory of Usmanu Danfodiyo University, Sokoto. *Cochlospermum tinctorium* roots were washed, air-dried and milled to powder using mortal and pestle and sieved to obtained fine powder and stored at room temperature with plastic packaging until use.

1.2 Test Bacteria Used for Antibacterial Screening

The test bacteria used in this research were obtained from Microbiology research laboratory, Usman Danfodiyo University Sokoto. The bacteria included Ten (10) food-borne isolates strains of *Staphylococcus aureus* and *Listeria monocytogene* isolated from onion, cabbage, lettuce and tomato.

1.3 Standardization of Bacterial Culture

The test bacteria cultures were sub-culture on nutrient agar and incubated at 37˚C for 24 hours. After incubation, a sterile wire loop was used to pick up the colonies of test bacterium and suspended in a test tube containing 10 mL of sterile normal saline. The turbidity of the innocula suspension was adjusted and standadized to that of 0.5 McFarland standard.

1.4 Determination of Antibacterial Activity of Plant Extract

The antibacterial activity of methanol extracts of *Cochlospermum tinctorium* was determined using agar well diffusion method. This was done by making well with equal size of 10.0 mm on freshly prepared Mueller Hinton. About 30 µL of standardized bacterial suspension was aseptically inoculated all over the media and allowed to settle for about 10 minutes. After which 0.5 mL of the following concentrations 10 mg/mL, 5.0 mg/mL and 5 mg/mL were placed into the wells and incubated at 37^{0}C for 24 hours.

1.5 Determination of Minimum Inhibitory Concerntration (MIC) and Minimum Bactericidal Concerntration (MBC) of the Extracts

The minimum inhibitory concerntration of the extracts was determined using the broth dilution method in nutrient broth. Five hundred micro-litres (500 µL) of the bacterial suspension were aseptically inoculated in each of the four tubes containing the extract in order of increasing dilution (10, 5, 2.5, 1.25 and 0.625 mg/mL). Thereafter, the test tubes were incubated at 37°C for 24 hours. After incubation, the test tube with the lowest concerntration of extracts without visible turbidity was taken to be the minimum inhibition concerntration (MIC) [16]. For determination of MBC, sample were taken from the broth with no visible growth in the MIC assay and subculture on freshly prepared nutrient agar and incubated at 37°C for 24 hours. The MBC was taken as the concentration of the extracts that did not show any visible growth on a new set of agar plates [17].

1.6 Thin-Layer Chromatography Profile of *Cochlospermum tinctorium* Root Extracts

Thin layer chromatography was run using TLC silica gel pre-coated plates in ascending manner. Capillary tube was used to spot the sample on the base line on a 10 cm by 4 cm TLC plates; the spots were developed in an air tight chromatin at room temperature. TLC separation of the *Cochlospermum tinctorium* was carried out using 100% methanol as the solvent system. The solvent front was allowed to travel at least 75% height on the TLC plate. Spots were visualized under day light, ultraviolet light (254 nm – 365 nm) and then by spraying with 10% tetraoxosulphate (IV) acid followed by heating in an oven for 4 minutes at 105˚C. The R_f values of distinct spots for the extract of *Cochlospermum tinctorium* root were calculated using the formula;

$$R_f = \frac{\text{Distance travelled by the spot}}{\text{Distance travelled by solvent}}$$

1.7 Column Chromatography Analysis of *Cochlospermum tinctorium* Extracts

One hundred and seven gram (107 g) of silica gel and mesh size was 70 – 230 was made into slurry with 100% methanol and was packed into a 2.5 cm x 63 cm glass column and allowed to stand for 24 hours to attain stability. Methanol extract was pre-adsorbed on 3 g of silica gel and loaded onto the column. The loaded sample was eluted gradient starting with 100% methanol, methanol: Ethyl acetate (80:20), methanol: ethyl acetate (60:40) and 100% ethyl acetate. All the fractions were collected separately and labelled as Fraction A, B, C, D, E and F, after which were subjected to antimicrobial screening and GC/MS analysis

1.8 Determination of Antibacterial Activity of Active Fractions of *Cochlospermum tinctorium* Extract

The antibacterial activity of active fractions of *Cochlospermum tinctorium* root powder was determined by well diffusion method. This was done by making well with equal size of 10.0 mm on freshly prepared Mueller Hinton. About 30 µL of standardized bacterial suspension was aseptically inoculated all over the media and allowed to settle for about 10 minutes. After which 0.5 mL of the following Fraction A, B, C, D, E and F were placed into the wells and incubated at 37˚C for 24 hours.

1.9 Gas Chromatography Mass SPECTOSCOPY (GC-MS) Analysis of the Active Fractions

GC-MS analysis was performed using GC-MS-QP2010 Plus (Shimadzu, Japan) and Gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument employing the following; Column Elite-1 fused silica capillary column (30m x 0.25mm 1D x µl df, composed of 100% Trisil). For GC-MS detection, an electron ionization system with ionization energy of 70eV was used. Helium gas (99.999%) was used as the carrier gas at constant flow rate 1ml/min and an injection volume of 2 µL was employed (Split ratio of 20:0) injector temperature 250˚C; ion-source temperature 200˚C. the oven temperature was programmed from 60.0 (for 0.00 minute) with an increase of 160˚C (Isothermal for 2.00 minutes) ending with a 2.00 minutes isothermal at 280˚C. Mass spectra were taken at 70eV; a scan interval of 0.5s and fragments from 45 to 700Da. Total GC running time was 19 minutes. The relative percentage amount of each component was calculated, by comparing its average peak area to the total areas, Software adopted to handle mass spectra and chromatogram was a turbomass. The detection employed the NIST Ver.2.0 year 2009 library [18].

1.10 Identification of Components

Interpretation on mass spectrum of GC-MS was done using the database of National Institute of Standard and Technology (NIST) having more than 62,000 patterns. The mass spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained.

2. RESULTS

The percentage yield of the crude extracts (g) obtained from the methanol extracts are presented on Table 1 the result indicates that methanol had the percentage yield of 5.17%.

The result of the thin layer chromatography (TLC) of *Cochlospermum tinctorium* crude methanol extract are presented on Table 2. The solvent system used was 100% methanol and four spots were visible and their Rf values are 0.02, 0.37, 0.44 and 0.80.

Table 1. Percentage yield of crude methanol extract of *Cochlospermum tinctorium* root powder

Solvent	Mass of sample (g)	Yield of the extract (g)	Percentage Yield (%w/w)
Methanol	300	15.5	5.17

Table 2. Thin layer chromatography (TLC) of the crude methanolic extract of *Cochlospermum tinctorium* root powder

Solvent system	Spots movement (cm)	Solvent front (cm)	R_f value
Methanol extract	0.2	9.8	0.02
	3.6	9.8	0.37
	4.3	9.8	0.44
	7.8	9.8	0.80

The antibacterial activity of the crude methanol extracts of the roots of *Cochlospermum tintorium* against antibiotic resistant *Staphylococcus aureus* and *Listeria monocytogene* (Table 4). The methanol extract revealed maximum zone of inhibition of 22.00 mm against antibiotic resistant *Staphylococcus aureus* isolated from tomato and 21.00 mm against *L. monocytogene* R1 at concerntration of 10 mg/ml, while the lowest zones of inhibition of 12.00 mm was recorded against *Staphyloccoccus aureus* isolated from spring onion and *L. monocytogene*.

The result of the column chromatography (CC) of *Cochlospermum tinctorium* crude methanol extract are shown on Table 3. The result indicates that ratio (80:20) had the highest number of active fractions of 3, followed by ratio (60:40) having 2, and lastly ratio (100:0) having 1 fraction only.

Table 3. Column chromatography (CC) of the crude methanolic extract of *Cochlospermum tinctorium* root powder

Solvent	Solvent ratio	Fractions
Methanol	80:20	3
	60:40	2
	0:100	0
	100:0	1

The result of the minimum inhibitory concerntration (MIC) of *Cochlospermum tinctorium* crude methanol extract against antibiotic resistant *Staphyloccoccus aureus* and *L. monocytogene* are presented on Table 5. It was observed that the *Staphyloccoccus aureus* SP1, SP2, and L showed MIC at 2.5mg/mL while *Staphyloccoccus aureus* T showed MIC at 1.25 mg/mL, the *L. monocytogene* R1, R2, R3, R4 showed MIC at 2.5 mg/mL while R5 showed MIC at 0.625 mg/mL. The minimum inhibitory

Table 4. Antibacterial activity of *Cochlospermum tinctorium* crude methanol extract against the antibiotic resistant *Staphylococcus aureus* and *Listeria monocytogene*

Test Organisms	Concentrations/Zone of inhibition in (mm)				
	10 mg/mL	5 mg/mL	2.5 mg/mL	Negative Control	Positive Control
Staphyloccoccus aureus SP1	19.0±0.6	16.0±0.2	12.0±0.9	0.00	22.0±0.3
Staphyloccoccus aureus SP2	20.0±0.9	18.0±0.4	13.0±0.8	0.00	20.0±0.8
Staphyloccoccus aureus SP2	19.0±0.5	15.0±0.6	12.0±0.6	0.00	20.0±0.4
Staphyloccoccus aureus L	20.0±0.4	16.0±0.1	14.0±0.2	0.00	21.0±0.6
Staphyloccoccus aureus T	22.0±0.6	20.0±0.6	17.0±0.4	0.00	24.0±0.6
L. monocytogene R1	21.0±0.5	18.0±0.5	13.0±0.7	0.00	25.0±0.3
L. monocytogene R2	20.0±0.6	18.0±0.3	14.0±0.3	0.00	26.0±0.5
L. monocytogene R3	21.0±0.1	19.0±0.4	14.0±0.5	0.00	26.0±0.4
L. monocytogene R4	19.0±0.4	16.0±0.6	12.0±0.6	0.00	24.0±0.2
L. monocytogene R5	20.0±0.3	15.0±0.6	14.0±0.3	0.00	28.0±0.6

Key: SP = Spring onion, R = Cabbage, L = Lettuce and T = Tomato. The result is presented as mean±SD concerntration of the crude methanol extract was obtained between 2.5-0.625 mg/mL for both *Staphyloccoccus aureus* and *L. monocytogene*.

The result of the minimum bactericidal concerntration (MBC) of *Cochlospermum tinctorium* crude methanol extract against antibiotic resistant *Staphyloccoccus aureus* and *L. monocytogene* are presented in Table 6. From the results obtained isolates *Staphyloccoccus aureus* SP1, SP2 and L showed MBC at 5 mg/mL while *Staphyloccoccus aureus* T showed MBC at 2.5 mg/mL, the *L. monocytogene* R1, R2, R3, R4 showed MIC at 5 mg/mL while R5 showed MBC at 2.5 mg/mL.

The results for the antibacterial activity of the active methanol extract of *Cochlospermum tinctorium* root powder against antibiotic resistant *Staphyloccoccus aureus* and *L. monocytogene* are presented in Table 7. The active methanol extract of *Cochlospermum tinctorium* root powder reveals maximum zone of inhibition 26.00 mm against *Staphyloccoccus aureus* L, 20.00 mm against *L. monocytogene* R4 and minimum inhibition of 15.00 mm against *Staphyloccoccus aureus* L, 12.00 mm against *L. monocytogene* R2.

Table 5. Minimum inhibitory concerntration (MIC) of *Cochlospermum tinctorium* crude methanol extract against antibiotic resistant *Staphyloccoccus aureus* and *L. monocytogene*

Test organisms	Concentrations of extracts				
	10 mg/mL	5 mg/mL	2.5 mg/mL	1.25 mg/mL	0.625 mg/mL
Staphyloccoccus aureus SP1	-	-	©	+	+
Staphyloccoccus aureus SP2	-	-	©	+	+
Staphyloccoccus aureus SP2	-	-	©	+	+
Staphyloccoccus aureus L	-	-	©	+	+
Staphyloccoccus aureus T	-	-	-	©	+
L. monocytogene R1	-	-	©	+	+
L. monocytogene R2	-	-	©	+	+
L. monocytogene R3	-	-	©	+	+
L. monocytogene R4	-	-	©	+	+
L. monocytogene R5	-	-	-	-	©

Key: SP = Spring onion, R = Cabbage, L = Lettuce and T = Tomato and © = MIC

Table 6. Minimum bactericidal concerntration (MBC) of *cochlospermum tinctorium* crude methanol extract against antibiotic resistant *Staphyloccoccus aureus* and *L. monocytogene*

Test isolate	Concentrations				
	10 mg/mL	5 mg/mL	2.5 mg/mL	1.25 mg/mL	0.6 mg/mL
S. aureus SP 1	-	¢	+	+	+
S. aureus SP 2	-	¢	+	+	+
S. aureus C	-	¢	+	+	+
S. aureus L	-	¢	+	+	+
S. aureus T	-	-	¢	+	+
L. monocytogene R1	-	¢	+	+	+
L. monocytogene R2	-	¢	+	+	+
L. monocytogene R3	-	¢	+	+	+
L. monocytogene R4	-	¢	+	+	+
L. monocytogene R5	-	-	¢	+	+

Key: SP = Spring onion, R = Cabbage, L = Lettuce and T = Tomato, ¢ = MBC

The result of the minimum inhibitory concerntration (MIC) of the active methanol fractions of *Cochlospermum tinctorium* root powder against antibiotic resistant *Staphyloccoccus aureus* and *L. monocytogene* are presented on Table 8. From the results obtained isolate *Staphyloccoccus aureus L* showed MIC at 4.0 mL, *L. monocytogene* R5 showed MIC at 5.0 mL, *L. monocytogene* R2 showed MIC at 3.0 ml and *Staphyloccoccus aureus* R showed MIC at 3.0 mL.

The result of the volatile organic compound profile of the active methanol fraction (A) of *Cochlospermum tinctorium* root powder tested against antibiotic resistant *Staphylococcus aureus* and *Listeria monocytogene* are presented on Table 9a. The chromatogram shows 23 peaks (compounds) in fraction A of which the highest peak intensity was observed at peak 3 (3-Tetradecanone- 20.99%) and the lowest at peak 15 (5-Hexyn-1-ol- 0.22%).

The result of the volatile organic compound profile of the active methanol fraction (E) of *Cochlospermum tinctorium* root powder tested against antibiotic resistant *Staphylococcus aureus* and *Listeria monocytogene* are presented on Table 9b. The chromatogram shows 11 peaks (compounds) in fraction E of which the highest peak intensity was observed at peak 11 (i-Propyl 9,12-octadecenadienoate - 69.12%) and the lowest at peak 3 (Silane, trimethyl(2-phenylethoxy)- 0.26%). Other compounds identified in fraction E include; Cyclotrisiloxane,hexamethyl-, 4-Isothiazole-carboxamide, .Omega.-Phenylacetic acid, Benzeneethanol, 4-hydroxy-, Pyrazolo[5,1-c]-as-triazine-, 1,2-Butadiene,1,1,4-triphenyl-3-trimethylsilyl-4-trimethylsilyloxy-, Diethyl Phthalate1, l-(+)-Ascorbic acid 2,6-dihexadecanoate, Heptanoic acid, 2-ethyl-.

3. DISCUSSION

The methanol extract yield obtained in this study appears promising for the plant. This is similar with the finding of Ibrahim et al. [19] who reported 5% yield for methanol extract of *Ceiba pentandra*. Plants are known to possess different chemical constituents and vary between plant species. The % yield obtained might be attributed to the variability in the constituents of the plant that have impact on the solubility of the constituents and the polarity of methanol [20]. The yield obtained is within ranges reported and suggest that the root of *C. tinctorium* possess posses an extractable yield of phyto-chemicals. The choice of methanol is due to the fact that it has been shown to be suitable for the isolation of lipophilic compounds [21]. The lipophilic activity has been shown to significantly affect the antibacterial activity of certain class of compounds [22].

The result of the column chromatography of *Cochlospermum tinctorium* crude methanol extract revealed that the methanol and ethyl acetate in ratio of 80:20 had the highest fractions of three compound. This indicated its suitability

Table 7. Antibacterial activity of active methanol fractions of *Cochlospermum tinctorium* root powder against antibiotic resistant *Staphyloccoccus aureus* and *L. monocytogene*

Fraction	Test organism	Zone of Inhibition (mm)		
Fraction A	*Staphyloccoccus aureus* L	22.0±0.6	26.0±0.5	23.0±0.6
Fraction B	*Staphyloccoccus aureus* L	15.0±0.3	16.0±0.6	16.0±0.2
Fraction D	*L. monocytogene* R5	14.0±0.8	14.0±0.9	13.0±0.3
Fraction E	*L. monocytogene* R4	20.0±0.6	19.0±0.4	21.0±0.8
Fraction F	*L. monocytogene* R2	13.0±0.5	13.0±0.1	12.0±0.4

Key: L = Lettuce, R = Cabbage. The result is presented as mean±SD

Table 8. The minimum inhibitory concerntration (MIC) of the active methanol active fractions of *Cochlospermum tinctorium* root powder

Fraction	Test organism	0.1 mL	0.2 mL	0.3 mL	0.4 mL	0.5 mL	0.6 mL	0.7 mL
Fraction A	*S. aureus* L	-	-	-	-	+	+	+
	L. monocytogene R5	-	-	-	-	-	+	+
Fraction E	*L. monocytogene* R2	-	-	-	+	+	+	+
	S. aureus R	-	-	-	+	+	+	+

Key: L= Lettuce, R = Cabbage, + = Positive, - = Negative

Table 9a. Volatile organic compound profile of the active methanol fraction A of *Cochlospermum tinctorium* root powder tested against antibiotic resistant *Staphyloccoccus aureus* and *L. monocytogene*

RT^{-1}	Compound	Molecular formular	Peak area normalised (%)
4.673	Tris (trimethylsilyl) amine	$C_9H_{27}NSi_3$	9.60
9.702	Undecane, 3-methylene-	$C_{12}H_{24}$	11.36
9.793	3-Tetradecanone	$C_{14}H_{28}O$	20.99
10.007	Undecyl acetate	$C_{13}H_{26}O_2$	7.82
10.926	1-Tridecene	$C_{13}H_{26}$	1.16
11.231	2-Heptanone, 4-methyl-	$C_8H_{16}O$	0.45
11.950	Saccharin	$C_7H_5NO_3S$	0.23
12.285	Heptanoic acid, 2-ethyl-, methyl ester	$C_{10}H_{20}O_2$	1.50
12.359	Tridecane, 3-methylene-	$C_{14}H_{28}$	1.80
12.427	3-Hexadecanone	$C_{16}H_{32}O$	2.05
12.584	1-Hexadecanol, acetate	$C_{18}H_{36}O_2$	20.82
12.947	Butanoic acid, 3-methyl-, 3,7-dimethyl-6-octenyl ester	$C_{15}H_{28}O_2$	0.99
13.022	3,3-Dimethyl-4-heptanol	$C_9H_{20}O$	0.68
13.436	(R)-(-)-(Z)-14-Methyl-8-hexadecen-1-ol	$C_{17}H_{34}O$	0.80
13.819	5-Hexyn-1-ol	$C_6H_{10}O$	0.22
14.311	Lauric acid, isopentyl ester	$C_{17}H_{34}O_2$	10.05
14.537	Heptanal n-Heptaldehyde	$C_7H_{14}O$	1.02
14.792	1-Hexadecanol, acetate	$C_{18}H_{36}O_2$	3.09
14.870	Stearic acid, ethyl ester	$C_{20}H_{40}O_2$	0.77
15.300	(R)-(-)-(Z)-14-Methyl-8-hexadecen-1-ol	$C_{17}H_{34}O$	0.76
16.010	Oleyl alcohol, trifluoroacetate	$C_{20}H_{35}F_3O_2$	0.60
16.318	Tridecane, 3-methylene-	$C_{12}H_{24}$	1.67
17.077	Oleyl alcohol, trifluoroacetat	$C_{20}H_{35}F_3O_2$	1.58

Table 9b. Volatile organic compound profile of the active methanol fraction (E) of *Cochlospermum tinctorium* root powder tested against antibiotic resistant *Staphyloccoccus aureus* and *L. Monocytogene*

RT^{-1}	Compound	Molecular Formular	Peak Area Normalised (%)
5.014	Cyclotrisiloxane, hexamethyl-	$C_6H_{18}O_3Si_3$	2.31
6.071	4-Isothiazolecarboxamide	$C_4H_4N_2OS$	0.59
6.490	Silane, trimethyl(2-phenylethoxy)-	$C_{11}H_{18}OSi$	0.26
6.670	.Omega.-Phenylacetic acid	$C_8H_8O_2$	0.38
8.654	Benzeneethanol, 4-hydroxy-	$C_8H_{10}O_2$	0.64
10.042	Pyrazolo[5,1-c]-as-triazine-	$C_7H_6N_4O_2$	0.58
10.234	1,2-Butadiene,1,1,4-triphenyl-3-trimethylsilyl-4-trimethylsilyloxy-	$C_{28}H_{34}OSi_2$	0.36
10.440	Diethyl Phthalate 1	$C_{12}H_{14}O_4$	2.83
13.666	1-(+)-Ascorbic acid 2,6-dihexadecanoate	$C_{38}H_{68}O_8$	22.02
13.934	Heptanoic acid, 2-ethyl-	$C_9H_{18}O_2$	0.92
15.064	i-Propyl 9,12-octadecenadienoate	$C_{21}H_{38}O_2$	69.12

in separation of different phyto-chemical constituents found within the plants. It was reported that the column chromatography is one of the most popular and widely used separation techniques used in characterizing both organic and inorganic material and thus suggesting its potential usefulness in chemical analysis of complex extract materials [23].

The results of thin layer chromatography revealed different R_f values. The differences in R_f value is an indication that different compounds were presence in the methanol extract of plant. The used of TLC is an inexpensive which provides answer as to how many components are in a mixture. It is also used to support the identity of a compound in a mixture when the Rf of a compound is compared with the Rf of a known compound. The TLC is one of the common practice for isolation and separation of the bioactive compounds that are then used for the determination of structure and biological activity [21]. It was reported that the component which shows less R_f value in a less polar solvent has high polarity and a high R_f values in less polar solvents shows that the compound is less polar [24]

The results of the antibacterial of crude methanol extracts showed that the *C. tinctorium* was active against the various test bacteria at different concentrations tested. However, the plant showed good inhibition toward test bacterial isolates. It was observed that at higher concentration of 10 mg/mL the inhibition activity fall within 19.00 – 21.00 mm which very closed to the control antibiotic with inhibition zone of 20 - 28mm. The reason for high antbacterial activity could be attributed to fact that *Staphyloccoccus aureus* and *L. monocytogene* are gram-positive bacteria whose outer peptidoglyan layer is not an effective permeability barrier. However, the high activity of crude methanol extract of the plant might attributed to the presence of varying different bioactive compounds which exerted their action in a different ways and thus resulting in inhibition the growth of bacteria. The mechanisms of action of plant constituents is not yet fully understood it is clear that the effectiveness of the extracts is largely depend on the type of solvent used. This observation is clearly indicated that the existence of non-polar residue in the plant extracts have contributed to its bactericidal and bacteristatic s activity [25]. Cowan [26] also reported that most antibiotics compounds already identified in the plants are reportedly aromatic or saturated organic molecules which can easily solubilized in the organic solvents. However, due to the emergence of antibiotic resistant, plants are being looked upon as an excellent alternate to combat the further spread of multidrug resistant microorganisms [27].

The minimum inhibitory concerntration of the crude methanol extract was obtained between 2.5 - 0.625 mg/mL for both *Staphyloccoccus aureus* and *L. Monocytogene* while the MBC was observed between 5.0 - 2.5 mg/mL. In this study we observed that the MIC value obtained were lower than the MBC values. This indicates that the plant extracts were bacteriostatic at lower concentration but bactericidal at higher concentration. Similar finding of Aliyu et al. [28] obtained MIC at 2.09 mg/mL against *Staphyloccoccus aureus* in the antibacterial activity of leaf extract of *Stereospermum kunthianum* (Bignoniaceae), and Kim *et al.* [29] obtained MIC at 2.0 mg/mL against *L. monocytogene* in the antibacterial activity of *Saposhnikovia divaricata*, *Peucedanum japonicum* and *Glehnia littoralis*. The Previous studies by Okemo et al. [30] suggested that at higher concerntration of plants extract the more rapidly organisms would be killed.

The active methanol extract of *Cochlospermum tinctorium* root powder reveals maximum zone of inhibition 26.00 mm against *Staphyloccoccus aureus* L and 21.00 mm was observed against *L. monocytogene* R4. The high activity of fraction A against *Staphyloccoccus aureus* and fraction E against *L. monocytogene* is an indication that the active compound presence in the plant exerted more antibacterial activity when it's in active and pure form. The low activity of active fraction B, D and F against the test bacteria indicated that the constituent might exert synergistic effect in inhibiting the growth of test organisms. This study is in close agreement with a previous studies of Arora et al. [31] that obtained 22.30 mm against *L. Monocytogene* in the antibacterial activity of seed, pomace and leaf extract of *Hippophae rhamnoides L*.

The GC-MS analysis of the active fractions showed the existence of various bioactive compounds with different chemical structures. The major compound in both fraction (A and E) are: 1-(+)-Ascorbic acid 2,6-dihexadecanoate, Diethyl phthalate, Undecyl acetate, 3-tetradecanone, 3-hexadecanone. Previous studies reported that 1-(+)-Ascorbic acid 2,6-dihexadecanoate which is identified in the aqeous extract of *Indigofera tinctoria* possess an antioxidant, anti-inflammatory and anti-nociceptive properties [32]. Diethyl phthalate was identified in the methanol extract of the flower of *Quisqualis indica* plant extract and it was found effective against *E. coli* and least effective against *S. pnemoniae, Staphyloccoccus aureus* [33,34]. Undecyl acetate was identified in the essential oil of *C. planchonii* and was effective against diarrhoea and some other infections [35]. 3-tetradecanone, 3-hexadecanone were identified in the essential oil of whole tubercle of *C. tinctorium* and was found to posess anti plasmodial properties [36].

4. CONCLUSION

From the above research it can be concluded that *Cochlospermum tinctorium* root powder has immense potential to be used in the area of pharmacology as it possess antimicrobial activity against the antibiotic resistant food-borne pathogens, thus could be exploited as alternative antimicrobial drugs for the treatment of diseases caused by those pathogens. Due to the presence of various compounds that are essential for good health, it can also be used to improve the health status of the mankind. The volatile organic compound profiling of the major compounds showed that they possess antimicrobial, anti-inflammatory and antinociceptive properties.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Mann Abdullahi, Muhammad G, Abdulkadir NU. Medicinal and economic plants of nupeland. Jube-Evans Books and Publication State, 1st edition. 2003;34-98.
2. Igoli JO, Igwe IC, Igoli NP. Traditional medicinal practices among the igede people of Nigeria. Journal of Herbs, Spices and Medicinal Plants. 2003;10(4):1-10.
3. Diallo B, Vanhaelen M, Kiso Y, Hikino H. Antihepatotoxic actions of *Cochlospermum tinctorium* Rhizomes. Journal of Ethnopharmacology. 1987;20:239-243.
4. Chaibenjawong P, Foster SJ. Desiccation-tolerance in *Staphylococcus aureus*. Arch. Microbiol. 2011;193:125-135.
5. Le LY, Baron F, Gautier M. *Staphylococcus aureus* and food poisoning. Genetics and Molecule Research. 2003;2:63-76.
6. Tango CN, Khan I, Park YS, Oh DH. Growth of *Staphylococcus aureus* in cookedready-to-eat ground fish as affected by inoculumsize and potassium sorbate as food preservative. Food Science Technology. 2016;71:400-408.
7. Scallan E, Hoekstra RM, Angulo FJ, Tauxe RV, Widdowson MA, Roy SL, Jones JL, Griffin PM. Foodborne illness acquired in the United States-major pathogens. Emerging Infectious Disease. 2011;17: 231-253.
8. Safety F. *Staphylococcus aureus* a problem when food is left out too long. Available:http://ohioline.Osu.Edu (Assessed on 05 July, 2015)
9. Jeyaletchumi P, Tunung R, Selina PM, Chai LC, Radu S, Farinazleen MG, Cheah YK, Mitsuaki N, Yoshitsugu N, Kumar MP. Assessment of *Listeria monocytogenes* in salad vegetables through kitchen simulation study. Journal of Tropical Agriculture and Food Science. 2012;40:55-62.
10. Azizoglu RA, Gorski L, Kathariou S. Listeria and produce: A troublesome liaison! Available:http://www.newfoodmagazine.com/advent-calendar/listeria-and produce/ (Assessed on 10 February 2017)
11. Ivanek R, Gröhn YT, Wiedmann M. Listeriamonocytogenes in multiple habitats and hostpopulations: Review of available data formathematical modeling. Food-borne Pathology of Disease. 2006;3:319-336.
12. Sauders BD, Wiedmann M. Ecology of *Listeria* spp and *L. monocytogenes* in the natural environment. Food Science and Technology. 2007;161:21.
13. Adzitey F, Huda N. *Listeria monocytogenes* in foods: Incidences and possible control measures. African Journal of Microbiology Research. 2010;4:2848-2855.
14. Wadhwa SG, Khaled GH, Edberg SC. Comparative microbial character of consumed food and drinking water. Critical Reviews in Microbiology. 2002;28:249-279.
15. Hoelzer K, Pouillot R, Dennis S. *Listeria monocytogenes* growth dynamics on produce: A review of the available data for predictive modeling. Food borne Pathogens and Disease. 2012;9:661-673.
16. Williams L, Wilkins S. Textbook of microbiology, 2nd Edition, New Delhi, India: Kluwer Health Publishers. 2007;30-31.
17. Akinjogunla, et al. Antimicrobial potential of *Nymphae lotus* (Nymphaeaceae) against wound pathogens. International Journal of Ethnopharcognosy. 2009;3(3):138-141.
18. Umar ZU, Mahaneem M. Analysis of phytochemical compound in water and methanol extracts of Malaysian propilis. International Journal of Pharma. and Bioscience. 2016;6(2):374-380.
19. Ibrahim R, Abubakar EM, Modibbo SM, Lamaran BG. Percentage yield and acute toxicity of plant extracts of *Ceiba pentandra* grown in Bauchi State, North Estern Nigeria. Journal of Pharmacognosy and Phytochemistry. 2017;6(5):1777-1779.
20. Hostettman K. Strategy for chemical evaluation of plant extracts. Pure Applied Chemistry. 1998;70(11):1-9.

21. Kinjumgiev A, Tsvetkova L, Sarkedjieve Y, Bankova V, Christo R, Propov S. Antibacterial, antifungal and antiviral activity of propilis for different geographical region. Journal of Ethnopharmacognosy. 1999;64:235-240.
22. Aliyu MS, Hauwa UA, Tijjani AB, Aliyu AB, Ya'u B. Phytochemical and antibacterial activity of *Stereospermum kunthianum* (Bignoniaceae). Nigerian Journal of Basic and Applied Science. 2017;17(2):235-239.
23. Kim M, Seo SK, Yun W. Antimicrobial and antioxidant activity of *Saposhnikovia divaricata*, *Peucedanum japonicum* and *Glehnia littoralis*. Indian Journal of Pharmaceutical Science. 2018;80(3):560-563.
24. Das Talukdar M, Dutta Choudhury, Chakraborty M, Dutta BK. Phytochemical screening and TLC profiling of plant extracts of Cyathea gigantea (Wall. Ex. Hook.) Haltt and Cyathea brunoruana Wall. Ex Hook (Cl and Bak). Assam University Journal of Science and Technology. 2010;5(1):70-74.
25. Antara S, Amla B. Evaluation of antimicrobila activity of different solvent extracts of medicinal plant: *Melia azedarach* L. International Journal of Current Pharmaceutical Research. 2012; 4(2):67-73.
26. Cowan M. Plants products as microbial as agents. Clinical Microbiology Review. 1999;12:564-582.
27. Rosina K, Barira Islam I, Mohd A, Shazi S, Anis AS, Manazir A, Mashiatullah S, Asad UK. Antimicrobial activity of five herbal extracts against multi drug resistant (MDR) strains of bacteria and fungus of clinical origin. Molecules. 2009;14:586-597.
28. Aliyu MS, Hauwa UA, Tijjani AB, Aliyu AB, Ya'u B. Phytochemical and antibacterial activity of *Stereospermum kunthianum* (Bignoniaceae). Nigerian Journal of Basic and Applied Science. 2017;17(2):235-239.
29. Kim M, Seo SK, Yun W. Antimicrobial and antioxidant activity of *Saposhnikovia divaricata*, *Peucedanum japonicum* and *Glehnia littoralis*. Indian Journal of Pharmaceutical Science. 2018;80(3):560-563.
30. Okemo PO, Mwatha WE, Chhabra SC, Fabry W. The kill kinetics of *Azadirachta indica* (Meliaceae) extracts in *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Candida albicans*. African Journal of Science and Technology. 2001;2:113-118.
31. Arora R, Suni M, Ashish Y, Ravi B, Tsering S. Antimicrobial activity of sed, pomace and leaf extracts of sea buckthorn (*Hippophae rhamnoides* L.) against foodborne and food spoilage pathogens. Africa Journal of Biotechnology. 2012; 11(45):10424-10430.
32. Ogunlesi M, Okiei W, Osibote EA. Analysis of the essential oil from the leaves of Sesamum radiatum, a potential medication for male infertility factor, by gas chromatography – mass spectrometry. African Journal of Biotechnology. 2010;9:1060-1067.
33. Kiruthika KA, Jaisheeba AA, Sornaraj R. Evaluation of antibacterial activity of some selected *Angiosperm* flower extract. International Journal of Chem Tech Research. 2011;3(4):1945-51.
34. Kumar M, Gitika Sharma A. *In vitro* antibacterial activity of flower extracts of *Quisqualis indica* Linn. against gram-positive and gram-negative bacteria International Journal of Advances in Pharmacy, Biology and Chemistry. 2014;3(3):781-5.
35. Lassina Ouattara, Jean Koudou, Louis CE, Obame Damintoti, Karou S, Alfred Traore, Jean Marie Bessiere. Chemical composition and antibacterial activity of *Cochlospermum* planchoni Hook. f. Ex planch essential oil from Burkina Faso. Pakistan Journal of Biological Sciences. 2007;10:4177-4179.
36. Benoit-Vical F, Valentin A, Mallie M, Bessiere JM. Antiplasmodial activity of *Cochlospermum planchonii* and *Cochlospermum tinctorium* tubercles essential oils. Journal of Essential Oil Research. 2001;13:65-67.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle3.com/review-history/46504